

MODEL INFORMED DRUG DEVELOPMENT AND PRECISION  
DOSING FOR DRUG-DRUG-GENE-INTERACTIONS

APPLICATION OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING

DISSERTATION

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## INCLUDED PUBLICATIONS

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PUBLICATION III - SIMVASTATIN: **Jan-Georg Wojtyniak**, Dominik Selzer, Matthias Schwab, and Thorsten Lehr. "Physiologically based precision dosing approach for drug-drug-gene interactions: a simvastatin network analysis." In: *Clinical Pharmacology & Therapeutics* (Dec. 2020). DOI: 10.1002/cpt.2111

## CONTRIBUTION REPORT

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Following the contributor roles taxonomy (CRediT) [4, 5], the author would like to declare his contributions to the publications included in this thesis.<sup>1</sup>

**PUBLICATION I - DATA DIGITIZING:** Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project Administration

**PUBLICATION II - ZOPTARELIN DOXORUBICIN:** Methodology, Software, Validation, Formal Analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization

**PUBLICATION III - SIMVASTATIN:** Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project Administration

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<sup>1</sup> For a description of the different taxonomy categories see appendix Chapter B

## ABSTRACT

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The global demand for pharmaceuticals is continuously growing. As a result, one can observe an increase in adverse drug reactions, which pose a critical risk to patients. The primary triggers for adverse drug reactions are drug-drug- and drug-gene-interactions. Model-informed drug discovery and development as well as model-informed precision dosing can help to mitigate the risks of drug-drug and drug-gene interactions.

Thus, this work aimed to improve and to apply physiologically-based pharmacokinetic modeling strategies in the context of model-informed drug discovery and development as well as model-informed precision dosing.

For this purpose, best practices for data digitization as an essential step in the development process of most physiologically-based pharmacokinetic models have been established. Moreover, models for zoptyarelin doxorubicin and simvastatin were developed and evaluated. The zoptyarelin doxorubicin model was used to guide the development process of this drug. In contrast, the simvastatin model was utilized in a drug-drug-gene interaction network to generate 10 368 dose recommendations for different interaction scenarios, which were made available in a digital decision support system.

In conclusion, the work can be seen as a beacon project to illustrate how physiologically-based pharmacokinetic modeling of drug-drug and drug-gene interactions can be applied in model-informed drug discovery and development as well as in model-informed precision dosing.

## ZUSAMMENFASSUNG

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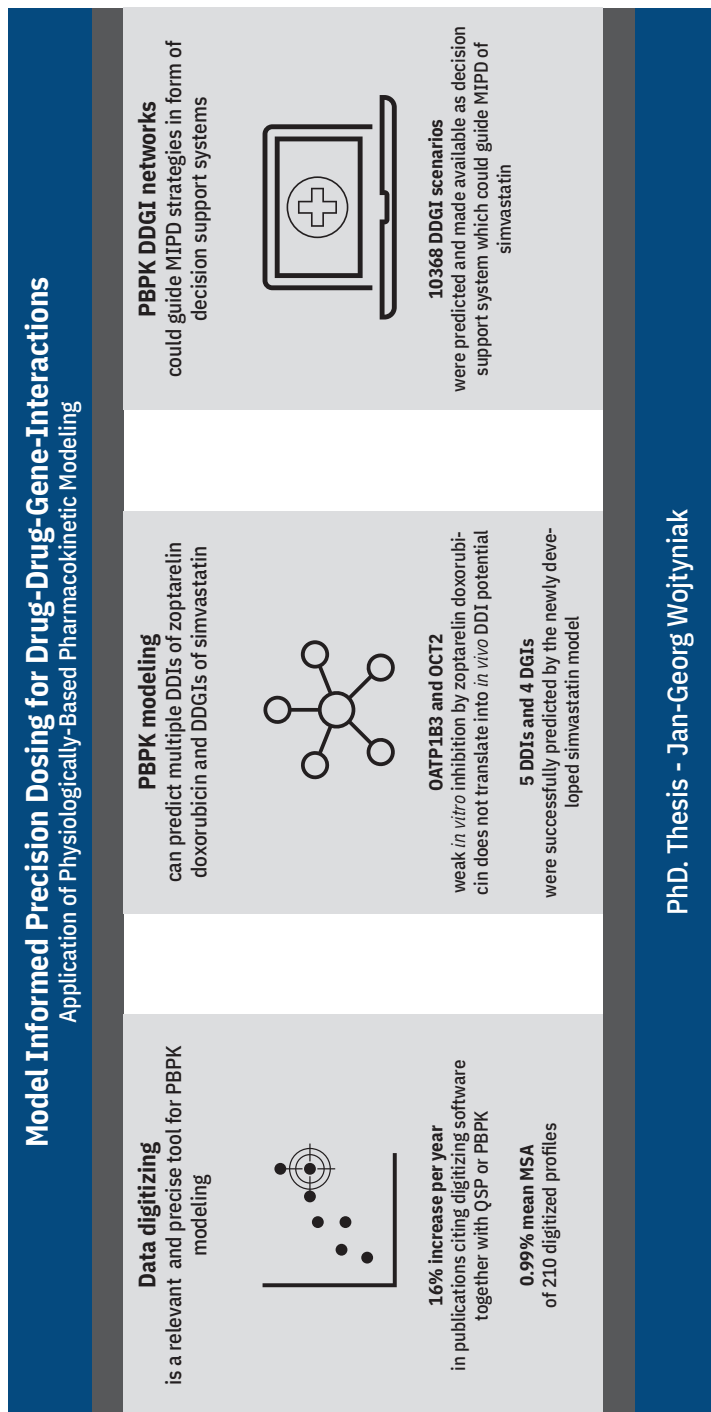
Der globale Arzneimittelbedarf steigt kontinuierlich an. Infolgedessen kommt es vermehrt zu unerwünschten Arzneimittelwirkungen, die eine Gefahr für Patienten darstellen. Eine wichtige Rolle beim Auftreten unerwünschter Arzneimittelwirkungen spielen Arzneimittel-Arzneimittel- und Arzneimittel-Gen-Wechselwirkungen. Um das Risiko solcher Wechselwirkungen zu minimieren, kann die modellgestützte Arzneimittelentwicklung und Präzisionsdosierung angewendet werden.

Das Ziel dieser Arbeit war es, physiologie-basierte pharmakokinetische Modelle zum Zweck der modellgestützten Arzneimittelentwicklung und Präzisionsdosierung einzusetzen.

Dafür wurde die Datendigitalisierung als wesentlicher Bestandteil der Entwicklung neuer physiologie-basierter pharmakokinetischer Modelle untersucht. Außerdem wurden Modelle für Zoptarelin Doxorubicin und Simvastatin entwickelt. Das Zoptarelin Doxorubicin Modell wurde verwendet, um die Entwicklung dieses Medikaments zu unterstützen. Mittels des Simvastatin Modells wurden in einem Interaktionsnetzwerk 10 368 Dosisempfehlungen für verschiedene Szenarien generiert und in einem digitalen Entscheidungsunterstützungssystem verfügbar gemacht.

Zusammenfassend kann die Arbeit als Leuchtturmprojekt gesehen werden, das zeigt, wie die physiologie-basierte pharmakokinetische Modellierung von Arzneimittel-Arzneimittel- und Arzneimittel-Gen-Wechselwirkungen in der modellgestützte Arzneimittelentwicklung und Präzisionsdosierung angewendet werden kann.





Graphical abstract. PBPK: Physiologically-Based Pharmacokinetic Modeling; QSP: Quantitative Systems Pharmacology; MSA: Median Symmetric Accuracy; DDI: Drug-Drug Interaction; DDDI: Drug-Drug-Gene Interaction; OATP1B3: Organic Anion-Transporting Polypeptide 1B3; OCT2: Organic Cation Transporter 2; MIPD: Model Informed Precision Dosing



*Ein Mensch, gestellt auf harte Probe,  
Besteht sie, und mit höchstem Lobe.  
Doch sieh da: es versagt der gleiche,  
Wird er gestellt auf eine weiche!*

— Eugen Roth - Ernst und heiter [6]

## DANKSAGUNG

---

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## ABBREVIATIONS

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<b>Notation</b>	<b>Description</b>
$K_M$	Michaelis-Menten Constant
ADE	Adverse Drug Event
ADR	Adverse Drug Reaction
AUC	Area Under the Plasma-Concentration Time Curve
$C_{max}$	Peak Plasma Concentration
CRediT	Contributor Roles Taxonomy
CYP	Cytochrome P450
CYP3A4	Cytochrome P450 3A4
DDGI	Drug-Drug-Gene-Interaction
DDI	Drug-Drug-Interaction
DGI	Drug-Gene-Interaction

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<b>Notation</b>	<b>Description</b>
DNA	Deoxyribonucleic Acid
DSS	Decision Support System
EMA	European Medicine Agency
FDA	Food and Drug Administration
GC-MS	Gas Chromatography–Mass Spectrometry
HMG-CoA	$\beta$ -Hydroxy $\beta$ -Methylglutaryl-Coenzyme A
HPLC	High-Performance Liquid Chromatography
LADME	Liberation, Absorption, Distribution, Metabolization, Excretion
LDL-C	Low-Density Lipoprotein Cholesterol
LHRHR	Luteinizing Hormone-Releasing Hormone Receptor
LLOD	Lower Limit of Detection
MID3	Model-Informed Drug Discovery and Development
MIPD	Model-Informed Precision Dosing
MSA	Median Symmetric Accuracy
NCA	Non-Compartmental Analysis
NCB	Net Clinical Benefit
NTE	New Therapeutic Entity
NVSS	National Vital Statistics System
OATP1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
OATP1B3	Solute Carrier Organic Anion Transporter Family Member 1B3
OCT2	Organic Cation Transporter 2
PBPK	Physiologically-Based Pharmacokinetic
PD	Pharmacodynamic
PGx	Pharmacogenomic
PK	Pharmacokinetic
PMx	Pharmacometrics
QC	Quality Control
QSP	Quantitative Systems Pharmacology
SA	Simvastatin Acid
SL	Simvastatin Lactone
USA	United States of America



## Part I

### INTRODUCTION AND AIMS

The chapter provides an overview of why drug-drug-gene interactions (DDGIs) place a heavy burden on our health care system. It also explains how DDGIs can be overcome with the help of model-informed drug discovery and development (MIDD), model-informed precision dosing (MIPD) and in particular with physiologically-based pharmacokinetic (PBPK) modeling. Finally, it specifies the aims of the presented work.



## INTRODUCTION

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### 1.1 ADVERSE DRUG REACTIONS AND ADVERSE DRUG EVENTS

Adverse drug reactions (ADRs) and adverse drug events (ADEs) are an essential and increasing burden for our healthcare and economic system [7, 8]. An ADR is “a response to a medicinal product which is noxious and unintended” while an ADE is “an injury resulting from medical intervention related to a drug” [8]. They are assumed to be a leading cause of morbidity and death worldwide as shown for instance by a meta-analysis of 39 prospective studies, which found that ADRs are responsible for 1.0% to 16.8% of admissions to hospitals in the United States of America (USA) [7].

Significantly related to the frequency of ADRs is the sharp increase in pharmaceuticals use as observed over the last decades. For instance, the number of prescriptions dispensed in the USA rose from 5.308 billion in 2014 up to 5.770 billion in 2018 [9]. Even more striking: when looking at the total nominal spending on medicines, they reached a volume of 482 billion \$ in 2018, as shown in Figure 1.1 [10].

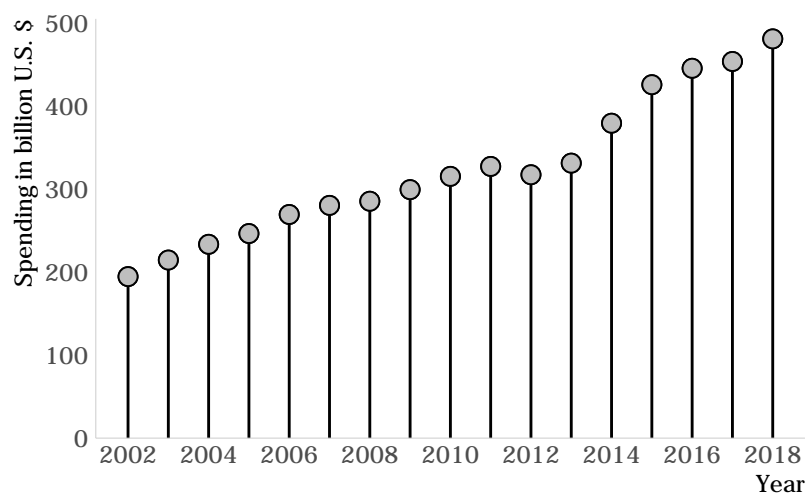


Figure 1.1: Total nominal spending on medicines in the USA from 2002 to 2018 [10].

As a result, it is estimated that 60% of the elderly take at least one prescription medicine per week [11]. In parallel, the incidence of ADRs is increasing too, as shown by an analysis from the USA National Vital Statistics System (NVSS) that showed an increase in ADR-related deaths from 8 to 12 per 1000 persons per year, during the timespan from 1999 to 2006 [8]. In contrast, it is estimated that 70% of ADRs, which lead

to an emergency department visit are preventable [8]. Reasons for ADRs are manifold and can be traced back to medication errors, drug interactions, diseases, or patient characteristics [8, 12]. Nevertheless, the impact of ADRs on our social and economic system remains vast and has even led to the development of a specialized branch of research called pharmacovigilance [12, 13].

## 1.2 DRUG-DRUG-, DRUG-GENE- AND DRUG-DRUG-GENE INTERACTIONS

To tackle ADRs, a drug therapy tailored to the patient's needs, characteristics, and circumstances is required [12, 14–16]. This therapy individualization is summarized under the term “precision dosing” [14–16]. Precision dosing is defined as “dose selection by a prescriber for an individual patient at a given time” [14]. Furthermore, it “focuses on the individualization of drug treatment regimens based on patient factors known to alter drug disposition and/or response” [16]. Very well known factors which are capable of altering drug disposition are drug-drug-interactions (DDIs), drug-gene-interactions (DGIs) and the combination of both, DDGIs [17–23].

DDI means that one drug, commonly called the perpetrator, alters the pharmacokinetic (PK) or pharmacodynamic (PD) profile of another drug, which is then called the victim compound [20]. DDIs do often happen in polypharmaceutical settings like in cancer treatments [24, 25]. A DGI, on the other hand, occurs “when an individual carrying one or more variant forms of a gene that codes for a drug-metabolizing enzyme or drug transporter with altered function receives a drug that is a substrate for the given enzyme or transporter” [17].

Unfortunately, although investigation of DDIs during drug development is mandatory and the knowledge about DGIs on pharmacotherapy is continuously growing, the development of precision dosing recommendations is lagging [16, 26, 27]. One reason for this is that DDI effects in certain situations cannot be investigated in a clinical trial [2]. For example, DDI studies of new therapeutic entities (NTEs) in oncology require special ethical considerations that may prevent a study from being conducted [2]. Thus, in such scenarios, advanced translational strategies are necessary to interpolate preclinical investigations to predict clinical effects [2].

Another reason is that clinical studies alone are often not sufficient to reflect the complicated situation of a post-approval setting. For instance, since the majority of polymorphisms are quite rare, most clinical trials compare DGIs individually rather than in combination to recruit a sufficient number of patients [3, 16]. The same applies to DDIs studies where mostly dedicated index drugs are used for effect quantification [27]. Moreover, commonly only homogeneous study populations are selected for clinical DDI studies, consisting of young and healthy individuals

without comorbidities or comedications. This is done to reduce the potential impact of covariates on study outcome and to ensure significant results [16, 28]. However, in a real-life polymedication environment, where people regularly take more than five drugs [29], DDIs and DGIs occur not purely as individual cases but rather in combination and as DDGIs [3, 21]. Therefore, novel approaches are needed to integrate the information on DDIs and DGIs obtained in clinical studies to apply them to more complex DDGI scenarios. This way precision dosing recommendations could be generated, and ADRs prevented [3, 20–23].

### 1.3 CASE EXAMPLES: ZOPTARELIN DOXORUBICIN AND SIMVASTATIN

Zoptarelin doxorubicin was an investigational chemotherapeutic agent designed for drug targeting of luteinizing hormone-releasing hormone receptor (LHRHR) positive tumors [2, 30]. It is a fusion molecule of the chemotherapeutic doxorubicin and a small peptide agonist to LHRHR [31]. The structure of zoptarelin doxorubicin is shown in Figure 1.2.

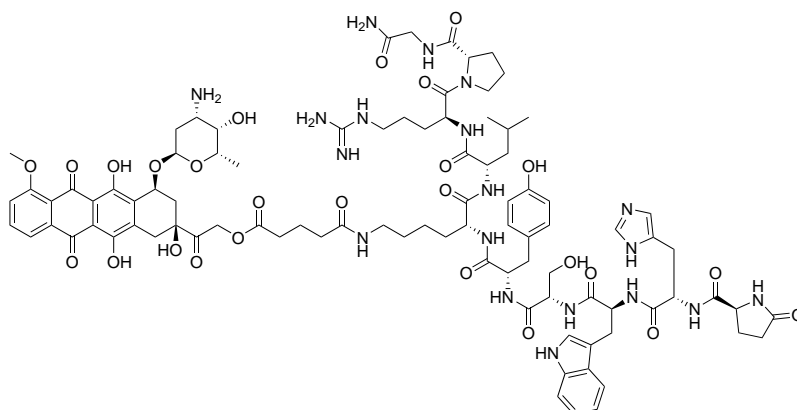


Figure 1.2: Molecular structure of zoptarelin doxorubicin [31].

It was hypothesized that by adding the LHRHR agonistic moiety, improved internalization of zoptarelin doxorubicin in LHRHR positive tumor cells and a reduced cardiotoxicity compared to unconjugated doxorubicin could be achieved [2, 30]. Zoptarelin doxorubicin is mainly metabolized by spontaneous and carboxylesterase-mediated hydrolysis [2]. In *in vitro* experiments, zoptarelin doxorubicin shows inhibitory effects on solute carrier organic anion transporter family member 1B3 (OATP1B3) and organic cation transporter 2 (OCT2) with  $IC_{50}$  values of  $16.5 \mu\text{mol l}^{-1}$  and  $3.26 \mu\text{mol l}^{-1}$ , respectively [2]. As a consequence, *in vivo* interactions with prominent substrates of those transporters like simvastatin (OATP1B3) and metformin (OCT2) were likely and had to be further investigated during the development process [2]. However, clinical DDI studies with deoxyribonucleic acid (DNA) intercalating agents are hardly feasible due to ethical aspects [2]. In the case of zoptarelin doxorubicin this could have led to a knowledge gap and

potential patient safety risks [2].

As already indicated, another good example compound which is prone to be influenced by DDGIs is simvastatin. Simvastatin is an oral  $\beta$ -hydroxy  $\beta$ -methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and counts to the most prescribed drugs in industrial nations [9]. Although simvastatin shows excellent cost-effectiveness and an optimal benefit-risk ratio [32, 33], over-dosage can lead to rhabdomyolysis which is a feared and potentially life-threatening ADR [34]. Multiple single DGIs and DDIs have been identified to change simvastatin PK and subsequently raise the risk of over-dosages [35–39]. This is because simvastatin has a complex PK with high inter-individual variability, which involves many different drug transporters and metabolic enzymes [39–41]. A simplified overview of processes relevant to simvastatin's PK is given in Figure 1.3.

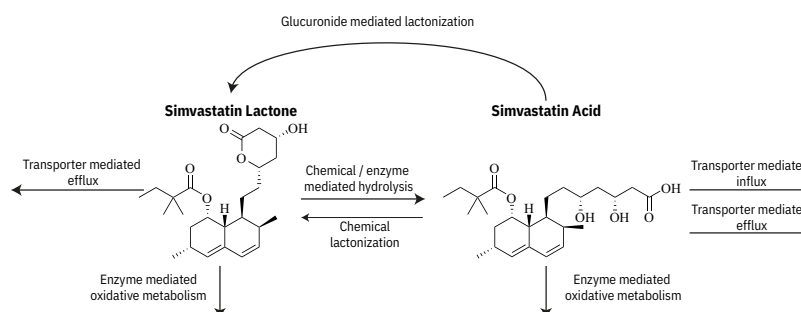


Figure 1.3: Simvastatin lactone and simvastatin acid molecular structure. Simvastatin is given as a prodrug (simvastatin lactone), which is hydrolysed by chemical and enzymatic processes to the more active form simvastatin acid [42]. Both are substrates for different transporters [43–45] and undergo further oxidative metabolism [42]. In addition, simvastatin acid can lactonize back to simvastatin lactone by chemical lactonization or via a glucuronide intermediate metabolite [42].

Hence, if a DGI or DDI alter transporter or enzyme activity, simvastatin's PK can change dramatically, as observed in several clinical trials [35–39]. To compare the effects more or less independent of the given dose it is helpful to look at the relative change of non-compartmental analysis (NCA) parameters like area under the plasma-concentration time curve (AUC) under DGI or DDI conditions compared to placebo as calculated in Equation 1.1.

By doing so, a relative change of  $-94\%$  (simvastatin acid (SA), AUC) under rifampicin co-treatment [46] and a relative change of  $+1117\%$  (SA, AUC) under clarithromycin co-treatment [47] is observed. Unfortunately, despite these already very strong observed effects for single DDIs, no recommendations for combined effects of DGIs and DDIs in the form of DDGIs for simvastatin were available [26]. That is because

investigation and evaluation of all possible combination scenarios, as outlined in Section 1.2, is of course not feasible with clinical trials alone [3, 16].

$$\%NCA_{change} = \frac{NCA_{obs,DDI/DGI} - NCA_{obs,placebo}}{NCA_{obs,placebo}} * 100 \quad (1.1)$$

where:

$\%NCA_{change}$	Relative change of the NCA estimate under DDI or DGI compared to placebo
$NCA_{obs,DDI/DGI}$	Observed NCA estimate under DDI or DGI conditions
$NCA_{obs,placebo}$	Observed NCA estimate under placebo conditions

#### 1.4 MODEL INFORMED DRUG DEVELOPMENT AND PRECISION DOSING - THE USE OF PHARMACOMETRICS

To overcome the knowledge gaps mentioned above MID3 and MIPD strategies can be applied [14–16, 48–52]. MID3 is a “quantitative framework for prediction and extrapolation, centered on knowledge and inference generated from integrated models of compound, mechanism and disease level data and aimed at improving the quality, efficiency and cost effectiveness of decision making” [52]. MIPD on the other hand is the targeted use of pharmacometrics (PMx) modeling techniques together with the individually measured patient and disease characteristics to find the optimal dose for a patient [53]. Thereby, PMx is a science that aims to quantify drug, disease, and clinical trial characteristics using mathematics and statistics [54]. Since its debut in the 1950s PMx has evolved into an essential cornerstone of pharmacotherapy, which, as shown in Figure 1.4, can be found in each phase of a drug’s life-cycle [51, 52, 54–57].

While PMx initially focused on empirical or semi-mechanistic models, the recently available computing power made it possible to develop physiologically more accurate models [58–67]. Subsequently, they are called physiologically-based pharmacokinetic (PBPK) models, and they are seen as one of the main pillars of the modeling and simulation revolution in pharmaceutical sciences [59, 60, 67]. PBPK models are multiple compartment systems and try to depict the physiological situation as detailed as necessary [59, 66]. Thus, their parameters and equations are based on real tissue characteristics like volume, surface area, or protein expression [59, 66]. Compartments are mathematically connected to represent the blood flow and to simulate the liberation, absorption, distribution, metabolization and excretion (LADME) behavior of drugs [59, 66]. PBPK models vary in complexity and can be

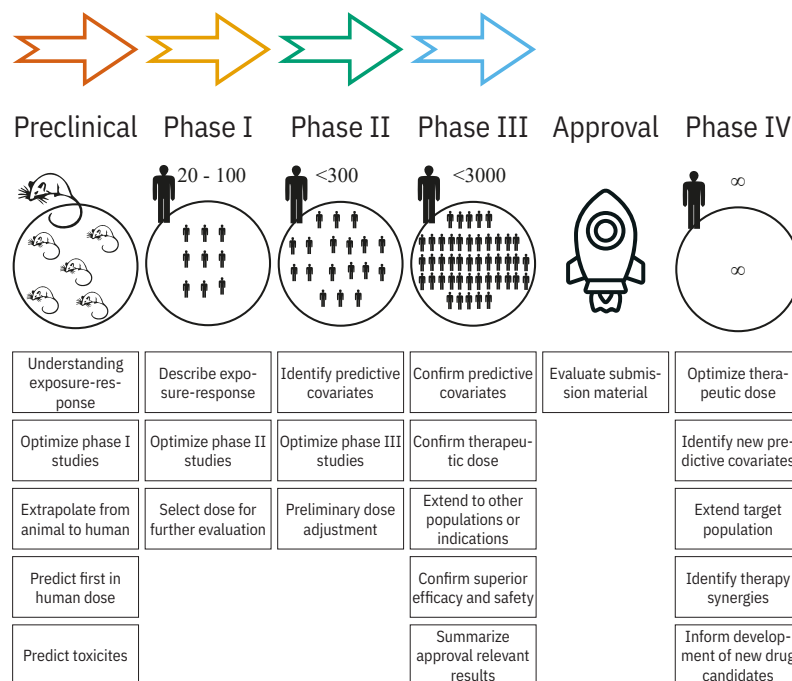


Figure 1.4: Different phases of drug development and tasks that can be informed by PMx techniques. Adapted from Mould and Upton [55].

established as minimal or whole-body PBPK models [61, 62, 64, 68]. Moreover, they can be integrated into systems biology networks [63, 69]. To establish a PBPK model, system and drug dependent parameters are required [59, 66]. System dependent parameters describe the physiological environment and include the aforementioned parameters like tissue characteristics [59, 66]. Drug dependent parameters are compound-specific parameters such as lipophilicity, acidity, or molecular weight [59, 66]. Figure 1.5 visualizes the step-by-step process for the development of a PBPK model, while Figure 1.6 summarizes the structure of a whole-body PBPK model and the different building blocks [1, 66, 67].

Since its conceptualization in 1937 [70], PBPK modeling has proven its usefulness in many different application areas [66, 70–72]. Issues that can be addressed with PBPK modeling are, for example, cross-species extrapolations or scaling to special populations like pediatric or organ impaired patients [66]. Due to these versatile application possibilities, especially for questions which for ethical reasons, or due to their feasibility can hardly be answered by clinical studies, PBPK modeling is recognized by regulatory agencies like the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) as valuable method [51, 66, 70–72]. They emphasize, for example, the use of PBPK modeling to explore and quantitatively predict the PK of drugs for DDIs and to support dose selection and labeling [27]. Although



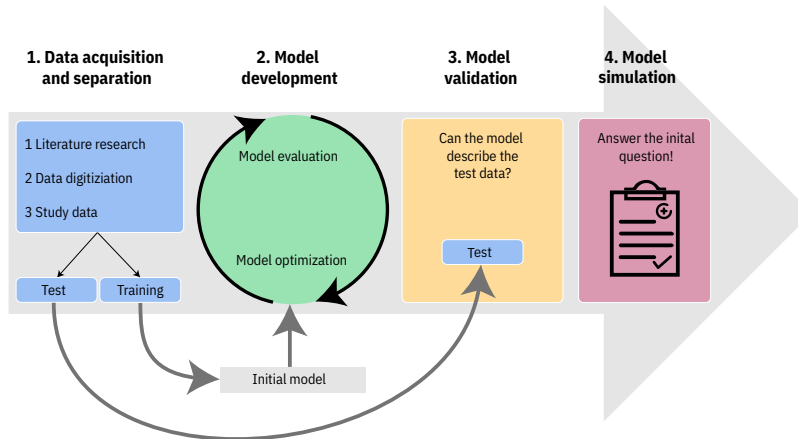


Figure 1.5: Stepwise PBPK model development workflow. In a first step, data are acquired. The data are then split into training and test dataset. Following, an initial model is set up and continuously refined. In step three, the model has to be validated and can lastly be used to answer the initial questions.

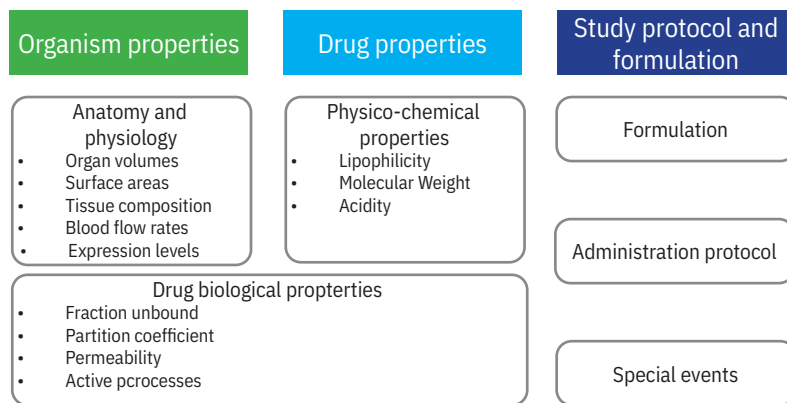
the potential of PBPK modeling is acknowledged, the technique is, as already mentioned, relatively new compared to other MID3 and MIPD strategies [3, 48, 65]. Thus, best practices for PBPK modeling are currently under development, and many work steps important for PBPK model building require a detailed evaluation [1].

#### 1.5 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING STRATEGIES FOR DRUG-DRUG-, DRUG-GENE- AND DRUG-DRUG-GENE INTERACTIONS

As mentioned, PBPK modeling is regularly used and emphasized for DDI assessment and predictions, for instance, during the drug development process [2, 51, 66, 70–72]. The convenient aspect of the PBPK approach is that the models can be developed individually for each substance [3, 74–79]. Subsequently, they can be connected as required to investigate the DDI of interest [2, 3, 74–79]. Thus, theoretically, networks of any size can be created even for very complicated DDIs [2, 3, 76–78]. The first examples of such DDI networks can already be found in the literature [3, 76–78]. Besides, PBPK modeling can also be used to predict DGIs [3, 76]. By combining the two approaches for DGIs and DDIs, complex interactions can be predicted similarly for DDGIs [3, 80]. Figure 1.7 illustrates the process with a puzzle in which the individual building blocks are developed separately and then put together for therapy optimization.

Thus, PBPK driven MIPD is a useful technique to derive therapy recommendations and this way to come as close as possible to the ultimate goal of a pharmacotherapy tailored to the patient [3, 15, 16, 50]. This

### Building blocks of a PBPK model



### Structure of a PBPK model

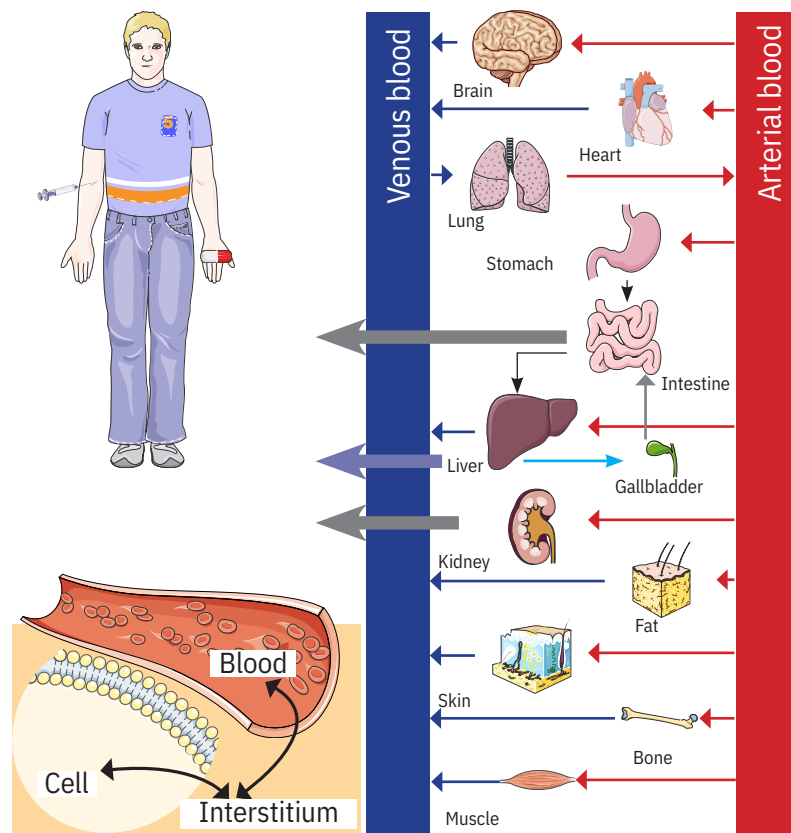


Figure 1.6: PBPK model building blocks and model structure. The upper part lists different sources of information necessary for the development of a PBPK model. The lower part of the figure visualizes the different compartments in a PBPK model, which represent the organ tissues. Each compartment is further subdivided into vascular, interstitial, and intracellular space. Adapted from Kuepfer et al. [66]. Illustrations of organs were taken from CC BY 3.0 Servier Medical Art by Servier [73].

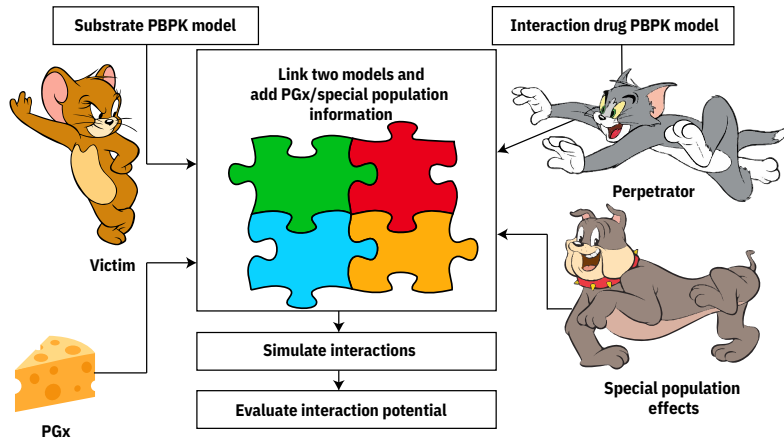


Figure 1.7: PBPK modeling approach for the prediction of DDGIs. Substrate and interaction PBPK models are developed individually and afterward connected. In addition, pharmacogenomic (PGx) and special population effects can be included. Tom and Jerry illustrations were taken from CC BY-NC 4.0 by pngimg.com [81].

way, ADRs could be prevented, lives saved and the healthcare system's burden reduced [15]. However, the pure distribution of PBPK models is not sufficient [16]. Rather, the recommendations derived from them must be made available in a way that they can be easily implemented in a clinical setting [16]. Thereby, decision support systems (DSSs) have been proven to be important tools to integrate the derived knowledge [16, 50]. Such systems allow clinicians to enter their patient characteristics, genotype information, and comedication data [3]. Subsequently, the system evaluates the input information, compares them with the MIPD analyses results, and generates an individual therapy recommendation [3].



One aim of this thesis was to improve current PBPK modeling strategies. Further, PBPK modeling of DDIs, DGIs, and DDGIs should be applied in the context of MID3 and MIPD for the example compounds zoptarelin doxorubicin and simvastatin, respectively. The thesis' aims were realized within the scope of the following projects:

**PROJECT I - DATA DIGITIZING:** The project aimed to assess the relevance of data digitizing for PBPK modeling and to establish recommendations for the digitization workflow. For this purpose, the general usage of data digitizing software in quantitative systems pharmacology (QSP) and PBPK modeling should be analyzed. Subsequently, the accuracy and precision of relevant digitizing software packages should be evaluated. Moreover, investigation of discrepancies between reported and graphically presented data as well as identification of covariates which might influence the digitizing process was aspired. Finally, recommendations regarding the creation of digitizable plots and the digitization process itself should be proposed.

**PROJECT II - ZOPTARELIN DOXORUBICIN:** Purpose of the second project was to support the drug development process of the anticancer drug zoptarelin doxorubicin. In detail, a whole-body PBPK model of zoptarelin doxorubicin and its active metabolite doxorubicin should be established. Further, the model should be used to assess the DDI potential for OATP1B3 and OCT2 substrates.

**PROJECT III - SIMVASTATIN:** The third project's objective was to establish dose recommendations for different DDGIs of simvastatin. In order to achieve this, the following specific goals have been defined. The first part aimed to develop a PBPK simvastatin DDGI network, including DDIs of five clinically relevant perpetrator drugs and the DGIs of four PGx relevant polymorphisms. The second objective was to use the developed network and to derive new dose recommendations in the context of MIPD. Finally, the results should be made publicly available as a web-based DSS for easy and quick access, especially for health care professionals.



## Part II

### INCLUDED MANUSCRIPTS

This chapter presents the scientific publications used for the presented work.





## RESULTS

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### 3.1 PUBLICATION I - DATA DIGITIZING: ACCURATE AND PRECISE DATA EXTRACTION FOR QUANTITATIVE SYSTEMS PHARMACOLOGY AND PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING

#### 3.1.1 *Reference*

**Jan-Georg Wojtyniak**, Hannah Britz, Dominik Selzer, Matthias Schwab, and Thorsten Lehr. "Data digitizing: accurate and precise data extraction for quantitative systems pharmacology and physiologically-based pharmacokinetic modeling." In: *CPT: Pharmacometrics & Systems Pharmacology* 9.6 (June 2020), pp. 322–331. DOI: 10.1002/psp4.12511

#### 3.1.2 *Author Contributions*

Following CRediT [4, 5], the contributions of the individual authors are listed in Table 3.1.<sup>1</sup>

Table 3.1: Author contributions for Publication I - Data Digitizing

Jan-Georg Wojtyniak	See included publications and contribution report on page vi
Hannah Britz	Conceptualization, Investigation, Writing - Review & Editing, Visualization
Dominik Selzer	Conceptualization, Software, Formal Analysis, Writing - Review & Editing
Matthias Schwab	Conceptualization, Writing - Review & Editing
Thorsten Lehr	Conceptualization, Investigation, Project Administration, Writing - Original Draft, Writing - Review & Editing

#### 3.1.3 *Copyright*

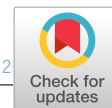
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<sup>1</sup> For a description of the different taxonomy categories see appendix Chapter B

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Citation: CPT Pharmacometrics Syst. Pharmacol. (2020) XX, 1–10; doi:10.1002/psp4.12

## ARTICLE

# Data Digitizing: Accurate and Precise Data Extraction for Quantitative Systems Pharmacology and Physiologically-Based Pharmacokinetic Modeling

Jan-Georg Wojtyniak<sup>1,2</sup>, Hannah Britz<sup>1</sup>, Dominik Selzer<sup>1</sup>, Matthias Schwab<sup>2,3,4</sup> and Thorsten Lehr<sup>1,\*</sup>

In quantitative systems pharmacology (QSP) and physiologically-based pharmacokinetic (PBPK) modeling, data digitizing is a valuable tool to extract numerical information from published data presented as graphs. To quantify their relevance, a literature search revealed a remarkable mean increase of 16% per year in publications citing digitizing software together with QSP or PBPK. Accuracy, precision, confounder influence, and variability were investigated using scaled median symmetric accuracy ( $\zeta$ ), thus finding excellent accuracy (mean  $\zeta = 0.99\%$ ). Although significant, no relevant confounders were found (mean  $\zeta \pm SD$  circles =  $0.69\% \pm 0.68\%$  vs. triangles =  $1.3\% \pm 0.62\%$ ). Analysis of 181 literature peak plasma concentration values revealed a considerable discrepancy between reported and *post hoc* digitized data with 85% having  $\zeta > 5\%$ . Our findings suggest that data digitizing is precise and important. However, because the greatest pitfall comes from pre-existing errors, we recommend always making published data available as raw values.

### Study Highlights

#### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ In quantitative systems pharmacology (QSP) and physiologically-based pharmacokinetic (PBPK) modeling, data digitizer becomes a valuable tool to translate literature data from a graphical representation into numerical values.

#### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study investigated the usage of digitizing software in QSP and PBPK modeling. Moreover, it evaluated the software accuracy, precision, confounder influence, and variability between software. In addition, the discrepancies between reported and graphically presented data were analyzed.

#### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The results of this study contributed to an improved understanding of the precision and accuracy of digitizing software.

#### HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ The study findings could help improve the quality of the QSP and PBPK models, which were developed based on digitized literature data. Furthermore, they can protect the modeler from using biased data that could subsequently lead to false *in silico* predictions and hence hamper the drug discovery and development process or, even worse, harm patients as a result of erroneously derived therapy recommendations.

During the past few years, quantitative systems pharmacology (QSP) and especially physiologically-based pharmacokinetics modeling (PBPK) have proven to be an important cornerstone of model-informed drug discovery and development.<sup>1</sup> However, for model development, time-dependent data of pharmacological relevant processes are a crucial requirement. Unfortunately, published data are typically presented in aggregate form as plots or graphs without providing access to the underlying raw, uncondensed data. As a result, researchers must extract the information of interest from the graphical representation to use the data for their modeling approaches. Despite the potential to automatically data-mine population average

pharmacokinetic (PK) data for certain applications,<sup>2</sup> data extraction from graphical representations still requires manual efforts. To illustrate the scale of this issue, it should be noted that PBPK projects not uncommonly rely on extracted data gathered from up to 50 articles.<sup>3–9</sup> Fortunately, several off-the-shelf digitization software packages that allow the extraction of numerical information from their two-dimensional graphical representation are currently available.<sup>10–13</sup> These software solutions have been in active use for some time for the well-established population PK approaches.<sup>14</sup> However, neither for them nor for QSP or PBPK modeling is information available regarding the importance and use of digitizing software. Moreover, to the best of our knowledge,

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there is no systematic evaluation of the accuracy and precision of these software solutions, nor have any interfering factors that could potentially bias the digitized output been identified. In addition, little is known about the extent of discrepancy between reported and graphically presented data that is typically only revealed after *post hoc* digitization and the nature of these errors and confounding factors when it comes to the digitization process. Consequently, these factors can potentially interfere with model development and evaluation processes and ultimately lead to false predictions and questionable model-based decisions.

Thus, the first objective of this analysis was to assess the general usage of the data digitizing software for QSP and PBPK modeling. Second, this analysis aimed to evaluate the accuracy and precision of a relevant digitizing software. Moreover, discrepancies between reported and graphically presented data were quantified, and the covariates influencing the digitizing process were identified. Finally, recommendations regarding the creation of digitizable plots and graphs and the digitization process itself were proposed.

## METHODS

### Literature search

In a first step, literature published between January 2005 and September 2019 were queried for terms regarding the most common digitizing software in combination with the two terminologies “systems pharmacology” and “physiologically based pharmacokinetic.” For this purpose, the software Publish or Perish<sup>15</sup> was used using the Google Scholar search engine for terminology queries. Google Scholar was used because the search engine allows a full-text analysis, which was a prerequisite because the use of digitizing software is, in most cases, only mentioned in the Methods sections of texts. For each digitizing software, the search query “systems pharmacology OR physiologically based pharmacokinetic AND software name” was used. The search was performed by one author (J.-G.W.) and reviewed by two other authors (H.B., D.S.). The search query results were not further edited or restricted by specific exclusion criteria. Moreover, no gray literature analysis was performed. Subsequently, the annual publications that contain the terms were used as a surrogate marker of importance. Furthermore, Poisson regression was applied to describe and predict the trends in software usage from 2005 to 2021. Moreover, a detailed search for publications from 2012 to 2019 in *CPT: Pharmacometrics & Systems Pharmacology (CPT:PSP)* was also performed. For this, the search query “systems pharmacology OR physiologically based pharmacokinetic AND software name” was used. To identify unreported uses of digitization, all publications from *CPT:PSP* containing only the terms “systems pharmacology OR physiologically based pharmacokinetic” without the name of a digitization software were selected. Following this, the cumulative publication frequency was calculated. Afterward, based on the assumption that data digitization is necessary for every project related to QSP or PBPK, the relative frequency of unreported digitizing software usage was estimated. Finally, to investigate the reporting rate of digitizing techniques in the methods, all published articles from *CPT:PSP* from 2018 were reviewed manually to identify articles related to PBPK that referenced a digitizing software and most likely had used literature data.

### Software evaluation

A study was performed to assess the accuracy and precision of the digitizing software GetData Graph Digitizer<sup>10</sup> and to identify the interfering factors that potentially have an influence on the digitized output. As study inclusion criteria, the subjects had to be at least 18 years old and be able to use the digitization software independently after a standardized software introduction. Furthermore, they had to give informed consent and, following this, were randomly split into two groups (group 1 and group 2). All subjects had to fill out a standardized questionnaire to collect demographics such as age and education. They were asked to digitize the same steady-state plasma concentration-time graph of a hypothetical drug (two-compartment model, absorption constant ( $K_a$ ) = 3 hour<sup>-1</sup>, plasma clearance (CL) = 4 L/hour, central volume of distribution ( $V_1$ ) = 20 L, intercompartmental clearance (Q) = 3 L/hour, peripheral volume of distribution ( $V_2$ ) = 70 L, oral bioavailability ( $F$ ) = 100%, dose = 1 mg simulated using Berkeley Madonna V 8.3.18 developed by Robert Macey and George Oster, University of California, Berkeley, CA) three times in a row. The two-compartment model was chosen as it can be easily parametrized and because the simulations can be easily reproduced. To minimize a possible bias attributed to learning effects, the plasma concentration-time profile in group 1 consists of 10 values marked as circles following 10 values marked as triangles (sample time points: 0, 1, 2, 3, 5, 7, 10, 13, 16, 20, 24, 25, 26, 27, 29, 31, 34, 37, 40, 44). The profile in group 2 was designed as triangles first and circles last. The random allocation sequence for the study subjects was generated with Excel 2016 (Microsoft, Redmond, WA) using a two-sized block randomization and implemented via consecutive questionnaire numbers. No blinding was performed. To validate that demographics are equally distributed within the groups, an analysis of variance (ANOVA) or chi-square goodness-of-fit tests were performed for continuous and categorical demographics, respectively. If the study data were missing, it was imputed with calculated median values for continuous variables and with calculated modal values for categorical variables. To evaluate a potential bias attributed to missing values, statistical analyses were performed with and without imputed values whenever necessary, and the results were compared afterward.

For comparing accuracy and precision, the scaled median symmetric accuracy ( $\zeta$ )<sup>16</sup> and  $\zeta$  standard deviation (SD) were calculated as shown in Eqs. 1–4. Scaling (minimum–maximum normalization<sup>17</sup>) as depicted in Eq. 1 was independently performed for time and concentration values.  $\zeta$  was calculated over the combined vector of scaled values for time and concentration values.

$$X_{\text{scaled}} = \frac{X - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}} + \varepsilon_{\text{machine}} \quad (1)$$

$$Q_i = \frac{X_{\text{scaled, digitized}}}{X_{\text{scaled, simulated}}} \quad (2)$$

$$\zeta = 100 * \left( \exp \left( \text{median} \left( \left| \log_e(Q_i) \right| \right) \right) - 1 \right) \quad (3)$$

$$SD = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (4)$$

With  $x_{\text{scaled}}$  = scaled value,  $x$  = original value,  $x_{\text{min}}$  = minimum of the original values,  $x_{\text{max}}$  = maximum of the original values,  $\epsilon_{\text{machine}}$  = machine epsilon,  $Q_i$  = accuracy ratio,  $x_{\text{scaled,digitized}}$  = scaled digitized value,  $x_{\text{scaled,simulated}}$  = scaled simulated value,  $n$  = number of  $\zeta$  values, and  $x_i = \zeta$ ,  $\bar{x}$  = mean  $\zeta$ .

Because  $\zeta$  for values equal zero is not defined, the machine epsilon (2.2E-16) was added to each value. Afterward, the impact of demographic variables (age, sex, education, average computer usage per day, experience with digitizing software, and right-handedness) and study-specific variables (digitizing time, symbol shape, type of computer used, and mouse usage) on  $\zeta$  were investigated using multiple linear regression. Moreover, to analyze if multiple digitization leads to better results, an ANOVA with different repetitions on  $\zeta$  was performed.

The required study sample size was calculated for comparing two sample means with an equal variance. Because no literature reference values were available, a mean  $\zeta$  of 5% and a variance of 2% for circle shapes were assumed. Furthermore, a 1.5% increase of  $\zeta$  in the triangle group was assumed compared with circles. From this, the necessity of at least 62 subjects was calculated to get a significant result with a statistical power of 80%, and a significance level of 5% and a dropout rate of 10% were assumed ( $28 \cdot 2 \cdot 1.1 = 61.6$ ).

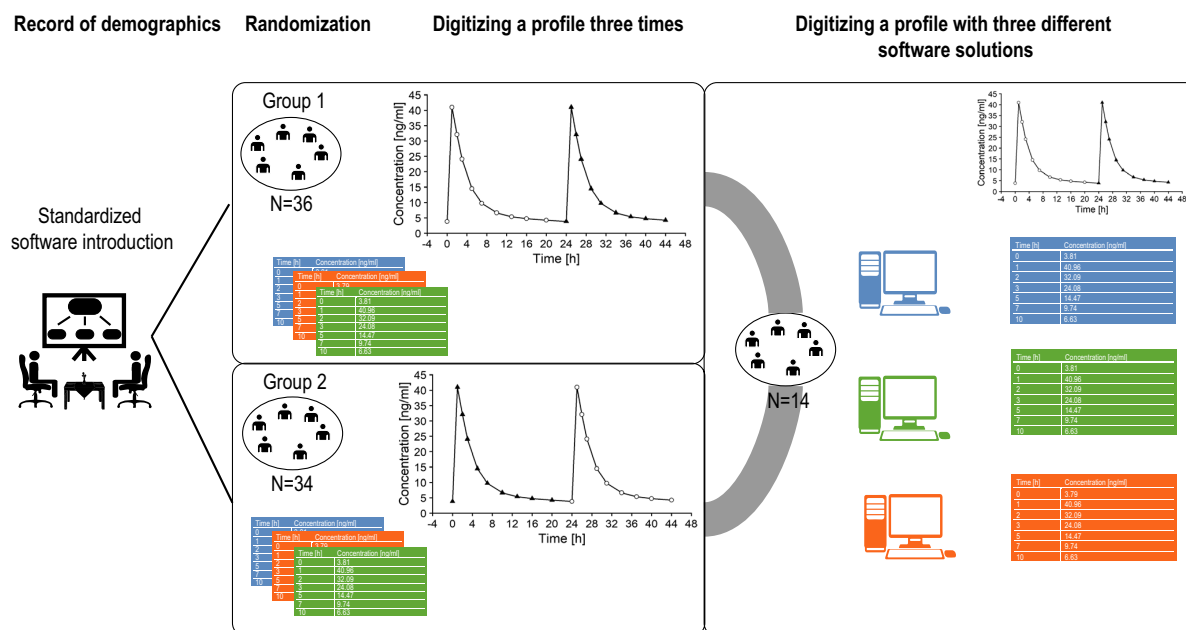
Subsequently, to investigate the impact of the use of digitized data on parameter estimation, the PK parameters of the hypothetical drug were estimated for each of the digitized profiles via nonlinear optimization using the lbfgsb3 R package.<sup>18</sup> Afterward, the relative deviation compared

to the parameters used for simulation were calculated and visualized.

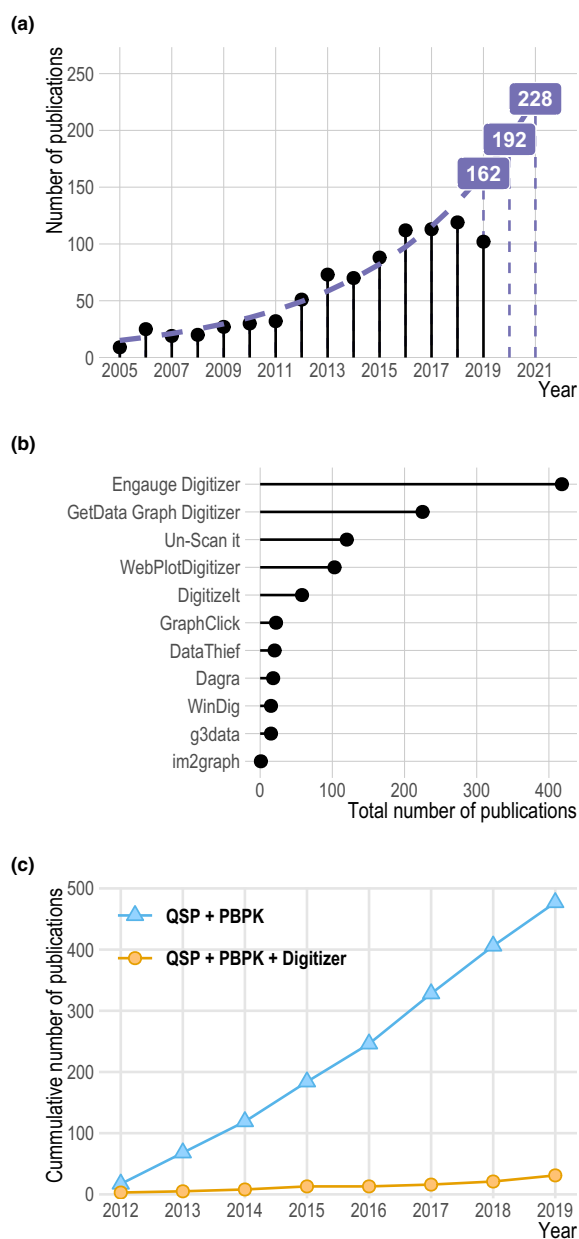
Consequently, a substudy with 14 subjects from the main study group was conducted to compare the accuracy and precision of the digitization software packages DataThief, Engauge Digitizer, and GetData Graph Digitizer. In this study, the subjects digitized the graph from group 1 with each digitization software. Afterward,  $\zeta$  and  $\zeta$  SD were calculated for the digitized profiles and subsequently analyzed via an ANOVA and pairwise  $t$ -test. The graphs of both groups and an overview of the whole study procedure are shown in **Figure 1**. The three digitization software packages DataThief, Engauge Digitizer, and GetData Graph Digitizer were selected based on the criteria of software availability, usability, included features, and the feedback from a small user survey (10 subjects from our group). A comprehensive list of the different digitization software features can be found in **Table S1**.

#### Analysis of published PK data

Finally, the extent of discrepancy between the reported and graphically presented data were investigated based on published sample time points and mean peak plasma concentration ( $C_{\text{max}}$ ) values. For this, digitized readouts as well as published raw values from single and multiple dose profiles that were previously digitized in-house with GetData Graph Digitizer were used. A complete list from all studies included can be found in the supplementary material in Tables S2 and S3. Unscaled  $\zeta$  was calculated individually for all available values. Following this, a stepwise multivariate linear regression analysis (forward inclusion  $P \leq 0.05$ , backward elimination  $P \leq 0.01$ ) was performed to investigate the relationship between the  $\zeta$  values and the portable



**Figure 1** Schematic overview of the study protocol as well as the concentration-time profiles digitized by study subjects.



**Figure 2** Results from literature search. **(a)** Number of publications containing the terms “systems pharmacology” or “physiologically based pharmacokinetic” and the names of the digitization software packages investigated during the past few years. Labels and the dashed purple line shows model-estimated values. Solid lollipops represent the observed values. **(b)** Total number of publications containing the terms “systems pharmacology” or “physiologically based pharmacokinetic” and the name of the digitization software package for each package investigated. The names investigated were “Engauge Digitizer,” “GetData Graph Digitizer,” “Un-Scan it,” “WebPlotDigitizer,” “Digitizelt,” “GraphClick,” “DataThief,” “Dagra,” “WinDig,” “g3data,” and “im2graph.” **(c)** Analysis of the cumulative publication frequency in *CPT: Pharmacometrics & Systems Pharmacology* of the terms “systems pharmacology” or “physiologically based pharmacokinetic” (blue solid line, triangles) when compared with “systems pharmacology” or “physiologically based pharmacokinetic” (orange solid line, circles) investigated during the the past few years. QSP, quantitative systems pharmacology; PBPK, physiologically-based pharmacokinetics.

Engauge Digitizer was most frequently cited, followed by the GetData Graph Digitizer (see **Figure b2**). For most software, an increase in use during the analyzed time span was observed. Arithmetic mean increase per year was 16%, with the highest increase for WebPlotDigitizer (87%) and the highest decrease for DataThief (–8%) (see **Figure S1**).

The Publish or Perish search query for QSP or PBPK in the journal *CPT:PSP* from 2012 to 2019 revealed 477 publications. In contrast, for the search terms QSP or PBPK and the names of the most important digitization software packages, only 31 entries were found. Based on the assumption that every QSP or PBPK project requires the use of digitization software, these findings led to an underreporting rate of 94%. **Figure 2c** shows the cumulative number of publications for both search queries. In addition, after the manual review of articles published in *CPT:PSP* in 2018, 20 original research articles that presented PBPK modeling results were found. Among those, 16 used concentration-time or other PK data for model development or validation. Among the 16 studies, 12 used literature data that most likely had to be digitized, 3 had access to individual level data, and 1 study used data from a database for their model development. Among the 12 studies that used literature information, 17% ( $n = 2$ ) reported that they had used a digitization software, leading to an underreporting rate of 83%. A detailed overview of the manual review process is shown in **Figure S2** and **Table S4**.

#### Software evaluation

Overall, 70 subjects (51% male) were enrolled in our study. Their mean age was 30 years (range of 18–65 years), and they engaged in a mean computer usage of 4.1 ( $\pm 3.0$ ) hours per day. Only 4% of them had worked with a digitizing software before. All subjects had an educational degree, with the lowest being lower secondary school-leaving certificate and the highest being a doctorate. Demographic characteristics and the number of subjects were equally distributed in both groups as summarized in **Table 1**. The ANOVA and chi-square goodness-of-fit tests revealed no significant differences in study demographics between the groups, with

document format (PDF) (scanned vs. not scanned), the publishing year, and the investigated parameter ( $C_{max}$  or sample time points). In addition, for values that revealed a  $\zeta$  greater than 5%, a root cause analysis was performed.

All graphical and statistical analysis as well as the sample size calculations were performed using R and R-Studio.<sup>19,20</sup>

## RESULTS

### Literature search

Digitizing software is increasingly used in QSP and PBPK modeling as shown in **Figure 2a**. The free and open-source

Table 1 Study demographics

Parameter and descriptive measures	Group 1, N = 36	Group 2, N = 34	Total, N = 70
Age, y, mean (SD)	30 (13)	30 (12)	30 (13)
Average computer usage per day, hour, mean (SD)	4.5 (3.1)	3.7 (2.9)	4.1 (3.0)
Mouse usage, count (%)	31 (86)	30 (88)	61 (87)
No experience with digitization software, count (%)	33 (92)	34 (100)	67 (96)
Right-handedness, count (%)	30 (83)	32 (94)	62 (89)
Male, count (%)	20 (56)	16 (47)	36 (51)

SD, standard deviation.

*P* values always greater than 0.08. The demographic information for all study participants was complete. Thus, no data had to be imputed.

Mean  $\zeta$  was small for all digitized profiles (0.99%), indicating excellent accuracy. Furthermore,  $\zeta$  SD was low (0.72%), revealing a good precision of the software. The regression analysis revealed a significant ( $P < 0.001$ ) effect of symbol shape and digitizing time on  $\zeta$ . These effects are visualized in **Figure 3**. Triangles had a 1.9-fold increased mean  $\zeta$  when compared with circles (1.3% vs. 0.69%) and, hence, were less accurately digitized. Furthermore, subjects digitizing slowly were more accurate than subjects digitizing faster (**Figure 3b**). Besides that, no other covariates had a significant effect on  $\zeta$ .

From the first to the last repetition, the mean digitizing time declined moderately (first, 3.01 minutes; second, 2.24 minutes; third, 2.16 minutes). No statistical difference in accuracy or precision was observed between the three replicates as shown in **Figure S3**.

Furthermore, the estimated PK parameters based on the digitized profiles revealed only small deviations when compared with the parameters used for profile simulation with a mean modulus of the relative deviation of 0.5%. The deviation of all parameters is visualized in **Figure 3c**.

An ANOVA analysis of the performed substudy revealed statistically significant differences in accuracy and precision among the investigated software (**Figure 3d**). GetData Graph Digitizer and Engauge Digitizer had a similar mean  $\zeta$  value (0.2%), whereas DataThief had a markedly increased value (0.5%). The  $\zeta$  SD was 0.1%, 0.2%, and 0.4% for GetData Graph Digitizer, Engauge Digitizer, and DataThief, respectively.

#### Analysis of published PK data

For investigating the literature profile quality, 181 mean  $C_{\max}$  values and 3499 sample time points of concentration-time profiles obtained from 81 literature studies published between 1984 and 2017, which were presented as graphs and as numeric values, were analyzed. Digitization was carried out as part of two of our in-house model developments (simvastatin, ketoconazole). The digitized profiles were originally derived from graphs that had either linear or logarithmic scaled axes and were depicted either as single or panel plots. Therefore, 3% of the sample time points and 85% of the mean  $C_{\max}$  values had a  $\zeta$  greater than 5%. The linear regression analysis revealed that besides the parameter investigated (sample time points or  $C_{\max}$ ), neither the PDF format (scanned vs. not scanned) nor the publishing year had a significant effect on  $\zeta$ .

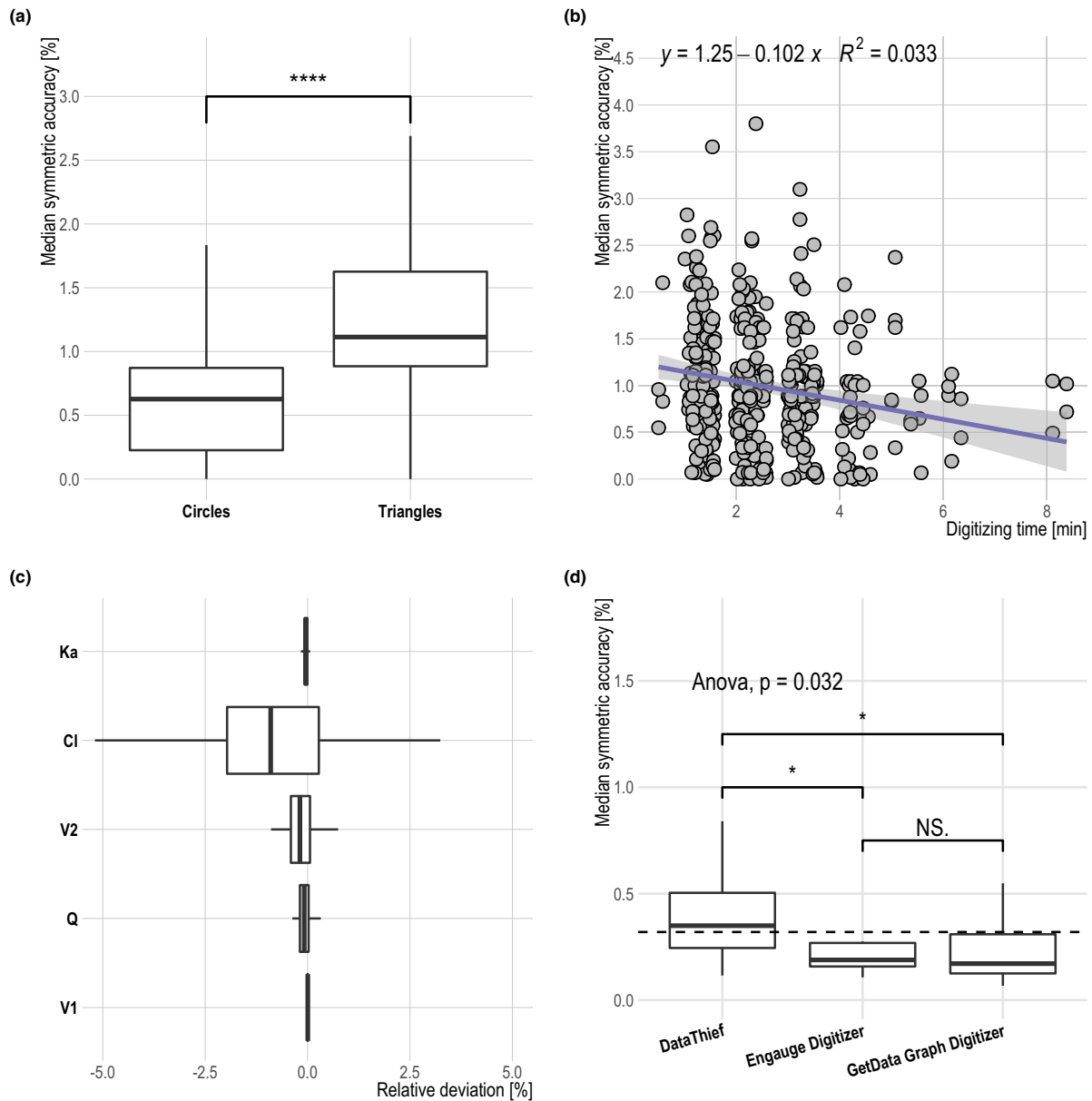
The subsequently performed root cause analysis found for all sample time points with  $\zeta$  greater 5% a justification, namely, either the x axis was not sufficiently resolved or the x axis in the graphic had an uneven resolution. In contrast, a reason for the discrepancy could be identified in only 40% of the mean  $C_{\max}$  values with  $\zeta$  greater than 5%. Specifically, they were caused either by poor graphic quality, incorrect labeling, or different types of central tendencies presented in the table and graphic. For the remaining digitized mean  $C_{\max}$  values, no justification could be found, leading to an assumption of either incorrectly stated mean  $C_{\max}$  values in the depicted concentration-time profile or in the presented table. An overview of the error frequencies and  $\zeta$  distribution is presented in **Figure 4**.

Finally, based on the most important study findings, a digitization algorithm as depicted in **Figure 5** was formulated that can help guide scientists through the digitization process.

## DISCUSSION

### Literature search

The reuse of data through digitization from published articles is an easy-to-use and attractive way for gathering necessary information, especially in QSP and PBPK modeling. This is also evident in the investigated publication frequencies of “systems pharmacology” or “physiologically based pharmacokinetic” in combination with the names of the investigated digitization software solutions. Thus, a remarkable, constant, and exponential increase in the number of literature references was observed. This was observed not only for the pooled number of publication frequencies but also for most of the software packages themselves. However, it should be mentioned that because of the large number of different software solutions, it is very unlikely that all digitizing software available was investigated. In addition, we assume that the actual number of unreported digitizing software usage is significantly higher and that the software is often not reported. This is supported by the cumulative number of publications from 2012 to 2019 in the journal *CPT:PSP*, where 477 publications citing “systems pharmacology” or “physiologically based pharmacokinetic” are published but only 31 publications additionally mention the name of a digitizer software. Subsequently, even if not every publication on the subject requires digitization software, this still suggests a massive underreporting. This assumption is further validated by the manual review of publications



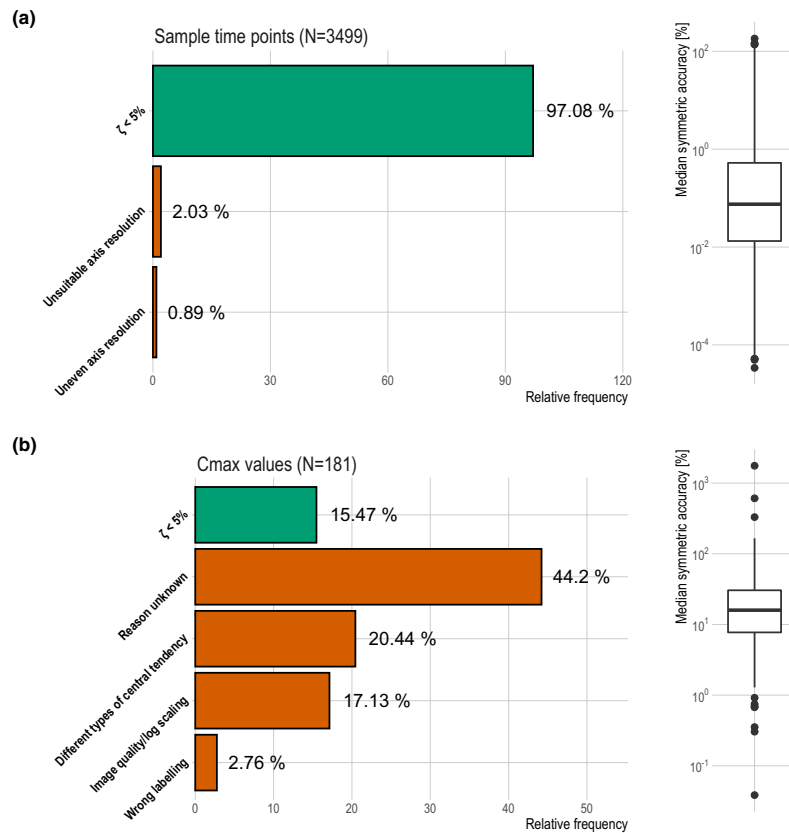
**Figure 3** Results from the multiple linear regression analysis. Influence of the symbol shape (a) on median symmetric accuracy is visualized as a boxplot with the Wilcoxon rank sum test. Effect of digitizing time (b) on median symmetric accuracy is depicted as a scatterplot with linear regression formula and coefficient of determination. (c) Relative pharmacokinetic parameter deviation of the estimated parameters when compared with the values used for profile simulation is shown as boxplots. Parameter estimation was performed for each digitization run. (d) Median symmetric accuracy for different digitization software shown as boxplots. ANOVA as well as pairwise Wilcoxon rank sum tests were performed. ANOVA  $P$  value is stated. For Wilcoxon rank sum test  $P$  values, the following annotation was used: \*\*\*\* $\leq 0.0001$ , \*\* $\leq 0.01$ ; \* $\leq 0.05$ ; NS  $> 0.05$ . In addition, in d arithmetic, the mean of all groups is shown as a dashed line. All boxplots visualize the following descriptive statistics: The median value, the interquartile range, and the 1.5-fold interquartile range. ANOVA, analysis of variance; NS, not significant.

from 2018 in *CPT:PSP* on PBPK modeling, revealing a reporting rate of 17%.

As a drawback of the performed literature search, one can state that the search query results were not further revised, and no gray literature analysis was carried out as

recommended for systematic reviews. However, the purpose of the literature search was not the development of a systematic and exhaustive review but, rather, the identification of general trends. For this reason, the methodology differs from that of a systematic review. For example, search





**Figure 4** Discrepancy between reported and graphically presented sample time points and mean  $C_{\max}$  values. Relative frequency of  $\zeta < 5\%$  and justifications for  $\zeta$  values  $\geq 5\%$  were presented as bar charts. Distribution of  $\zeta$  values were in addition shown as boxplots. **(a)** depicts the results for digitized sample time points while **(b)** displays the digitized mean  $C_{\max}$  values.  $C_{\max}$ , peak plasma concentration.

queries were not carried out in various databases such as PubMed or Embase as recommended for systematic reviews. Instead, the search engine Google Scholar was used, whose algorithm screens many different databases and sources; moreover, this is better suited to get an overview of the frequency of use in literature.

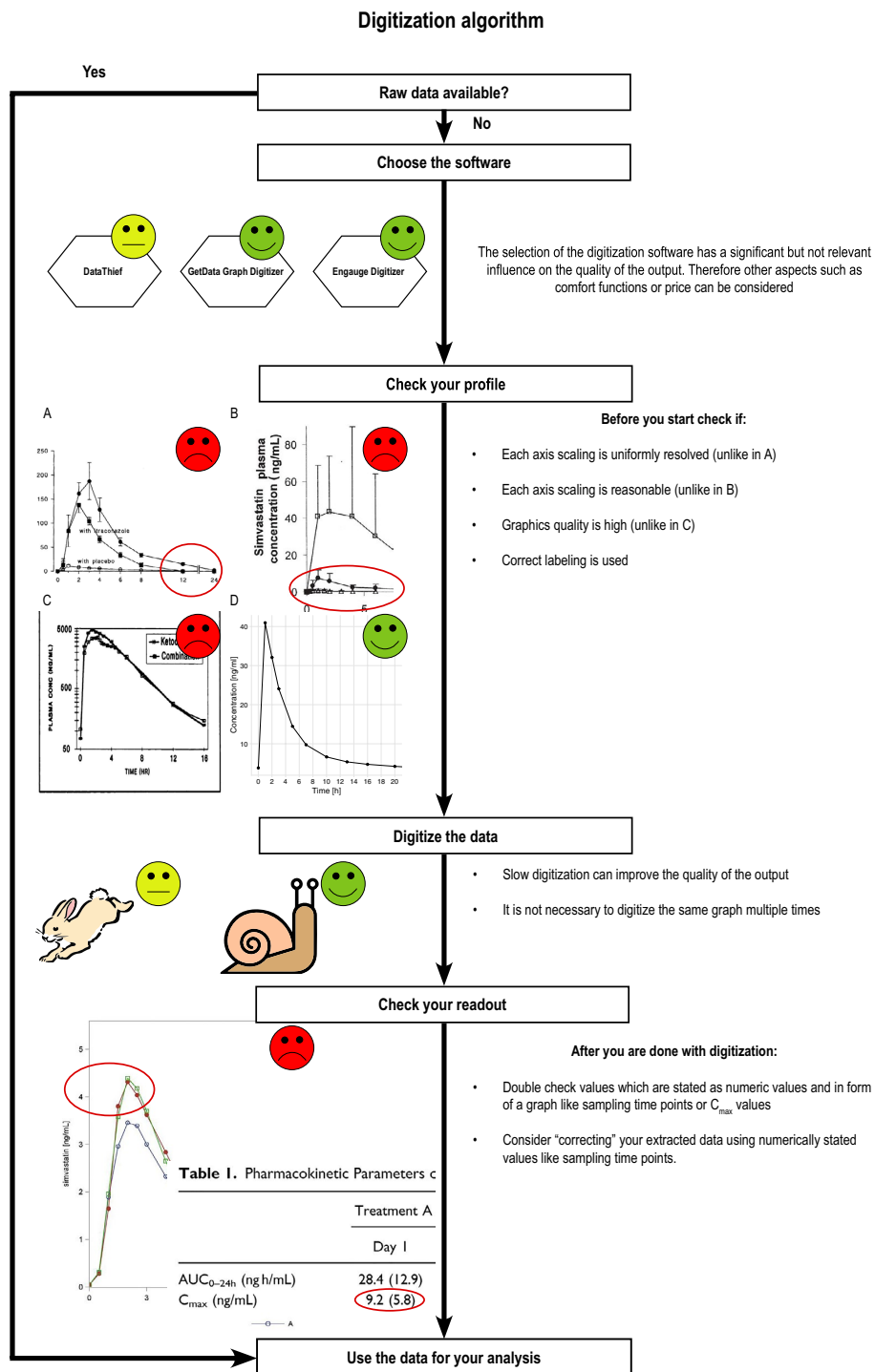
Nevertheless, it can be assumed that the usage of digitizing software in QSP and PBPK modeling will further increase in the next few years as shown in **Figure 2**.

### Software evaluation

For the study with 70 subjects,  $\zeta$  was chosen as an error metric because of its intuitive interpretation as a relative error.<sup>16</sup> With a mean  $\zeta$  of 0.99% and a mean  $\zeta$  SD of 0.725%, the digitized readouts were tremendously accurate and precise. This is also reflected in the accuracy of the PK parameters subsequently fitted to the digitized profiles showing a mean modulus of relative deviation of 0.5% when compared with the original values. This further suggests the assumption that the accuracy of individual digitized values is less important because they are not independently analyzed in the model but as time-dependent series of values. Apart from this, the symbol shape and digitizing time revealed a significant effect on the accuracy ( $\zeta$ ), leading, for example, to a 1.9-fold

lower mean  $\zeta$  value for circles when compared with triangles. Nevertheless, these effects are still negligible, considering the overall small  $\zeta$ . Although the average digitizing time declined with each repetition, no advantages in accuracy were observed if the same graph was digitized more than once. Based on these results, we recommend that one-time, slow-paced digitizing is sufficient for a proper readout.

The additionally performed substudy revealed significant differences in accuracy comparing DataThief and the two software products Engauge Digitizer and GetData Graph Digitizer. Here, DataThief showed a 1.5-fold decline in accuracy when compared with Engauge Digitizer and GetData Graph Digitizer. As mentioned previously, this effect is still negligible because the mean  $\zeta$  for all three software packages was still less than 0.6%. This led to the assumption that although significant differences between the software exist, accuracy is still excellent, and thus, other software features are more important. For example, the freely available and open-source software Engauge Digitizer is still under active development on GitHub, providing a wide range of functionalities and available in different languages for multiple operating systems. Although this might raise the question of whether, apart from the software, other factors such as the operating system also have an impact on



**Figure 5** Proposed digitization algorithm to improve the daily digitizing and graph creation practice in the fields of quantitative systems pharmacology and physiologically-based pharmacokinetics. Examples are taken from refs. 22 to 25. AUC<sub>0-24h</sub>, area under the plasma concentration-time curve calculated from 0 to 24 hours post dose; C<sub>max</sub>, peak plasma concentration.

accuracy, at least for Engauge, this is very unlikely because it is programmed in Qt, an operating system-independent programming language.<sup>12,21</sup>

#### Analysis of published PK data

Data that were redundantly presented as numeric values as well as in a graphs or plots were analyzed using  $\zeta$  as an error metric for the differences between reported values and the corresponding digitized graphical representation. If  $\zeta$  was  $> 5\%$ , the graphs and plots were further explored to determine the article properties that may impede researchers from retrieving correct readouts.  $\zeta$  of the digitized sample time points were in good agreement with the results derived from the previously conducted study. However, a few sources of errors could be identified. Specifically, the resolution of the axes seemed to have an important influence on the quality of the digitized readout. If one of the axis resolutions is uneven or the resolution does not allow cleanly distinguishing between individual measuring points, the result can be falsified. Surprisingly, with 80% of the 181 mean  $C_{\max}$  values having a  $\zeta > 5\%$  and a maximum  $\zeta$  of 1760%, alarmingly large differences between the published numerical values and the values in graphs were found. Even worse, as shown in **Figure 4**, after the performed root cause analysis for 40% of the  $C_{\max}$  values with  $\zeta$  greater 5%, no justification could still be identified. This leads to the assumption that either the wrong graph was plotted or a wrong  $C_{\max}$  was reported. Based on these findings, we strongly recommend that published data should additionally always be made available as raw data. Furthermore, if such access is available, digitizing reported and graphically presented data should be avoided; instead, raw data should be used. Moreover, if access to raw data is not available, researchers should check that each axis scaling is uniformly and optimally resolved, the graphics quality is high, and the correct labeling is used. In addition, they should try to double check their digitized values based on values that are additionally published in a numeric form. However, although following the last recommendation may prevent the use of corrupted data, there is no option to correct the readout if the errors that are already present before digitization get detected. Consequently, it is very likely that many profiles cannot be reused after all. For this reason, it is hoped that in the long run, all data published in condensed form as graphs will also be made available to scientists as raw values.

In summary, it was found that digitizing software has become more popular, especially in QSP and PBPK modeling. The presented results indicate that they are a great tool to gather data from graphical representations with excellent accuracy and precision. Moreover, neither user-dependent nor software-dependent relevant confounders could be identified. Although the digitizing time, symbol shape, and software used had a statistically significant influence on digitizing accuracy, the impact on the routine digitizing practice seems negligible. Digitizing a graph more than once did not improve the quality of the readout and thus is redundant. However, it was also found that the greatest danger of incorrectly derived analysis results based on digitized data does not come from the process of digitizing but from pre-existing errors in the published data. Overall, the results of this study

are the results of the first systematic investigation on the accuracy and precision of digitizing software. Hopefully, the derived recommendations as summarized in **Figure 5** may guide and improve the daily digitizing and graph creation practice in the field of QSP and PBPK modeling and eventually enhance the quality of models developed based on digitized readouts.

**Supporting Information.** Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website ([www.psp-journal.com](http://www.psp-journal.com)).

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[Correction added on 19 June 2020 after first publication: Funding Information section was included.]

**Conflict of Interest.** The authors declared no competing interests for this work.

**Author Contributions.** J.-G.W., H.B., D.S., M.S., and T.L. wrote the manuscript. J.-G.W., H.B., D.S., M.S., and T.L. designed the research. J.-G.W., H.B., and T.L. performed the research. J.-G.W. and D.S. analyzed the data.

**Data Availability Statement.** The datasets generated and analyzed during the current study as well as the scripts for reproducing the analysis results are available on request from the corresponding author.

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3.2 PUBLICATION II - A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPk) PARENT-METABOLITE MODEL OF THE CHEMOTHERAPEUTIC ZOPTARELIN DOXORUBICIN — INTEGRATION OF IN VITRO RESULTS, PHASE I AND PHASE II DATA AND MODEL APPLICATION FOR DRUG-DRUG INTERACTION POTENTIAL ANALYSIS

3.2.1 *Reference*

Nina Hanke, Michael Teifel, Daniel Moj, **Jan-Georg Wojtyniak**, Hannah Britz, Babette Aicher, Herbert Sindermann, Nicola Ammer, and Thorsten Lehr. "A physiologically based pharmacokinetic (PBPk) parent-metabolite model of the chemotherapeutic zoptarelin doxorubicin — integration of in vitro results, Phase I and Phase II data and model application for drug-drug interaction potential analysis." In: *Cancer Chemotherapy and Pharmacology* 81.2 (Feb. 2018), pp. 291–304. DOI: 10.1007/s00280-017-3495-2

3.2.2 *Author Contributions*

Following CRediT [4, 5], the contributions of the individual authors are listed in Table 3.2.<sup>2</sup>

Table 3.2: Author contributions for Publication II - Zoptarelin Doxorubicin

Nina Hanke	Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing
Michael Teifel	Project Administration, Data Curation, Investigation, Validation, Writing - Review & Editing
Daniel Moj	Validation, Software, Visualization, Writing - Original Draft, Writing - Review & Editing
Jan-Georg Wojtyniak	See included publications and contribution report on page vi
Hannah Britz	Methodology, Software, Validation, Formal Analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization
Babette Aicher	Project Administration, Writing - Review & Editing
Herbert Sindermann	Data Curation, Investigation, Validation, Writing - Review & Editing

<sup>2</sup> For a description of the different taxonomy categories see also appendix Chapter B

Nicola Ammer	Project Administration, Writing - Review & Editing
Thorsten Lehr	Project Administration, Writing - Original Draft, Writing - Review & Editing

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# A physiologically based pharmacokinetic (PBPK) parent-metabolite model of the chemotherapeutic zoptarelin doxorubicin—integration of in vitro results, Phase I and Phase II data and model application for drug–drug interaction potential analysis

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## Abstract

**Purpose** Zoptarelin doxorubicin is a fusion molecule of the chemotherapeutic doxorubicin and a luteinizing hormone-releasing hormone receptor (LHRHR) agonist, designed for drug targeting to LHRHR positive tumors. The aim of this study was to establish a physiologically based pharmacokinetic (PBPK) parent-metabolite model of zoptarelin doxorubicin and to apply it for drug–drug interaction (DDI) potential analysis.

**Methods** The PBPK model was built in a two-step procedure. First, a model for doxorubicin was developed, using clinical data of a doxorubicin study arm. Second, a parent-metabolite model for zoptarelin doxorubicin was built, using clinical data of three different zoptarelin doxorubicin studies with a dosing range of 10–267 mg/m<sup>2</sup>, integrating the established doxorubicin model. DDI parameters determined in vitro were implemented to predict the impact of zoptarelin doxorubicin on possible victim drugs.

**Results** In vitro, zoptarelin doxorubicin inhibits the drug transporters organic anion-transporting polypeptide 1B3 (OATP1B3) and organic cation transporter 2 (OCT2). The model was applied to evaluate the in vivo inhibition of these transporters in a generic manner, predicting worst-case scenario decreases of 0.5% for OATP1B3 and of 2.5% for OCT2 transport rates. Specific DDI simulations using PBPK models of simvastatin (OATP1B3 substrate) and metformin (OCT2 substrate) predict no significant changes of the plasma concentrations of these two victim drugs during co-administration.

**Conclusions** The first whole-body PBPK model of zoptarelin doxorubicin and its active metabolite doxorubicin has been successfully established. Zoptarelin doxorubicin shows no potential for DDIs via OATP1B3 and OCT2.

**Keywords** AEZS-108 · AN-152 · Doxorubicin · PBPK modeling · Drug–drug interaction · Targeted chemotherapy

## Introduction

Zoptarelin doxorubicin (also known as AEZS-108, AN-152 and ZEN-008) is a fusion molecule of the chemotherapeutic doxorubicin and an LHRHR agonist [1]. The DNA intercalating agent doxorubicin is chemically linked to the carrier molecule D-Lys6-LHRH, which enables specific binding and selective uptake of zoptarelin doxorubicin by tumors expressing receptors for LHRH (“drug targeting”), followed by the intracellular release of the active component doxorubicin. The rationale for the synthesis and development of this hybrid molecule is to increase the cytotoxic specificity, while decreasing the general toxicity when compared to doxorubicin alone.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00280-017-3495-2>) contains supplementary material, which is available to authorized users.

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In vitro, zoptarelin doxorubicin has shown stronger anti-proliferative effects in human LHRHR positive ovarian and endometrial cancer cells compared to doxorubicin [2], as well as higher cytotoxic potency in LHRHR expressing human oral and laryngeal carcinoma cells [3]. In nude mice bearing subcutaneous human LHRHR positive endometrial and ovarian tumors, equimolar doses of zoptarelin doxorubicin were significantly more effective in tumor growth inhibition compared to doxorubicin. Furthermore, in the high-dose study arm, seven of the ten mice treated with doxorubicin died, while all ten mice treated with zoptarelin doxorubicin survived [4]. Growth of subcutaneous human urinary bladder cell tumors in nude mice was more potently inhibited by zoptarelin doxorubicin compared to doxorubicin [5].

In clinical Phase I and Phase II studies, zoptarelin doxorubicin has shown therapeutic activity in patients with LHRHR positive ovarian and endometrial cancer [6, 7]. The PK properties of zoptarelin doxorubicin have been assessed in the first-in-human, dose escalation Phase I study in patients. Plasma half-life and clearance were calculated to be approximately 2 h and 1 L/(min\*m<sup>2</sup>), with the reservation that in this early study the measured plasma concentrations showed a high variability [8]. Due to the size and hydrophilicity of zoptarelin doxorubicin (decapeptide coupled via a glutaryl linker to doxorubicin), passive distribution into tissues is limited, but cellular entry is expected to be facilitated by target binding to LHRHR, followed by internalization of the drug–receptor complex and intracellular cleavage to release the doxorubicin moiety within the target cells. In aqueous solution and in blood plasma, zoptarelin doxorubicin is subject to spontaneous and carboxylesterase-mediated hydrolysis into doxorubicin and probably D-Lys6-LHRH-glutarate. Metabolite profiles in liver microsomal incubations suggest a minor role of oxidative metabolism compared to hydrolysis.

Doxorubicin PK studies in patients show that doxorubicin follows a multiphasic disposition after intravenous infusion. The initial distribution half-life of approximately 5 min indicates rapid tissue uptake, while a terminal half-life of 20–48 h reflects slow elimination from tissues. Steady-state distribution volumes exceed 20–30 L/kg revealing extensive drug uptake into tissues. Plasma clearance is in the range of 8–20 mL/min/kg and is governed by metabolism and biliary excretion [9–11].

To evaluate the zoptarelin doxorubicin DDI potential in vitro, a DDI screening on cytochrome P450 (CYP) enzymes and recommended drug transporters has been performed. In these assays, zoptarelin doxorubicin showed no inhibition or induction of CYP enzymes, but in the transporter studies, zoptarelin doxorubicin inhibited OATP1B3 and OCT2 with IC<sub>50</sub> values of 16.5 and 3.26 μmol/L, respectively. Doxorubicin itself and D-Lys6-LHRH-glutarate inhibited OATP1B3 with IC<sub>50</sub> values > 100 μmol/L

and OCT2 with IC<sub>50</sub> values > 200 μmol/L. Based on these results, in vivo interactions with drugs that are substrates of OATP1B3 (e.g. simvastatin) or OCT2 (e.g. metformin) could not be ruled out. As these victim drugs are widely used, their co-administration with zoptarelin doxorubicin would be very likely, creating a need to investigate the impact of these potential DDIs. However, clinical DDI studies involving DNA intercalating agents are, for ethical reasons, difficult to conduct. PBPK modeling offers an excellent alternative to dedicated clinical DDI studies and is recommended and supported by the FDA (US Food and Drug Administration) and EMA (European Medicines Agency) to predict the magnitude of in vivo DDIs from in vitro results [12, 13].

The objectives of this modeling investigation were (1) to establish the first whole-body PBPK model of zoptarelin doxorubicin and its active metabolite doxorubicin, (2) to apply the zoptarelin doxorubicin model for a general assessment of the DDI potential with OATP1B3 and OCT2 victim drugs and (3) to predict the magnitude of zoptarelin doxorubicin DDIs with simvastatin and metformin in worst-case scenarios.

## Materials and methods

### Clinical studies used

The results of three different clinical studies with PK blood sampling were available for model development (Table 1). Study 1 (ZEN-008-Z023) is a Phase I first-in-human sequential group dose escalation and PK study, performed in 17 female patients with LHRHR positive tumors. Zoptarelin doxorubicin was administered as a 2-h intravenous infusion, once every 21 days, in doses of 10, 20, 40, 80, 160 or 267 mg/m<sup>2</sup> [8]. Data of two patients were excluded due to bioanalytical issues. Study 2 (AEZS-108-046) is a combined Phase I/II study, with PK sampling performed in a sub-set of 14 male or female patients with locally advanced unresectable or metastatic LHRHR positive urothelial carcinoma who failed platinum-based chemotherapy. Zoptarelin doxorubicin was administered as a 2-h infusion every 21 days in doses of 160, 210 or 267 mg/m<sup>2</sup> (results not published, yet). Data of four patients were excluded because of sample hemolysis. Study 3 (AEZS-108-053) is a Phase I cardiac safety and PK study comparing zoptarelin doxorubicin and doxorubicin therapy in 21 and 11 female patients, respectively, with locally advanced recurrent or metastatic cancer. Zoptarelin doxorubicin was administered as a 2-h infusion of 267 mg/m<sup>2</sup>. Doxorubicin was administered as a 1-h infusion of 60 mg/m<sup>2</sup> (results not published, yet). Data of two patients were excluded due to sampling issues. Details on the patient demographics of these studies (age, weight, body surface area) are listed in Table 1.



**Table 1** Studies used for zoptarelin doxorubicin PBPK model development and evaluation

Dose (mg/m <sup>2</sup> )	Administration	n	Women (%)	Age (years)	Weight (kg)	BSA (m <sup>2</sup> )	Dataset	Study references
<b>Doxorubicin</b>								
36	iv (96 h), SD	7	50 <sup>a</sup>	30.0 <sup>a</sup>	64.0 <sup>a</sup>	1.73 <sup>a</sup>	Training	[15]
30	iv (bolus), QD	7	50 <sup>a</sup>	30.0 <sup>a</sup>	64.0 <sup>a</sup>	1.73 <sup>a</sup>	Training	[16]
60 <sup>a</sup>	iv (1 h), SD <sup>a</sup>	3 <sup>a</sup>	100 <sup>a</sup>	71.0 (67–74) <sup>a</sup>	67.0 (58–84) <sup>a</sup>	1.64 (1.57–1.77) <sup>a</sup>	Training	[18]
60	iv (1 h), SD	9	100	59.9 (44–74)	64.1 (41–84)	1.63 (1.28–1.81)	Training	Study 3 (AEZS-108-053)
<b>Zoptarelin doxorubicin</b>								
10	iv (2 h), SD	1	100	58.0	84.0	1.89	Training	Study 1 (ZEN-008-Z023)
20	iv (2 h), SD	1	100	48.0	65.0	1.70	Training	Study 1 (ZEN-008-Z023)
40	iv (2 h), SD	1	100	69.0	145.0	2.48	Training	Study 1 (ZEN-008-Z023)
80	iv (2 h), SD	1	100	44.0	55.0	1.63	Training	Study 1 (ZEN-008-Z023)
160	iv (2 h), SD	6	100	59.3 (55–69)	83.2 (58–107)	1.89 (1.60–2.12)	Test	Study 1 (ZEN-008-Z023)
267	iv (2 h), SD	5	100	48.8 (31–63)	66.9 (59–85)	1.73 (1.64–1.89)	Test	Study 1 (ZEN-008-Z023)
160	iv (2 h), SD	3	0	64.0 (63–65)	78.3 (69–90)	1.97 (1.84–2.07)	Test	Study 2 (AEZS-108-046)
210	iv (2 h), SD	3	29	66.0 (55–83)	89.6 (64–121)	2.02 (1.71–2.38)	Test	Study 2 (AEZS-108-046)
267	iv (2 h), SD	4	25	69.0 (62–87)	70.0 (52–86)	1.81 (1.51–1.98)	Test	Study 2 (AEZS-108-046)
267	iv (2 h), SD	21	100	61.6 (46–78)	71.4 (45–108)	1.73 (1.35–2.13)	Training	Study 3 (AEZS-108-053)

Values given for age, weight and BSA are arithmetic means, minima and maxima

<sup>a</sup>Assumed, BSA: body surface area, iv: intravenous, n: number of individuals studied, QD: once daily, SD: single dose, test: test dataset (model evaluation), training: training dataset (model development and parameter optimization)

To supplement the measurements of the doxorubicin arm of Study 3, published human in vivo data of doxorubicin in plasma, white blood cells, urine and feces were added, to build the “training dataset” for the development and parameter optimization of the doxorubicin model. As training data for zoptarelin doxorubicin model development and parameter optimization, the four lowest dose applications of Study 1 (10, 20, 40, 80 mg/m<sup>2</sup>) plus the measurements of Study 3 (267 mg/m<sup>2</sup>, highest clinical dose) were chosen. Evaluation of the zoptarelin doxorubicin model was carried out with the clinical data of the remaining dosing groups of Study 1 as well as the complete clinical Study 2 as the “test dataset”.

## Software

PBPK modeling was performed with PK-Sim® and MoBi® (Open Systems Pharmacology Suite, Version 7.0.0, Bayer AG, Leverkusen, Germany). Parameter optimization was accomplished using the Monte Carlo algorithm of the “Parameter Identification Toolbox” in MATLAB® (Version R2013b, The MathWorks, Inc., Natick, MA, USA). Sensitivity analysis was performed within PK-Sim®. Graphics and PK parameter analyses were compiled with MATLAB® R2013b.

## Doxorubicin model development

Model development was started with the establishment of a model of the active metabolite doxorubicin. To limit the

parameters to be optimized during model development, the minimal number of processes necessary was implemented into the model. For the doxorubicin model these are (1) doxorubicin binding to DNA, (2) an unspecific metabolic hepatic clearance and (3) an unspecific elimination to bile. Glomerular filtration and enterohepatic cycling were enabled, as they are active under physiological conditions. A diagram of the PBPK model structure is given in Zoptarelin Doxorubicin Supplementary Fig. 1.

To model the binding of doxorubicin to DNA as the cause of the extensive distribution into and slow elimination from body tissues, a binding partner was implemented into the DNA-rich organs, with published values for  $K_d$  and  $k_{off}$  [14]. In the literature, there are reports of doxorubicin concentration measurements in plasma and white blood cells [15, 16] that were utilized to inform the distribution (cellular permeability, see below) and DNA binding processes. As there is no white blood cell (WBC) compartment in PK-Sim, the red blood cell (RBC) compartment was used as a substitute to represent the nucleated white blood cells. The volume of this red blood cell compartment is larger than the physiological volume of the white blood cells; therefore, a relative concentration of DNA binding sites (that are absent in the anucleate RBCs) was implemented into the RBC compartment and estimated. The DNA binding site reference concentration (concentration in the tissue with the highest concentration of binding sites) was also optimized.

To account for hepatic metabolism to doxorubicinol and other metabolites, an unspecific metabolic first-order clearance was implemented into the liver and optimized.

To model biliary excretion, an unspecific first-order transport from liver to bile was implemented and estimated. As the lipophilicity of doxorubicin is very low ( $\log P = 1.27$ ), calculated passive cellular permeability is low. However, doxorubicin has been reported to be a substrate of diverse transporters, including the human isoforms of OATP1A and OATP1B [17]. To accurately describe the available clinical data, passive cellular permeability was increased, to compensate for active transport processes that have not been implemented into the model.

To obtain values for the parameters that could not be adequately informed from literature or in-house preclinical studies, optimization was performed by simultaneously fitting the model to the data of the doxorubicin arm of Study 3 (9 patients), measured doxorubicin plasma and white blood cell intracellular concentration–time profiles of Speth et al. [15, 16] (two studies with mean values of 7 patients each) and published fraction of doxorubicin dose administered excreted unchanged to urine and feces information [18].

### Zoptarelin doxorubicin model development

The final doxorubicin model was then used in the establishment of the zoptarelin doxorubicin model, together with clinically observed plasma concentration–time profiles of zoptarelin doxorubicin and doxorubicin following intravenous administration of zoptarelin doxorubicin. The following processes were implemented into the zoptarelin doxorubicin model: (1) zoptarelin doxorubicin binding to the LHRHR target, (2) internalization of zoptarelin doxorubicin by LHRHR and (3) hydrolysis of zoptarelin doxorubicin to release the active doxorubicin moiety within blood plasma as well as intracellularly. A diagram of the PBPK model structure is given in Zoptarelin Doxorubicin Supplementary Fig. 1.

To model the binding of zoptarelin doxorubicin to its target LHRHR, this receptor was implemented and values for  $K_d$ ,  $k_{off}$  as well as the LHRHR reference concentration were estimated. Expression of LHRHR is described in the literature to occur in non-malignant pituitary, ovary, testis, prostate and breast cells, as well as in cancer cells of diverse origin [19, 20]. In the model, LHRHR was implemented into the gonadal compartment (approximate organ volume of 0.013 L). To compensate for the missing pituitary, prostate, breast and, most notably, cancer cell expression, as these tissues are not represented in standard PK-Sim individuals, LHRHR expression was further added at a 50% expression level to the lung compartment (approximate organ volume of 1 L). The lung was chosen as a well perfused organ with no special pharmacokinetic function in this analysis (as would

have been the case with liver or kidney). Implementation of zoptarelin doxorubicin binding to LHRHR into the model clearly improved the shape of the simulated zoptarelin doxorubicin plasma concentration–time curves.

Internalization of zoptarelin doxorubicin was implemented as a cellular uptake facilitated by LHRHR (into gonads and lung), followed by intracellular hydrolysis to release the doxorubicin moiety. As  $K_M$  value of this uptake process an  $IC_{50}$  value of 7.45 nmol/L was used, measured in a radio ligand displacement assay with very low concentrations of the radiolabeled ligand [21]. Therefore, it was assumed that  $IC_{50} = K_i$  and this value was used as  $K_M$  value for the internalization process. A very similar  $IC_{50}$  value of 10 nmol/L has been described in the literature for the binding of the endogenous agonist LHRH to LHRHR [22]. The internalization turnover number was estimated.

To model the hydrolysis of zoptarelin doxorubicin to D-Lys6-LHRH-glutarate and doxorubicin, a hydrolytic clearance was implemented into blood plasma, gonads and lung. The hydrolysis rate in plasma was optimized, informed by the measured concentrations of zoptarelin doxorubicin being hydrolyzed and of doxorubicin resulting from this hydrolysis. The hydrolysis rate in gonads and lung was assumed to be the same as in plasma.

Parameter optimization was performed by simultaneously fitting the model to measured zoptarelin doxorubicin and doxorubicin plasma concentration–time profiles after administration of zoptarelin doxorubicin obtained in Study 1 (10, 20, 40, 80 mg/m<sup>2</sup>) and Study 3 (267 mg/m<sup>2</sup>).

### Virtual population characteristics

To predict the variability of the simulated plasma concentration–time profiles, virtual populations of 100 individuals were generated according to the population demographics of each respective dosing group of the Studies 1, 2 and 3. The ICRP (International Commission on Radiological Protection) database in PK-Sim [23] was used for generation of virtual Caucasian populations. In the generated virtual populations, age, height, weight, corresponding organ volumes, tissue compositions, blood flow rates, etc. are varied by an implemented algorithm within the limits of the ICRP database. In addition, the zoptarelin doxorubicin hydrolysis rate, the reference concentrations of the binding partners LHRHR and DNA, as well as the doxorubicin hepatic and biliary clearance rates were set to be log-normally distributed with variabilities of 25%CV (relative standard deviation). To create a virtual population for the DDI predictions, reflecting an even larger demographic variability and representing the target cancer patient population, preliminary demographics of a large clinical Phase III study (Study 4, AEZS-108-050 [24]) were used.

### Model evaluation

Model performance was evaluated by comparison of the predicted concentration–time profiles of the virtual populations to the plasma concentrations observed in the clinical studies, which had not been used during parameter optimization (test dataset). All population predictions compared to observed plasma concentration–time profiles are documented in the “Results” section or in the supplementary material, together with predicted compared to observed  $AUC_{last}$  and  $C_{max}$  values of all studies. Furthermore, the biological plausibility of optimized parameters was checked and sensitivity analyses were conducted for the doxorubicin and zoptarelin doxorubicin models.

Sensitivity of the final models to single parameters (local sensitivity analysis) was investigated, measured as changes of the AUC extrapolated to infinity ( $AUC_{inf}$ ) of a simulation of the highest applied dose. All parameters relevant to the respective model were included into the analysis, optimized parameters as well as parameters fixed to literature values. Parameters were defined as relevant if they have been optimized (see Zoptarelin Doxorubicin Supplementary Tables 4 and 5), if they might have a strong influence due to calculation methods used in the model (lipophilicity, fraction unbound), if they are related to optimized parameters (doxorubicin-DNA  $K_d$ , doxorubicin-DNA  $k_{off}$ , doxorubicin blood/plasma ratio) or if they had significant impact in former models (solubility, intestinal permeability, EHC continuous fraction, cellular permeability, blood/plasma ratio, GFR fraction). A sensitivity value of  $-1.0$  signifies that a 10% increase of the examined parameter causes a 10% decrease of the simulated  $AUC_{inf}$ .

### General assessment of the zoptarelin doxorubicin DDI potential

To obtain a general statement on the DDI potential of zoptarelin doxorubicin, the final model was applied to predict the in vivo inhibition of OATP1B3 and OCT2 in a generic manner (independent of the victim drug affected by this inhibition), by calculating the relative change of these transporters’  $K_M$  values due to inhibition by zoptarelin doxorubicin.

Assuming a competitive inhibition and Michaelis–Menten kinetics, we expect a change in the  $K_M$  value of the transport of the affected victim drugs, but not of the maximal transport rate, as competitive inhibition can be overcome by high victim drug concentrations. Therefore, the inhibition is characterized by the relative change of  $K_M$  according to Eq. 2:

$$K_M \text{ apparent, victim drug } (\mu\text{mol/L}) = K_M \text{ victim drug} * \left( 1 + \frac{\text{inhibitor concentration}}{\text{inhibitor } K_i} \right) \quad (1)$$

$$K_M \text{ apparent, victim drug } (\%) = 100\% * \left( 1 + \frac{\text{inhibitor concentration}}{\text{inhibitor } K_i} \right) \quad (2)$$

Simulations to assess the DDI potential of zoptarelin doxorubicin were performed for the highest clinical dose of 267 mg/m<sup>2</sup> zoptarelin doxorubicin as intravenous infusion over 2 h. OATP1B3 is predominantly expressed at the basolateral membranes of hepatocytes located around the central vein, facilitating the uptake of organic anions for hepatic clearance [25]. To estimate the effect of zoptarelin doxorubicin on OATP1B3, predicted population interstitial unbound concentrations of zoptarelin doxorubicin in the liver were used as input for Eq. 2. OCT2 is mainly expressed at the basolateral membrane of renal tubule cells, facilitating the uptake of organic cations from the blood for subsequent renal secretion [26]. To estimate the impact of zoptarelin doxorubicin on OCT2, predicted population interstitial unbound concentrations of zoptarelin doxorubicin in the kidney were employed.

The zoptarelin doxorubicin  $K_i$  values for inhibition of OATP1B3 and OCT2 were calculated from  $IC_{50}$  values determined in vitro (16.5 and 3.26  $\mu\text{mol/L}$ ), the substrate concentrations applied in these assays (0.05  $\mu\text{mol/L}$  estradiol-17beta-glucuronide and 10.0  $\mu\text{mol/L}$  metformin) and the OATP1B3 and OCT2 transport  $K_M$  values for these substrates (15.8 [27] and 990.0  $\mu\text{mol/L}$  [28]), according to the Cheng-Prusoff equation for competitive inhibition [29]:

$$K_i = \frac{IC_{50}}{1 + \text{substrate concentration} / K_M} \quad (3)$$

$K_i$  values for pure competitive inhibition are independent of the affected victim substrate, the substrate concentration and the assay conditions [30]. Therefore, the relative changes of  $K_M$  calculated from Eq. 2 are in theory applicable to all putative zoptarelin doxorubicin victim drugs transported by OATP1B3 and OCT2.

### Specific assessment of the zoptarelin doxorubicin DDI potential

To evaluate the in vivo interaction potential of zoptarelin doxorubicin with actual OATP1B3 and OCT2 victim drugs, the model was coupled to PBPK models of simvastatin and metformin (for details on the simvastatin and metformin PBPK models see the Simvastatin and Metformin Supplementaries). Simvastatin acid, the pharmacologically active metabolite of the prodrug simvastatin, is recommended by the FDA as a victim drug for the clinical investigation of OATP1B1/1B3 DDIs [31]. Metformin is recommended by the FDA as well-established substrate of the cationic

transport system for the use in clinical studies of DDIs involving OCT2/MATE [31].

Simvastatin is administered in the form of the inactive lactone that is hydrolyzed after ingestion to the active simvastatin acid. Only the acid form is transported by OATP1B1 and OATP1B3 from blood plasma into hepatocytes. The model applied for DDI prediction is a whole-body parent-metabolite PBPK model of simvastatin lactone and simvastatin acid. Because of the overlapping substrate specificities of OATP1B1 and OATP1B3, it is difficult to pinpoint the exact contribution of each isoform to simvastatin acid transport [32]. As the goal of this analysis was to assess worst-case scenarios, a combined OATP1B1/3 transport was modeled and this whole transport was inhibited with the zoptarelin doxorubicin  $K_i$  determined for OATP1B3, even though OATP1B1 was not affected *in vitro*. This approach results in an overprediction of the impact of OATP1B3 inhibition, but avoids underprediction of the DDI potential due to misspecification of the OATP1B3 contribution.

As worst-case co-administration scenarios, simultaneous administrations of 267 mg/m<sup>2</sup> zoptarelin doxorubicin with 80 mg simvastatin (once daily, day 5) or 1000 mg metformin (three times daily, day 5) were simulated, and victim drug plasma concentrations with and without co-administration of zoptarelin doxorubicin were assessed in population predictions. Different time intervals between the start of zoptarelin doxorubicin infusion and the day 5 morning dose of the victim drugs were simulated, to find the administration schemes resulting in highest drug–drug interaction impact for worst-case scenario assessment.

## Results

A comprehensive parent-metabolite PBPK model for the prediction of zoptarelin doxorubicin and doxorubicin concentrations following different intravenous doses of zoptarelin doxorubicin has been successfully developed.

A schematic representation of the parent-metabolite model structure is shown in Zoptarelin Doxorubicin Supplementary Fig. 1. All drug-dependent parameters of the final model, taken from literature or preclinical studies as well as all optimized parameter values, are given in Zoptarelin Doxorubicin Supplementary Table 4. All system-dependent parameters of the final model, particularly expression levels of the implemented binding partners in the different tissues with their geometric standard deviations of lognormal distribution in virtual populations, are given in Zoptarelin Doxorubicin Supplementary Table 5. No other system-dependent parameters were changed or adjusted.

## PBPK model development and performance

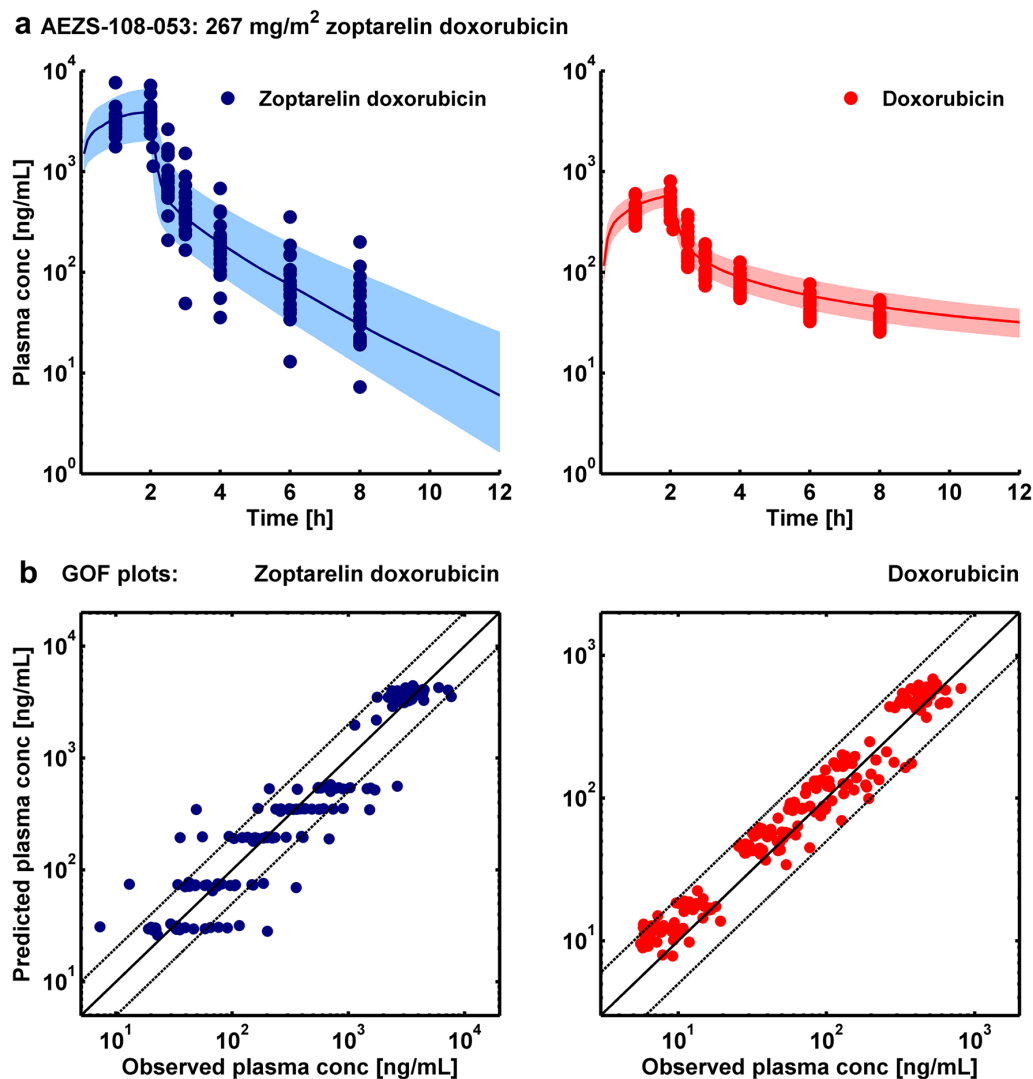
The established doxorubicin and zoptarelin doxorubicin PBPK models show excellent descriptive and predictive performance.

The data used for doxorubicin model development consisted of individual plasma concentration–time profiles following application of 60 mg/m<sup>2</sup> of doxorubicin to a total of nine patients. These measurements were supplemented by literature data of white blood cell concentrations and excretion to urine and feces information (Table 1). Predicted and observed doxorubicin plasma concentrations of Study 3 as well as fractions excreted to urine and feces following administration of doxorubicin are presented in Zoptarelin Doxorubicin Supplementary Fig. 2 (training dataset). Predicted and observed doxorubicin plasma and white blood cell concentrations following administration of doxorubicin as published by Speth et al. are shown in Zoptarelin Doxorubicin Supplementary Fig. 3 (training dataset). These concentrations were fitted with lower weight compared to the measurements of Study 3, given the age of the data and the assumption underlying the blood cell concentrations that 10<sup>9</sup> cells equal a volume of 1 mL, knowing that white blood cells are very diverse in size and shape. Prediction of the doxorubicin concentrations resulting from administration of zoptarelin doxorubicin is presented in Figs. 1 and 2 as well as in Zoptarelin Doxorubicin Supplementary Figs. 4 and 5.

The data used for zoptarelin doxorubicin model establishment included individual plasma concentration–time profiles collected in three clinical trials, following application of seven different doses of zoptarelin doxorubicin in a range of 10–267 mg/m<sup>2</sup>. Plasma concentrations of zoptarelin doxorubicin and doxorubicin were collected in a total of 46 patients (Table 1). Model performance of the final zoptarelin doxorubicin model is demonstrated in Fig. 1 and Zoptarelin Doxorubicin Supplementary Fig. 4 for the studies used during parameter optimization (training dataset) and in Fig. 2 and Zoptarelin Doxorubicin Supplementary Fig. 5 for the independent clinical data (test dataset).

As can be seen in Fig. 1, the inter-individual variability of the measured concentrations is wider for zoptarelin doxorubicin than for doxorubicin. This is unexpected, as the variability of its main ADME mechanism, namely the hydrolysis of zoptarelin doxorubicin to doxorubicin, affects both analytes. The more pronounced variability of the parent compound concentrations in blood plasma, where zoptarelin doxorubicin and doxorubicin are sampled, might result from its very low permeability compared to doxorubicin, which extensively distributes into body tissues [11]. The predicted variability in the population simulations is also wider for zoptarelin doxorubicin.

Furthermore, the variance of the measured concentrations in the very first clinical Study 1 is higher than in the



**Fig. 1** Training dataset: **a** Population simulations (semilog scale) compared to observed data of zoptarelin doxorubicin (blue) and doxorubicin plasma concentrations (red) following intravenous administration of 267 mg/m<sup>2</sup> zoptarelin doxorubicin. Clinical data (Study 3,  $n=21$ ) are shown as dots. Population simulation medians are shown as lines; the shaded areas depict the 5th–95th percentile popula-

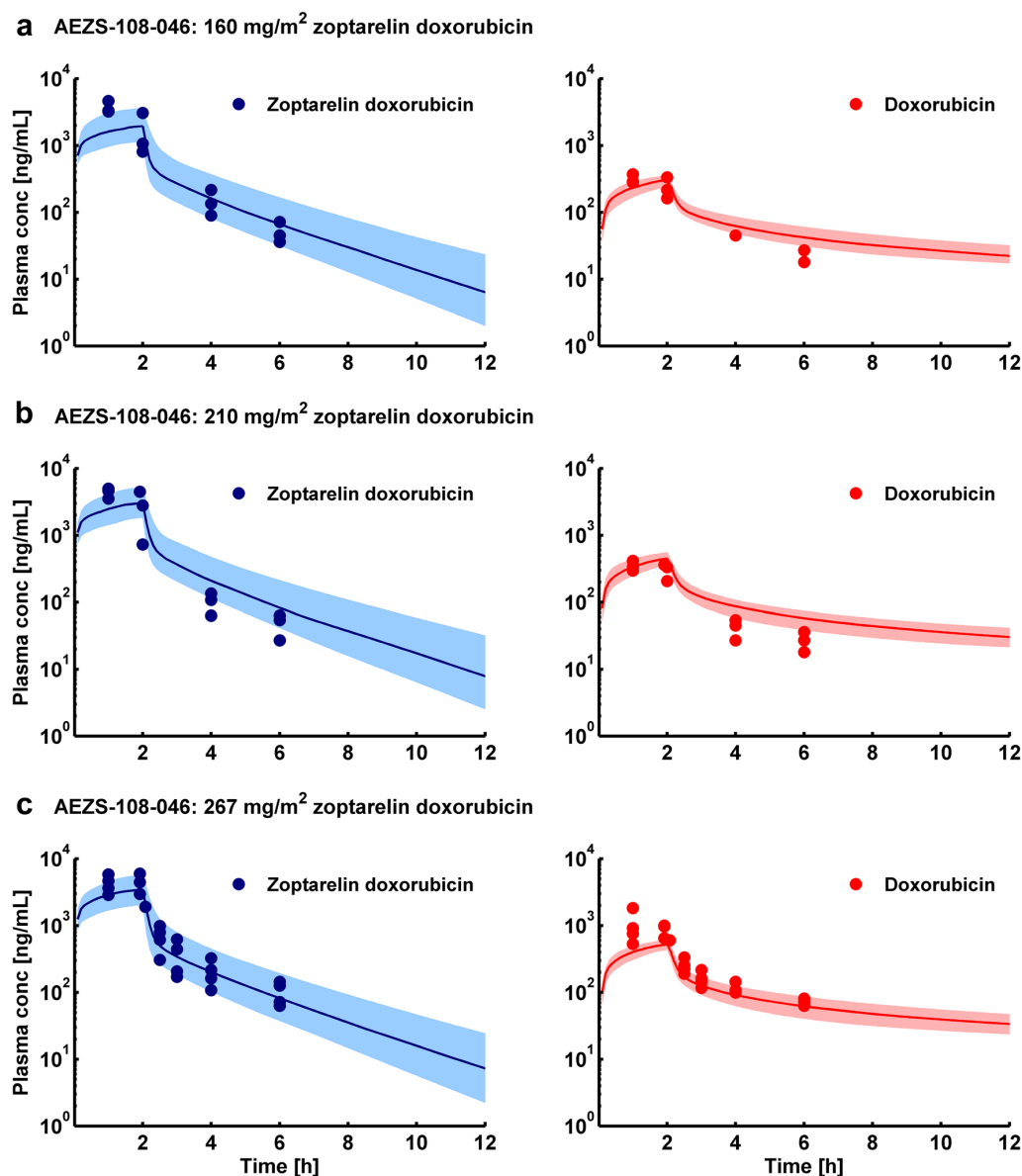
tion prediction intervals. **b** Goodness of fit (GOF) plots (log scale) demonstrating the correlation of individual predicted with observed zoptarelin doxorubicin (blue) and doxorubicin plasma concentrations (red) of the study shown above. The solid lines represent the line of unity; the dashed lines indicate twofold deviation

following trials (see Zoptarelin Doxorubicin Supplementary Fig. 5, test dataset). This high variability might be the result of errors in sampling time or of hydrolytic cleavage of zoptarelin doxorubicin prior to freezing of some of the blood samples [8]. These issues could be resolved and, therefore, did not affect later measurements.

Precision of model parameter estimates is shown in the tables listing the drug-dependent and system-dependent

zoptarelin doxorubicin PBPK model parameters (Zoptarelin Doxorubicin Supplementary Tables 4 and 5).

Using the final model, pharmacokinetic parameters ( $AUC_{last}$  and  $C_{max}$ ) of all dosing groups have been calculated from population simulations as mean values with standard deviation and compared to observed values (see Zoptarelin Doxorubicin Supplementary Tables 1 and 2). Prediction errors for  $AUC_{last}$  and  $C_{max}$  values are also



**Fig. 2** Test dataset: Population simulations (semilog scale) compared to observed data of zoptarelin doxorubicin (blue) and doxorubicin plasma concentrations (red) following intravenous administration of 160, 210 or 267 mg/m<sup>2</sup> zoptarelin doxorubicin. Clinical data (Study

2,  $n=3$ ,  $n=3$  and  $n=4$ ) are shown as dots. Population simulation medians are shown as lines; the shaded areas depict the 5th–95th percentile population prediction intervals

given in Zoptarelin Doxorubicin Supplementary Tables 1 and 2. Plots of predicted versus observed  $AUC_{last}$  and  $C_{max}$  values with twofold prediction success limits are shown in Zoptarelin Doxorubicin Supplementary Fig. 6.

### PBPK model sensitivity analysis

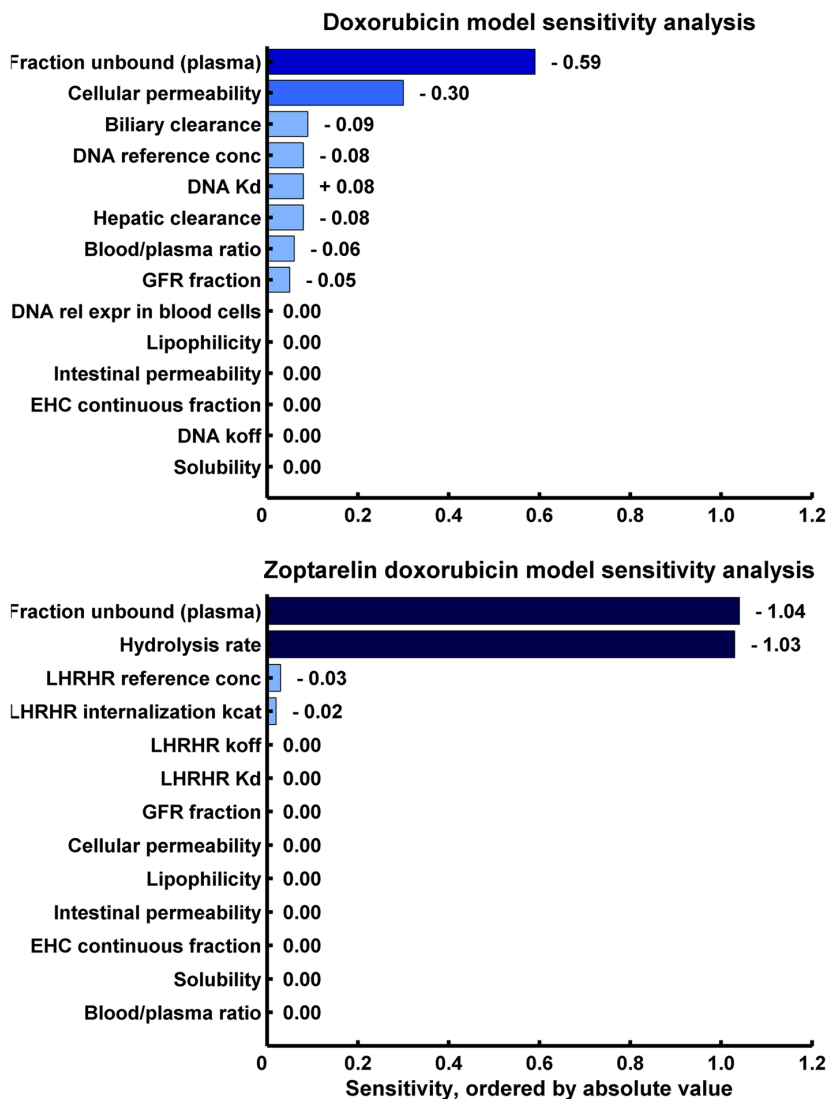
Sensitivity analyses were conducted for the doxorubicin and the zoptarelin doxorubicin model, with simulations of

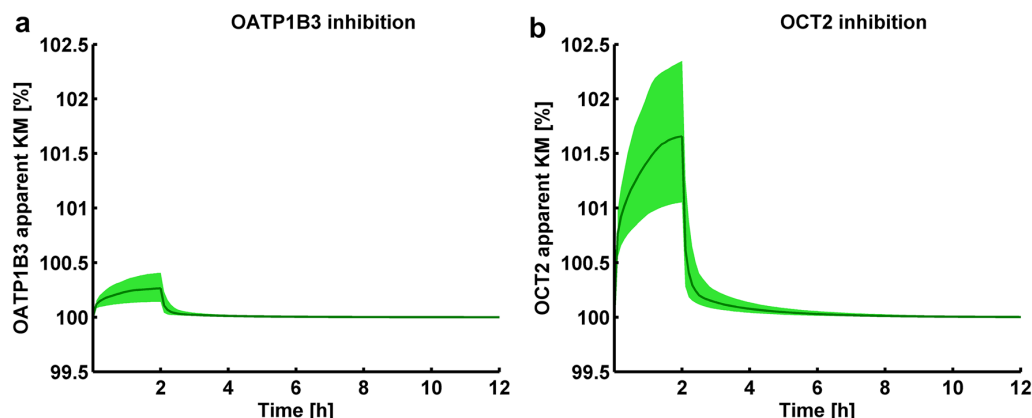
single intravenous administrations of 60 mg/m<sup>2</sup> doxorubicin (1-h infusion) and of 267 mg/m<sup>2</sup> zoptarelin doxorubicin (2-h infusion), respectively. The investigated model parameters and results are listed in Fig. 3. The doxorubicin model is sensitive to the values of fraction unbound in plasma (sensitivity value of  $-0.6$ ) and cellular permeability (sensitivity value of  $-0.3$ ). The zoptarelin doxorubicin model is sensitive to the values of fraction unbound in plasma (sensitivity value of  $-1.0$ ) and zoptarelin doxorubicin hydrolysis rate (sensitivity value of  $-1.0$ ).

### General assessment of the zoptarelin doxorubicin DDI potential

To obtain a general assessment of the in vivo DDI potential of zoptarelin doxorubicin via OATP1B3 and OCT2, independently of the substrate affected by this inhibition, the relative changes of the apparent  $K_M$  values for these two transporters were calculated according to Eq. 2. As input inhibitor concentrations, predicted population interstitial unbound concentrations of zoptarelin doxorubicin in the liver and the kidney, respectively, were used (267 mg/m<sup>2</sup> zoptarelin doxorubicin, 2-h infusion). Zoptarelin doxorubicin  $K_i$  values were calculated to be 16.45  $\mu\text{mol/L}$  for

**Fig. 3** Doxorubicin and zoptarelin doxorubicin model sensitivity analysis results. Conc: concentration, EHC: enterohepatic circulation, GFR: glomerular filtration rate,  $k_{\text{cat}}$ : catalytic rate constant,  $K_d$ : dissociation constant,  $k_{\text{off}}$ : dissociation rate constant, LHRHR: luteinizing hormone-releasing hormone receptor, rel expr: relative expression, normalized to tissue with highest expression





**Fig. 4** Zoptarelin doxorubicin DDI potential: Maximum impact of zoptarelin doxorubicin on OATP1B3 and OCT2. **a** Relative change of OATP1B3 apparent  $K_M$  during inhibition by 267 mg/m<sup>2</sup> zoptarelin doxorubicin. **b** Relative change of OCT2 apparent  $K_M$  during inhibition

by 267 mg/m<sup>2</sup> zoptarelin doxorubicin. Population simulation medians are shown as lines; the shaded areas depict the 5th–95th percentile population prediction intervals

OATP1B3 and 3.23  $\mu$ mol/L for OCT2 (see “Materials and methods”). The resulting relative changes of apparent  $K_M$  values amount to less than 0.5% for OATP1B3 and to less than 2.5% for OCT2, as illustrated in Fig. 4.

To rate the impact of a 2.5% change in  $K_M$ , the relation of initial reaction velocity  $v_0$  and  $K_M$  can be applied:

$$v_0 = \frac{V_{\max} * \text{substrate concentration}}{K_M + \text{substrate concentration}} \quad (4)$$

For substrate concentrations significantly below  $K_M$  and unchanged maximal reaction velocity  $V_{\max}$  (competitive inhibition assumed), a 2.5% increase of  $K_M$  results in a 2.5% decrease of initial reaction velocity. For higher substrate concentrations, the influence of increased  $K_M$  will be even smaller.

### DDI potential of zoptarelin doxorubicin with simvastatin and metformin

For specific DDI predictions with simvastatin and metformin, victim drug steady-state plasma concentrations after administration of the highest common doses of 80 mg simvastatin (once daily, day 5) or 1000 mg metformin (three times daily, day 5) with and without co-administration of 267 mg/m<sup>2</sup> zoptarelin doxorubicin were simulated.

Testing of different time intervals between the start of zoptarelin doxorubicin infusion and administration of the victim drugs showed highest DDI impact on simvastatin acid, when the zoptarelin doxorubicin infusion is started 2 h after the administration of simvastatin (zoptarelin doxorubicin  $C_{\max}$  at the time of simvastatin acid  $C_{\max}$ ); and highest DDI impact on metformin, when the zoptarelin doxorubicin

infusion is started 1 h before the administration of metformin (zoptarelin doxorubicin  $C_{\max}$  at the time of metformin  $C_{\max}$ ).

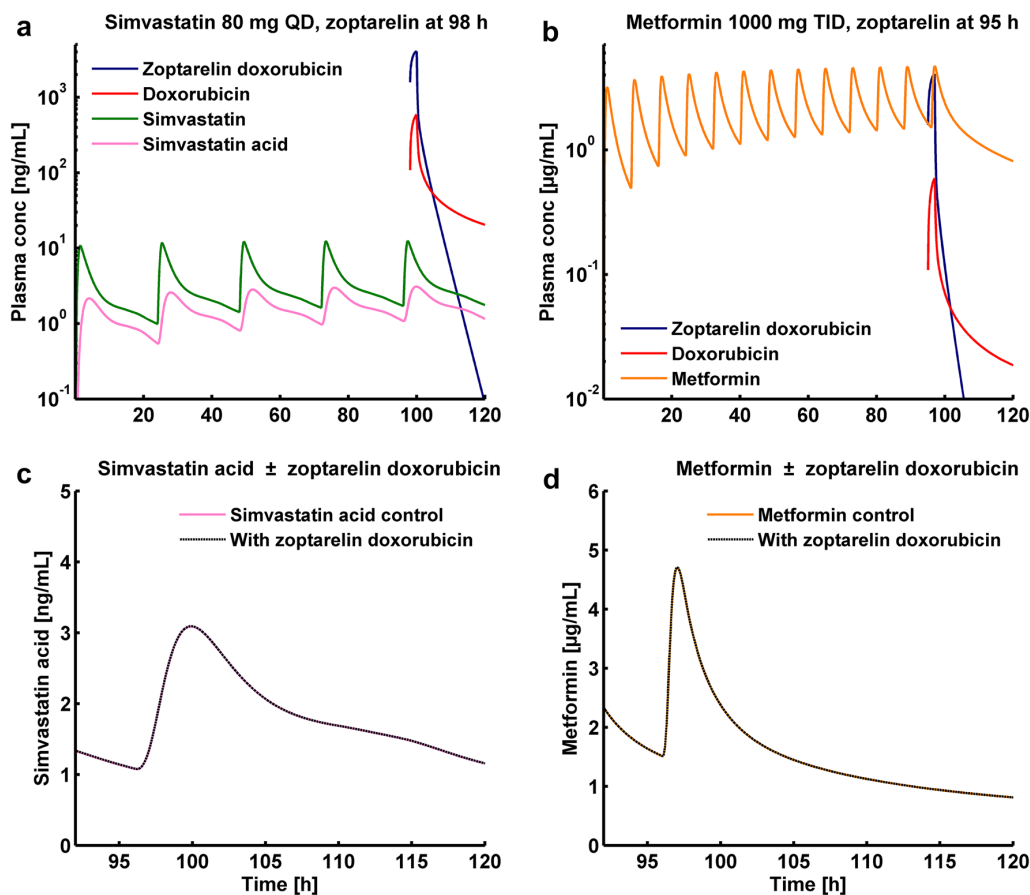
The identified administration regimens for maximum DDI impact and the resulting plasma concentrations of zoptarelin doxorubicin, simvastatin acid and metformin are illustrated in Fig. 5a, b. Victim drug plasma concentrations of simvastatin acid and metformin, with and without co-administration of zoptarelin doxorubicin are presented in Fig. 5c, d. DDI impact on victim drug AUC and  $C_{\max}$  values, simulated with the different tested dosing regimens is shown in Zoptarelin Doxorubicin Supplementary Table 3. The identified worst-case co-administration scenarios result in a 0.114% increase of the plasma AUC<sub>96–120</sub> of simvastatin acid and a 0.096% increase of the AUC<sub>96–104</sub> of metformin, due to liver and kidney uptake inhibition.

## Discussion

### Doxorubicin model

The presented doxorubicin model is the first whole-body PBPK model developed with clinical data from humans and a mechanistic implementation of the binding of doxorubicin to DNA. This binding is essential to describe the pharmacokinetics of doxorubicin, as it is the driving force behind the unusual distribution behavior of this drug [33]. The developed model accurately describes doxorubicin plasma concentrations following direct administration of doxorubicin and also very successfully predicts the concentrations of doxorubicin released following administration of a variety of different doses of zoptarelin doxorubicin.





**Fig. 5** Zoptarelin doxorubicin DDI potential: Specific DDI worst-case scenario predictions with simvastatin acid and metformin as OATP1B3 and OCT2 victim drugs. **a** Predicted zoptarelin doxorubicin (blue), doxorubicin (red), simvastatin (green) and simvastatin acid (pink) plasma concentrations (semilog scale) of a typical individual during administration of 80 mg simvastatin every 24 h, plus 267 mg/m<sup>2</sup> zoptarelin doxorubicin once, on the morning of day 5. The last administration of simvastatin is at 96 h; zoptarelin doxorubicin infusion (2 h) is started 2 h later at 98 h, resulting in simultaneous peak plasma concentrations of zoptarelin doxorubicin and simvastatin acid at 100 h. **b** Predicted zoptarelin doxorubicin (blue), doxorubicin (red) and metformin (dark yellow) plasma concentra-

tions (semilog scale) of a typical individual during administration of 1000 mg metformin every 8 h, plus 267 mg/m<sup>2</sup> zoptarelin doxorubicin once, on the morning of day 5. The last administration of metformin is at 96 h; zoptarelin doxorubicin infusion (2 h) is started 1 h earlier, at 95 h, resulting in simultaneous peak plasma concentrations of zoptarelin doxorubicin and metformin at 97 h. **c** Overlay of predicted simvastatin acid plasma concentrations using the administration protocol shown in **a**, without (pink) and during co-administration of zoptarelin doxorubicin (dashed black line). **d** Overlay of predicted metformin plasma concentrations using the administration protocol shown in **b**, without (dark yellow) and during co-administration of zoptarelin doxorubicin (dashed black line)

The sensitivity of the doxorubicin model to the value of fraction unbound is to be expected, as this parameter determines the doxorubicin concentration available for all pharmacokinetic processes. The value used in the model has been carefully determined in vitro at Aeterna Zentaris and has not been optimized. The doxorubicin fraction unbound measured in-house (26.3%) is in very good accordance with the literature (25%, [34]). The moderate sensitivity of the model to the cellular permeability value underlines the influence of this parameter. Adjustment of this value greatly improved

the model performance and, therefore, it has been included into the set of optimized parameters.

Several other PBPK models of doxorubicin have been developed so far, mostly established from animal data with the benefit of measured doxorubicin concentrations in different tissues [35–38]. Among those is a very nice model of doxorubicin in mice, that has been extrapolated to humans including evaluation of the predicted serum concentrations with actual clinical data, as well as mechanistic modeling of DNA binding to describe the tissue distribution of doxorubicin [35]. The only other model directly developed from

human data lacks a mechanistic implementation of the tissue binding, but features a physiologically based description of the effects of aging on the distribution clearance of doxorubicin [36].

In the presented doxorubicin model, DNA binding sites have so far only been implemented into 8 of the 22 model compartments, resulting in an overestimation of the doxorubicin accumulation in these tissues and an underestimation of the doxorubicin concentration in the tissues without binding partner (not counting the blood cell compartment, as the DNA concentration within this volume has been separately adjusted to match literature data). Although the DNA binding site reference concentration of the virtual patients has been optimized ( $K_d$  and  $k_{off}$  have been fixed to literature values), the obtained value is biologically plausible. A rough estimate of the number of DNA base pairs per human is  $6.0 \times 10^{22}$  ( $6.0 \times 10^9$  base pairs per cell,  $1.0 \times 10^{13}$  cells per human). This equals 0.1 mol of base pairs per human. As the doxorubicin binding partner was implemented only into the 8 most important tissues of the model patient, these DNA binding sites are distributed into 4 L of tissue with equal expression of this binding partner, resulting in a reference concentration of 0.025 mol/L. The optimized value of 0.046 mol/L is in the same order of magnitude. Furthermore, there are reports of binding of doxorubicin not only to DNA, but (with a lower affinity) also to cardiolipin and DNA-associated enzymes, which have not been implemented into the model [9, 39]. To predict the doxorubicin concentrations and pharmacodynamics within a distinct organ, the distribution of the DNA binding sites will have to be implemented in an anatomically correct way, as has been proposed by Gustafson et al. [35].

Despite minor limitations, this model is a suitable basis for further refinement and subsequent extrapolation to vulnerable populations receiving doxorubicin treatment such as children, elderly and patients with organ impairment.

### Zoptarelin doxorubicin model

The presented zoptarelin doxorubicin model is the first PBPK model of zoptarelin doxorubicin and accurately describes and predicts plasma concentrations of zoptarelin doxorubicin and its active metabolite doxorubicin following infusion of different doses of zoptarelin doxorubicin. This is remarkable, as the model has been developed with data collected in three clinical trials investigating patients with different types of cancer.

As for the doxorubicin model, the sensitivity of the zoptarelin doxorubicin model to the value of fraction unbound was to be expected and the value used in the model has also been carefully measured in-house. The relatively high sensitivity of the model to the zoptarelin doxorubicin hydrolysis rate value emphasizes the impact of this parameter on the

elimination of zoptarelin doxorubicin and on the predicted AUC.

The primary aim of this PBPK analysis was to assess the DDI potential of zoptarelin doxorubicin with OATP1B3 and OCT2 victim drugs. Future applications of the presented model could include the implementation of a tumor compartment to enable the prediction of zoptarelin doxorubicin and doxorubicin concentrations in the target tissue and to answer questions regarding efficacy and pharmacodynamics of zoptarelin doxorubicin. For a first estimate, the model can be employed to simulate the internalization and intracellular concentrations of zoptarelin doxorubicin as well as the resulting concentrations of doxorubicin in the gonads. The lack of a tumor compartment (compensated by a low expression of LHRHR in the lung) does not impact the results and interpretation of the presented PBPK analysis of the interaction with OATP1B3 and OCT2, as these DDIs are determined by the concentrations in liver and kidney.

### Zoptarelin doxorubicin DDI potential

Zoptarelin doxorubicin shows no inhibition or induction of cytochrome P450 enzymes in vitro, as well as no inhibition of investigated transporters other than OATP1B3 ( $IC_{50} = 16.5 \mu\text{mol/L}$ ) and OCT2 ( $IC_{50} = 3.26 \mu\text{mol/L}$ ). P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter 1 (OAT1), OAT3 and OATP1B1 are not inhibited in vitro ( $IC_{50}$  values  $> 200 \mu\text{mol/L}$ ). The predicted maximum relative changes of transport rate during treatment with the highest clinical dose of zoptarelin doxorubicin are 0.5% for OATP1B3 and 2.5% for OCT2 at the end of the infusion. In line with this general interaction potential assessment, no impact of zoptarelin doxorubicin on plasma concentrations of the OATP1B3 and OCT2 victim drugs simvastatin acid and metformin was found in worst-case scenario simulations. These results are in accordance with the expectations due to low interstitial concentrations of zoptarelin doxorubicin in relation to the zoptarelin doxorubicin  $K_i$  values for inhibition of OATP1B3 and OCT2. As zoptarelin doxorubicin (MW = 1893.06 g/mol) is a 10-amino acid polypeptide linked to doxorubicin and positively charged at two amino groups at physiological pH, its passive permeability is low, leading to low interstitial concentrations.

This example demonstrates that PBPK modeling is a valuable technique to analyze the risk of investigational drugs suspected to cause drug–drug interactions in vivo. In vitro results and pharmacokinetic data from early clinical studies are used to establish mechanistic and physiologically based models that allow the in vivo prediction of drug–drug interactions. This approach is supported by drug approval agencies [12, 13] and can help to minimize patient risk, costs

and time needed for drug development. Furthermore, PBPK modeling has the capability to generate information whenever the conduct of clinical trials is not ethical, as is the case in all frail populations such as children, elderly and patients.

## Conclusion

This is the first report of a whole-body PBPK model of zoptarelin doxorubicin and its active metabolite doxorubicin. The model was applied for the evaluation of the zoptarelin doxorubicin drug–drug interaction potential (1) by a general assessment of the OATP1B3 and OCT2 inhibition potential of zoptarelin doxorubicin in vivo and (2) by specific DDI simulations of the impact of zoptarelin doxorubicin on simvastatin acid and metformin exposure in worst-case scenarios. No DDI potential of zoptarelin doxorubicin was detected in these analyses.

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## Compliance with ethical standards

**Funding** This study was funded by Aeterna Zentaris.

**Conflict of interest** Nina Hanke, Daniel Moj, Jan-Georg Wojtyniak and Hannah Britz declare that they have no conflict of interest. Michael Teifel, Babette Aicher, Herbert Sindermann and Nicola Ammer are employees of Aeterna Zentaris. Thorsten Lehr has received research grants from Aeterna Zentaris.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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### 3.3 PUBLICATION III - PHYSIOLOGICALLY BASED PRECISION DOSING APPROACH FOR DRUG-DRUG-GENE INTERACTIONS: A SIMVASTATIN NETWORK ANALYSIS

#### 3.3.1 *Reference*

**Jan-Georg Wojtyniak**, Dominik Selzer, Matthias Schwab, and Thorsten Lehr. "Physiologically based precision dosing approach for drug-drug-gene interactions: a simvastatin network analysis." In: *Clinical Pharmacology & Therapeutics* (Dec. 2020). DOI: 10.1002/cpt.2111

#### 3.3.2 *Author Contributions*

Following CRediT [4, 5], the contributions of the individual authors are listed in Table 3.3.<sup>3</sup>

Table 3.3: Author contributions for Publication III - Simvastatin

Jan-Georg Wojtyniak	See included publications and contribution report on page vi
Dominik Selzer	Formal Analysis, Methodology, Writing - Review & Editing
Matthias Schwab	Conceptualization, Writing - Review & Editing
Thorsten Lehr	Conceptualization, Project Administration, Formal Analysis, Methodology, Writing - Review & Editing

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<sup>3</sup> For a description of the different taxonomy categories see also appendix Chapter B



# Physiologically Based Precision Dosing Approach for Drug-Drug-Gene Interactions: A Simvastatin Network Analysis

Jan-Georg Wojtyniak<sup>1,2</sup> , Dominik Selzer<sup>1</sup>, Matthias Schwab<sup>2,3,4</sup> and Thorsten Lehr<sup>1,\*</sup>

Drug-drug interactions (DDIs) and drug-gene interactions (DGIs) are well known mediators for adverse drug reactions (ADRs), which are among the leading causes of death in many countries. Because physiologically based pharmacokinetic (PBPK) modeling has demonstrated to be a valuable tool to improve pharmacotherapy affected by DDIs or DGIs, it might also be useful for precision dosing in extensive interaction network scenarios. The presented work proposes a novel approach to extend the prediction capabilities of PBPK modeling to complex drug-drug-gene interaction (DDGI) scenarios. Here, a whole-body PBPK network of simvastatin was established, including three polymorphisms (*SLCO1B1* (rs4149056), *ABCG2* (rs2231142), and *CYP3A5* (rs776746)) and four perpetrator drugs (clarithromycin, gemfibrozil, itraconazole, and rifampicin). Exhaustive network simulations were performed and ranked to optimize 10,368 DDGI scenarios based on an exposure marker cost function. The derived dose recommendations were translated in a digital decision support system, which is available at [simvastatin.precisiondosing.de](http://simvastatin.precisiondosing.de). Although the network covers only a fraction of possible simvastatin DDGIs, it provides guidance on how PBPK modeling could be used to individualize pharmacotherapy in the future. Furthermore, the network model is easily extendable to cover additional DDGIs. Overall, the presented work is a first step toward a vision on comprehensive precision dosing based on PBPK models in daily clinical practice, where it could drastically reduce the risk of ADRs.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Drug-drug interactions (DDIs), drug-gene interactions, and drug-drug-gene interactions (DDGIs) are well known triggers of adverse drug reactions that might be preventable by precision dosing. One example compound prone to DDGIs is simvastatin.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ How physiologically based pharmacokinetic (PBPK) modeling can be utilized for model-informed precision dosing (MIPD) of complex DDGIs.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study presents whole-body PBPK models for simvastatin lactone and simvastatin acid, including variation of four

pharmacogenes and was tested against four DDI perpetrator drugs and one DDI victim. In addition, the model was used to develop a digital decision support system based on dose recommendations for 10,368 simulated interaction scenarios.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The presented dose recommendations might help to better assess risks of simvastatin therapy in pharmacogenomic and polypharmacy context. Furthermore, the study highlights and guides how PBPK can help to bring MIPD into daily clinical practice.

Adverse drug reactions (ADRs) are a burden to our health care and economic systems. The US Food and Drug Administration (FDA) assumes that annually > 2,216,000 serious ADRs in hospitalized patients lead to over 106,000 deaths in the United

States—ranking them as the fourth leading cause of death.<sup>1,2</sup> The associated costs are tremendous and are estimated to add up to US \$200 billion per year.<sup>1</sup> This situation is likely to become more acute as a result of ever-growing prescription use. According to

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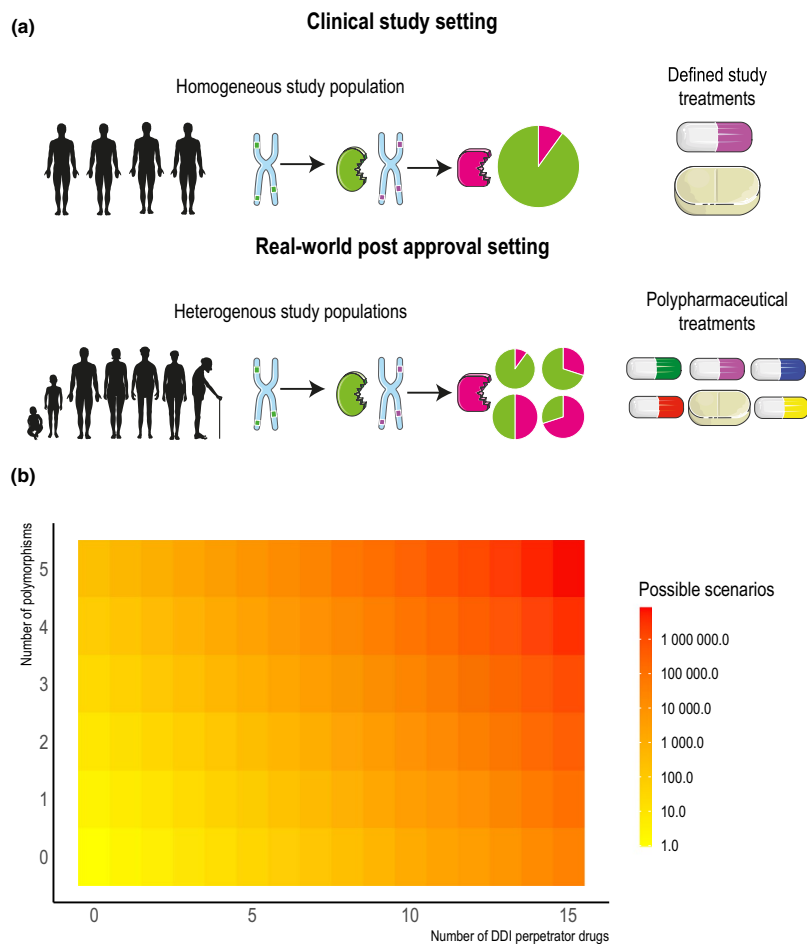
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the Centers for Disease Control and Prevention, the proportion of Americans taking > 5 prescription drugs on a regular basis has almost tripled in the past 20 years.<sup>3</sup>

Drug-drug interactions (DDIs) and drug-gene interactions (DGIs) are the most common reasons for ADRs.<sup>2,4,5</sup> Unfortunately, in current clinical practice DDIs and DGIs are considered separate entities that are typically handled in a nonholistic fashion.<sup>4</sup> However, as shown recently, 19% of potentially clinically significant interactions occur as a combination of DDIs and DGIs (drug-drug-gene interaction (DDGIs)).<sup>4,5</sup> Tackling DDIs, DGIs, or DDGIs using guidelines on dose adaptation could reduce the number of ADRs substantially, because it is assumed that 80% of ADRs

are dose-related and, hence, could be prevented.<sup>6,7</sup> This concept can be summarized as precision dosing for DDIs and DGIs.<sup>8</sup>

The current approach of developing such guidelines would be the investigation of DDGIs in clinical trials analogous to presently conducted trials on DDIs and DGIs.<sup>8,9</sup> Those studies are typically performed in healthy volunteers, in a homogenous study population and with a controlled treatment plan (Figure 1a).<sup>8</sup> Consequently, they do not reflect the situation in multimorbid patients, affected by polypharmacy and genetic polymorphisms, which is the patient group most susceptible to ADRs (Figure 1b).<sup>1,8</sup> Moreover, due to the combinatorial explosion of all possible DDGIs, exhaustive studies might not be feasible at all (Figure 1c).



**Figure 1** Difference between a clinical study setting and a real-world post-approval setting. (a) The upper part shows the research situation in a clinical setting. A homogenous study population receives a defined treatment regimen in a standardized procedure. The subsequently obtained results are used for the development of therapy recommendations for the post-approval setting. The lower part depicts the real-world postapproval setting with a higher variability in demographics, variant distribution, and a higher degree in polypharmacy compared with the clinical study population. As a result, as shown in (b) various possible DDGI scenarios are conceivable depending on the amount of concomitantly used perpetrator drugs and occurring polymorphisms. For the calculation it was assumed that each perpetrator has two DDI states (preparator is given or perpetrator is not given) and each clinically relevant polymorphism could have three independent phenotypes. Following the number of possible scenarios was calculated with  $n_{\text{scenarios}} = 2^x \text{perpetrator} * 3^y \text{polymorphism}$ . The increase of possible DDGI scenarios is shown as a heatmap. The number of possible DDGI scenarios is shown on a log-scale. Some figure elements are taken from smart.servi.com (CC BY 3.0). DDGI, drug-drug-gene interaction; DDI, drug-drug interaction.

To overcome this problem, a promising approach would be the application of whole-body physiologically based pharmacokinetic (PBPK) modeling.<sup>4</sup> PBPK models hold the capability to predict the DDGI potential of drugs *in silico* and to develop alternative precision dosing regimens for patients.<sup>4</sup> The reliability of this technique has already been demonstrated in several DDI and DGI studies and is acknowledged by regulatory agencies.<sup>10–12</sup> However, although PBPK modeling has been accepted as a useful option to predict the extent of DDGIs, examples on how PBPK modeling can be used for model informed precision dosing (MIPD) are still scarce.<sup>12</sup>

Thus, the aim of this work was to illustrate the complexity of DDIs, DGIs, and DDGIs based on the example of simvastatin. Simvastatin was selected as it is among the most prescribed drugs in industrial nations and is highly susceptible to potentially life-threatening ADRs due to its complex pharmacokinetics (PKs).<sup>13–18</sup> Moreover, this work should provide guidance for the development of an PBPK-based MIPD approach. Therefore, a comprehensive simvastatin DDGI PBPK network model was implemented to serve a web-based decision support system that offers quick and easy access to optimized dose recommendations for individual patients.

## METHODS

### Software

PBPK model development was performed with PK-Sim and MoBi (version 8 – Build 21) as part of the Open Systems Pharmacology Suite.<sup>19</sup> Model parameter identification was accomplished using Monte-Carlo optimization. Local sensitivity analysis was also performed within PK-Sim. Published plasma concentration-time profiles were digitized using GetData Graph Digitizer (version 2.26.0.20, S. Fedorov).<sup>20</sup> Graphics and statistical analysis were produced and implemented using R (version 3.6.3).<sup>21</sup>

### Simvastatin PBPK model building

The simvastatin model was developed in a stepwise procedure. In a first step, physicochemical parameters of simvastatin lactone (SL) and simvastatin acid (SA) as well as information on absorption, distribution, metabolism, and excretion processes were extracted from literature. Subsequently, mean plasma concentration-time profiles of SL and SA after oral single dose and multiple dose administration were digitized from published studies and separated into training and test datasets for model development and evaluation, respectively. Model input parameters, which were not available as PK-Sim reference values or that could not be informed from published literature values were optimized by fitting the model to measured plasma concentration-time profiles from the training dataset. PBPK study simulations were built based on healthy individuals with the reported mean values for age, weight, height, and genetic background, as stated in the corresponding study protocol, respectively. If parameter information was lacking, a PK-Sim mean individual (healthy male European, 30 years of age, body weight of 73 kg, a height of 176 cm, and based on the International Commission on Radiological Protection database) with wild type genotype was substituted. For all simulated individuals, glomerular filtration and enterohepatic cycling was implemented. A detailed description of the model development process, including information about digitized studies and model parameters can be found in the **Supplementary Material, chapter 2**.

### DGI implementation and DDI network development

DGI effects were implemented assuming a changed enzyme turnover number ( $k_{cat}$ ) compared with wild type. Here, the homozygous wild type  $k_{cat}$  as well as  $k_{cat}$  for homozygous polymorphic individuals were

estimated during model training (see **Supplementary Material, chapter 1.1.2** and **chapter 2.4**).

A DDI network was built to further evaluate the performance of the developed model. Thus, previously developed models of clarithromycin, gemfibrozil, itraconazole, rifampicin, and midazolam were coupled with the simvastatin model.<sup>12,22,23</sup> Population mean profiles as well as area under the curve (AUC) and peak plasma concentration ( $C_{max}$ ) values were predicted and compared against observed study data to evaluate the network quality.<sup>20</sup>

A detailed overview on the implementation of the DDI network, including relevant interaction parameters from *in vitro* experiments as well as the mathematical implementation of the drug interaction processes, is provided in the **Supplementary Material in chapter 1** and **chapter 3**.

### PBPK network evaluation and sensitivity analysis

PBPK model evaluation was performed using different statistical and graphical evaluation techniques. Predicted plasma concentration-time profiles were compared with observed profiles. Moreover, goodness-of-fit plots for predicted vs. observed plasma concentrations were examined. Mean relative deviation<sup>24</sup> and median symmetric accuracy<sup>25</sup> were calculated for all differences between observed and predicted plasma concentrations. In addition, the performance was evaluated by comparison of the noncompartmental analysis parameters AUC from last dose to last observation and  $C_{max}$ . AUC was computed using a linear-up log-down method. Geometric mean fold errors (GMFEs) were derived for differences between observed and predicted AUC and  $C_{max}$  values. For DGI and DDI predictions, AUC effect ratios were compared, in which a deviation of the observed from the predicted effect ratio less than two times was considered sufficient. Finally, local sensitivity analysis of the final model to single parameter changes was calculated as relative changes of the AUC of one dosing interval in steady-state conditions. A detailed overview of performance measurements and the local sensitivity analysis can be found in the **Supplementary Material, chapter 1.4**.

### Dose optimization

Simvastatin dose optimization for several DDGI scenarios, including individual DDIs and DGIs was performed. As reference, plasma concentration-time profiles for SL and SA in a mean individual after administration of 5 mg up to 80 mg (5 mg steps) SL once daily for 7 days were simulated and SL and SA AUCs from the time of the last dose up to 24 hours postdose derived. In a second step, a DDGI matrix was set up covering every possible combination of the three polymorphisms *SLCO1B1* (rs4149056), *ABCG2* (rs2231142), and *CYP3A5* (rs776746) and comedication with the four perpetrator drugs clarithromycin, itraconazole, gemfibrozil, and rifampicin. DDGI scenarios were simulated with administered SL doses according to the reference (7 days + 24 hours postdose) and reasonable perpetrator dosing regimens (see **Table 1**). For each simulation, SL and

**Table 1** Investigated perpetrator regimens

Perpetrator	Half-life, monotherapy	Regimen
Clarithromycin	3.3–4.9 hours	500 mg b.i.d.
Itraconazole	~ 24 hours	200 mg daily
Rifampicin	2.5 hours	600 mg daily concomitant with simvastatin
Rifampicin	2.5 hours	600 mg daily 17 hours after simvastatin dosing
Gemfibrozil	7.6 hours	600 mg b.i.d.



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SA AUCs were calculated. Following, relative AUC deviations of SL and SA from the reference values were computed for each DDGI scenario with an exposure marker cost function as shown in Eq. 1:

$$\text{Exposure Marker} = \frac{|AUC_{SL-DDGI} - AUC_{SL-ref}|}{AUC_{SL-ref}} + \frac{|AUC_{SA-DDGI} - AUC_{SA-ref}|}{AUC_{SA-ref}} \quad (1)$$

With Exposure Marker = Relative differences of SL and SA exposure per simvastatin dose as a cost function for dose optimization (the smaller the better),  $AUC_{SL-DDGI}$  = AUC for SL under DDGI condition,  $AUC_{SL-ref}$  = reference AUC for SL,  $AUC_{SA-DDGI}$  = AUC for SA under DDGI condition,  $AUC_{SA-ref}$  = reference AUC for SA.

For each DDGI scenario, the exposure marker cost function was minimized to identify the simvastatin dose with the smallest exposure deviation (matching exposure). For different therapeutic dose levels of simvastatin (20 mg, 40 mg, and 60 mg) relative frequency of recommended doses and the relationship between the number of DDGIs and the optimal dose level were analyzed. Moreover, a hierarchical Euclidian distance cluster analysis stratified against the DDIs and DGI was performed to identify patterns for generalized dose recommendations. Clustering was computed with complete linkage using the *hclust* function in R.

Results from the dose optimization were transferred into a DDS web application implemented with the R package “shiny,” allowing users to easily filter simulation analysis tailored to DDGIs and simvastatin doses of interest.

## RESULTS

## Simvastatin PBPK model building and evaluation

We successfully developed a whole-body PBPK model of SL and SA. For placebo and DGI model development and evaluation mean data from 57 studies were extracted including 59 SL and 57 SA plasma-concentration time profiles, which represent information from 1,271 study participants. For DGI implementation, plasma-concentration time profiles or AUC and  $C_{max}$  values for *SLCO1B1* (rs4149056) c.521C/C, c.521T/C, c.521T/T, *ABCB1* (rs1128503 rs2032582 and rs1045642) c.1236T-c.2677T-c.3435T, c.1236C-c.2677G-c.3435C, *ABCG2* (rs2231142) c.421A/A, c.421C/A, c.421C/C, and *CYP3A5* (rs776746) *CYP3A5\*3\*3*, *CYP3A5\*3\*1*, *CYP3A5\*1\*1* were used for model development and optimization. System-dependent parameters like reference concentrations and enzyme expression profiles were taken from the PK-Sim database or extracted from literature as described in **Supplementary Material, chapter 1.3**. Doses available for model development and evaluation ranged from 10 mg to 80 mg simvastatin after single and multiple doses.

Extensive model evaluations, as described in the **Supplementary Material, chapter 2.3**, revealed good model performance for placebo PK profiles. Mean ratios predicted vs. observed AUCs were 1 for SL and 0.9 for SA. Mean predicted vs. observed  $C_{max}$  ratios were 0.9 and 0.8 for SL and SA, respectively. GMFE values were 1.3 for SL AUC, 1.5 for SL  $C_{max}$ , 1.5 for SA AUC, and 1.7 for SA  $C_{max}$ , respectively.

## DGI model evaluation

The model was capable to precisely describe and predict the DGI profiles in the training and test datasets. The average AUC ratio was 1.0 for SL and 0.7 for SA, whereas the mean  $C_{max}$  ratio was

0.8 for SL and 0.6 for SA. For DGI the GMFE values were 1.3 for SL AUC and 1.4 for SL  $C_{max}$ , 1.8 for SA AUC, and 2.2 for SA  $C_{max}$ , respectively (see **Supplementary Material, chapter 2.4.6**). **Figure 2a** shows an example prediction of SA for *SLCO1B1* (rs4149056) c.521C/C and c.521T/T genotype.

## DDI network development

A DDI network was built by coupling models for clarithromycin, gemfibrozil, itraconazole, rifampicin, and midazolam with the newly derived simvastatin model (see **Supplementary Material, chapter 3**). **Figure 2b** shows an example prediction of SL under clarithromycin cotreatment. Mean predicted vs. observed AUC ratios for SL, SA, and midazolam were 1.2, 1.5, and 0.9, respectively. Average predicted vs. observed  $C_{max}$  ratios for SL, SA, and midazolam were 0.9, 1.1, and 1, respectively. GMFE values were 1.3 for both SL AUC and  $C_{max}$ , 1.7 and 1.8 for SA AUC and  $C_{max}$ , as well as 1.1 for both midazolam AUC and  $C_{max}$ .

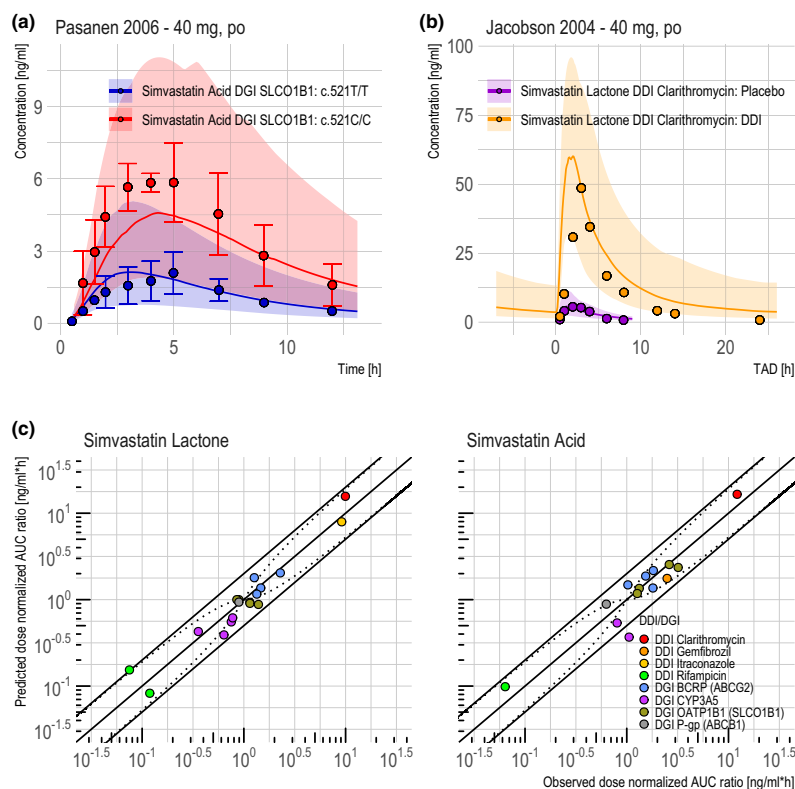
Moreover, predicted DDI and DGI effect ratios were in good agreement with observed effect ratios, as shown in **Figure 2c**. Overall, only 1 of 18 AUC effect ratios for SL and 1 of 14 AUC effect ratios for SA showed a deviation from the observed effect ratio greater than twofold. **Figure 3** summarizes the metabolic and transportation processes involved in the DDI network and visualizes the relationships between the included compounds and processes.

## Dose optimization

For each simvastatin therapeutic dose (5 to 80 mg in 5 mg steps) 648 DDGIs were optimized, which led to a total of 10,368 DDGI dose recommendations derived from the exposure marker cost function as described in Eq. 1. Cluster analysis revealed that cluster groups differ vastly with several subclusters and no observable pattern (**Figure 4a**). Thus, no generally applicable rule could be established on how to dose simvastatin.

Descriptive statistics revealed that for 13% (60 mg simvastatin therapeutic dose) to 25% (20 mg simvastatin therapeutic dose) of the investigated DDGI scenarios no alternative simvastatin dose could be found. Median optimal dose levels over all investigated DDGIs were 5 mg, 10 mg, and 20 mg for simvastatin therapeutic doses of 20 mg, 40 mg, and 60 mg, respectively. Analyses of the number of DDGIs against the optimal doses revealed a trend for all therapeutic dose levels: a greater number of DDGIs leads toward a lower optimal dose. For a therapeutic dose level of 40 mg, results are visualized in **Figure 4a** (cluster analysis), **Figure 4b** (relative frequency of optimal doses), and **Figure 4c** (number of DDGIs against optimal dose values).

DDGI network simulations were processed and transferred into a web-based interactive decision support system, which can be accessed at [simvastatin.precisiondosing.de](http://simvastatin.precisiondosing.de). The system allows users to investigate simvastatin DDGI situations of interest and explore different scenarios. Here, the user can select a given simvastatin dose, the active comedication, and the *SLCO1B1*, *ABCG2*, and *CYP3A5* genotype. Then, the application presents the optimization results, including recommended dose compared with therapeutic dose and allows the further investigation



**Figure 2** Example profiles and model evaluation plots for the developed simvastatin PBPK DDGI network. **(a)** Example profiles of the observed vs. predicted simvastatin acid plasma concentration-time profiles for SLC01B1 (rs4149056) c.521C/C, and c.521T/T genotypes.<sup>54</sup> **(b)** Example profiles of the predicted vs. observed simvastatin lactone plasma concentration-time profiles with and without clarithromycin co-treatment.<sup>55</sup> In **a** and **b** dots are observed mean values extracted from literature. Error bars display the observed SDs. Solid lines show the predicted median profile of 100 simulated individuals. Shaded area depicts the predicted 90% confidence interval. **(c)** Depicts the observed vs. predicted dose normalized AUC effect ratios (dose normalized AUC under DDI/DDGI conditions divided by dose normalized AUC under placebo conditions). Solid lines show the line of identity as well as the twofold deviations. Dotted lines are the quality limits as proposed by Guest *et al.*<sup>56</sup> AUC, area under the curve; DDGI, drug-drug-gene interaction; DDI, drug-drug interaction; PBPK, physiologicallybased pharmacokinetic.

of SL and SA exposures for the placebo situation, the investigated DDGI situation, and the situation after dose optimization. **Figure 5** depicts case examples analyzed with the support system.

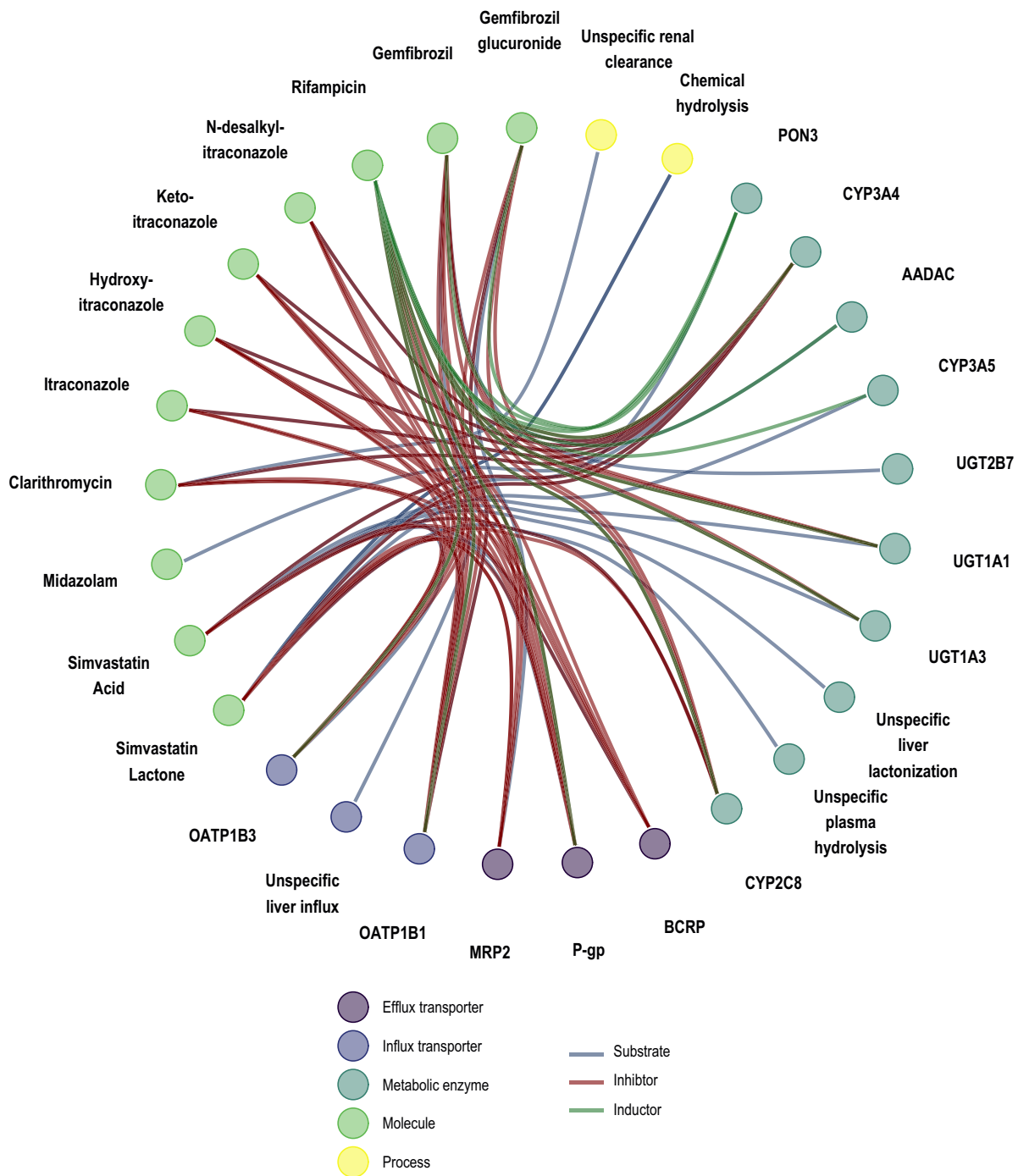
## DISCUSSION

PBPK modeling is increasingly applied during preclinical and clinical development allowing prospective prediction of drug exposure for various scenarios. Investigation of DDIs for regulatory labeling recommendations and problems regarding organ impairment, drug absorption, and pediatric starting dose selection demonstrated the usefulness of this class of mechanistical models in the past.<sup>26</sup> Because DDIs and DGIs can be considered as major drivers of ADRs<sup>2,4,5</sup> the application of PBPK-based MIPD to reduce the incidence of ADRs seems sensible. However, efforts toward the application of physiologically based models for MIPD are still scarce.<sup>12</sup> In this work, we investigated the adaption of PBPK modeling approaches for precision dosing regarding DDGI-sensible compounds in

complex interaction networks and the integration of finding into a decision support system.

Current techniques to address MIPD typically include Bayesian adaptive control methods.<sup>8,27</sup> However, these approaches are limited to interactions, which are already studied and implemented in the model.<sup>8</sup> An extension with other, clinically untested perpetrator or victim drugs or further genetic polymorphisms, is challenging or even impossible. In contrast, PBPK models are well-suited to tackle this limitation and are emphasized by regulatory agencies to investigate new, untested scenarios.<sup>4,8,10–12</sup> At the moment, most whole-body PBPK models purely account for interindividual variability by adapting the physiology of the underlying virtual patient. Hence, the estimation of individual parameters, as it is accomplished in Bayesian methods, is hardly feasible. Consequently, future developments should focus on connecting approaches like maximum *a posteriori* estimation to the realm of PBPK modeling in order to allow PBPK Bayesian techniques to come within reach, combining the best from both worlds. As an application example, such models could use the interindividual variability of a metabolic enzyme

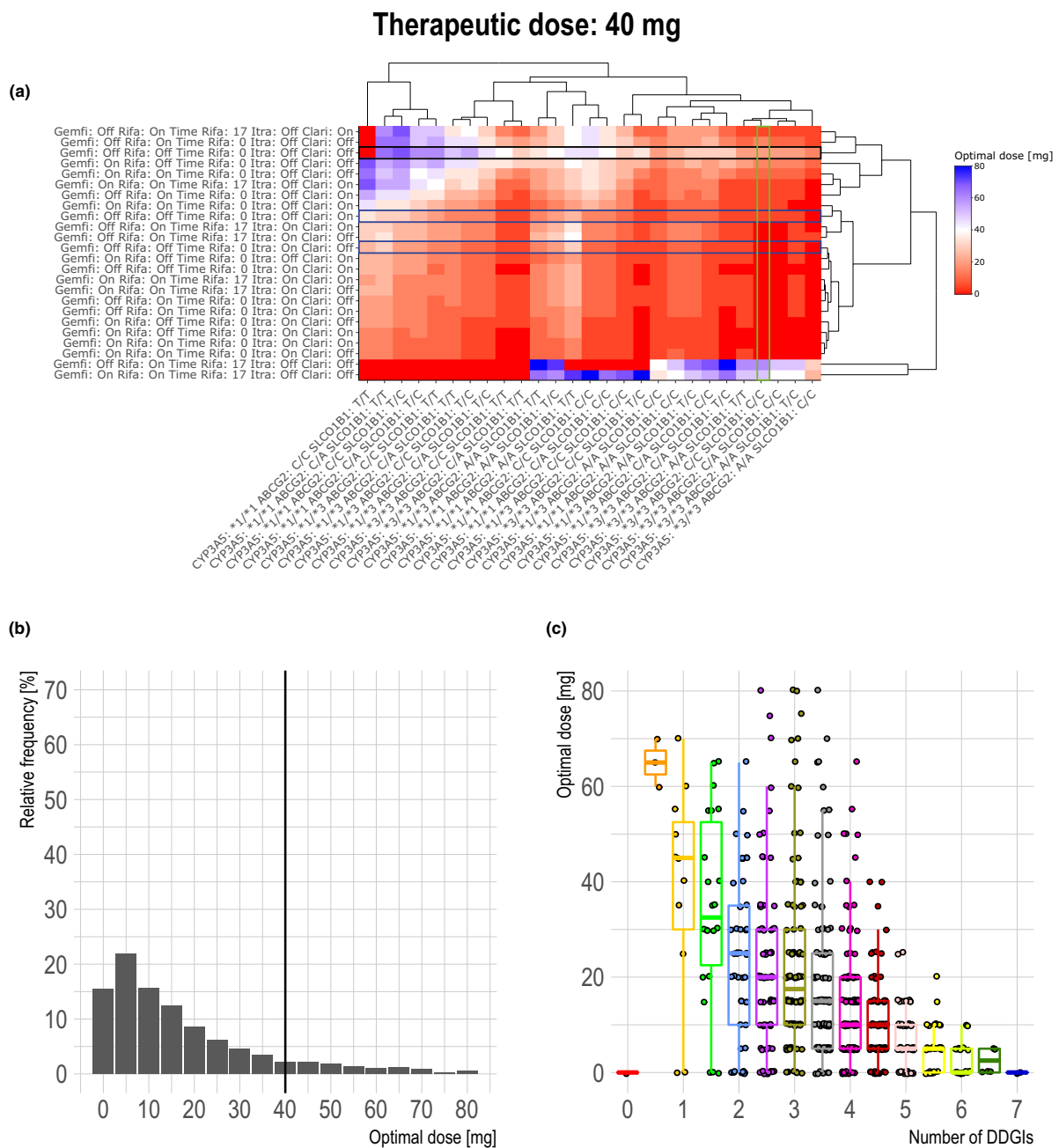
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**Figure 3** Relationships between the PK pathways of the compounds included in the presented DDGI network. For the six different drugs simvastatin, itraconazole, rifampicin, gemfibrozil, clarithromycin, and midazolam metabolic, inhibitory and inductive effects are shown as lines. DDGI, drug-drug-gene interaction; PK, pharmacokinetic.

and, based on the measured individual plasma concentration of a harmless reference substance, predict the optimal treatment regime for another compound that is metabolized by this very enzyme. This will require further technical development, the availability of

sufficient individual data, and additional physiological knowledge, but could consequently improve the precision of the PBPK-based MIPD approach. Fortunately, the continuous research efforts, as for example, shown by the open systems pharmacology community,

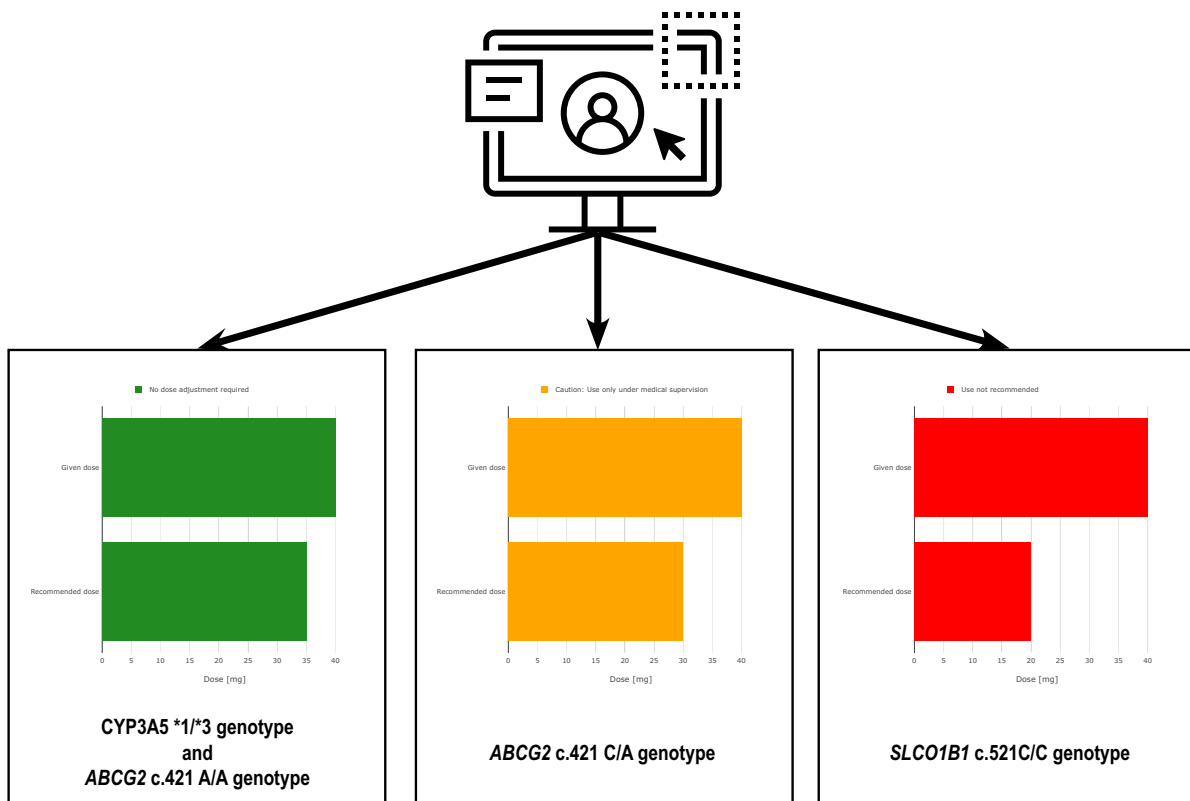


**Figure 4** Results from the dose optimization analysis. **(a)** Heatmap of the performed cluster analysis. Investigated DGIs are shown on the x-axis, whereas DDIs are listed on the y-axis. A color-coding as described on the right side of the plot depicts the optimal doses for each DDGI combination. Cluster analysis results are shown as dendrograms on the right and top site of the heatmap. Furthermore, simvastatin treatment without additional DDI-partner, single cotreatment with itraconazole or clarithromycin and the DGI situation for SLC01B1 (rs4149056) c.521C/C are highlighted with rectangles. **(b)** Shows the relative distribution of optimal doses for simvastatin. Solid lines depict the therapeutic dose. **(c)** Boxplots visualizing the number of DDGIs against the optimal simvastatin doses. All boxplots show the following descriptive statistics: The median value, the interquartile range, and the 1.5-fold interquartile range. All analyzes are shown for a therapeutic dose level of 40 mg simvastatin. DDGI, drug-drug-gene interaction; DDI, drug-drug interaction; DGI, drug-gene interaction.

constantly extend the library of available PBPK models and model features.<sup>28</sup> This progressive trend is encouraging that current knowledge and technical gaps can steadily be narrowed.

We demonstrated the applicability of physiologically based precision dosing using the example of simvastatin. Because it is among the drugs most frequently involved in major interactions,

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**Figure 5** Case examples analyzed with the developed decision support system. Therapeutic simvastatin dose was 40 mg and SL and SA exposure deviation were equally weighted. From left to right, different recommendations for action are given depending on the deviation between the optimal dose under DDGI condition compared with the therapeutic dose. DDGI, drug-drug-gene interaction; SA, simvastatin acid; SL, simvastatin lactone.

simvastatin is a perfect candidate to showcase the feasibility of physiologically based dose recommendations for known DGIs and comedication with frequently used DDI partners.<sup>5</sup> For simvastatin, only two whole-body or semimechanistic PBPK models are described in the literature yet.<sup>29,30</sup> Despite good predictive performance for the area of application, they typically focused on a single polymorphism (e.g., *SLCO1B1* (rs4149056)) and one DDI CYP3A4 inhibition effect. In contrast, our work vastly broadens the area of application by the successful development of a newly built whole-body simvastatin PBPK model covering multiple crucial PK processes. Subsequently, the model was connected to a comprehensive DDGI network to extensively study and simulate complex DDGI scenarios. The final model covered four important polymorphisms in the *ABCB1*, *SLCO1B1*, *ABCG2*, and *CYP3A5* genes<sup>20,31–35</sup> relevant for simvastatin's PK and was tested using previously developed and evaluated models for the perpetrators itraconazole, rifampicin, clarithromycin, gemfibrozil, and the victim midazolam.<sup>10–12,23</sup> The simvastatin network showed overall good descriptive and predictive performance and was hence used for further dose optimization analysis. Despite good performance, the model has some limitations, which are primarily caused by insufficient or lacking model input data. For example, for all studies where no information about the genotype was provided,

homozygous wild type genotypes were assumed. Fortunately, the prediction of included placebo profiles with unknown genotype showed that this assumption is sufficient to achieve good model accuracy. Information about the known polymorphism in *ABCB1* was rare and could only be included in the model training dataset and not for testing. Moreover, for some simvastatin PK pathways no data regarding their significance or activity could be gathered (see **Supplementary Material, chapter 2**). Those pathways and associated processes could either not be included in the model or their affinity ( $K_m$ ) or activity ( $k_{cat}$ ) values had to be estimated. Here, additional *in vitro* studies could help to fill this knowledge gaps in the future and, subsequently, further improve the model quality.<sup>36</sup>

Although precision dosing is considered a public health need, the amount and availability of recommendations for adjustments in case of DDGIs, including DDIs and DGIs, are lagging behind. For simvastatin, 5 pharmacogenes are listed on pharngkb.org as level 2 variants, which equals at least moderate evidence for a significant influence on the pharmacotherapy.<sup>15</sup> Yet, only for one polymorphism in *SLCO1B1* (rs4149056) recommendations on how to adapt the dose are on hand.<sup>15,37,38</sup> For the poor function *SLCO1B1* genotype (c.521C/C) low dosing, prescription of an alternative statin or routine creatine kinase surveillance is typically

recommended.<sup>37,38</sup> Our developed model-based dose recommendations agree on the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline by also recommending an alternative drug for the *SLCO1B1* c.521C/C polymorphism (see **Figure 5**).<sup>37,38</sup> The FDA drug label of simvastatin (Zocor) contraindicates the concomitant use of strong CYP3A4 inhibitors like itraconazole or clarithromycin.<sup>39</sup> This is also reflected by the presented PBPK DDGI network as shown in **Figure 4a** (red highlighting rectangles) for single clarithromycin or itraconazole cotreatment. Except for DDGIs with some CYP3A5 activity (\*1/\*1 or \*1/\*3), the model always predicts that no optimal simvastatin dose could be found (optimal dose = 0 mg). This is not surprising, because the originally published clarithromycin and itraconazole models did not include CYP3A5 inhibition (see **Supplementary Material, chapter 3**).<sup>10,23</sup> Although there are hints of CYP3A5 inhibition by itraconazole or clarithromycin in past studies, information available were too sparse to include this process in the models.<sup>40,41</sup> This lack of information is most likely due to the fact that the *CYP3A5*\*3/\*3 nonexpressor genotype is the major genotype in many populations without recent African ancestry. Although only 10–25% of Europeans have detectable levels of hepatic CYP3A5, this rate increases to 55–95% in African Americans.<sup>42–44</sup> For scenarios where CYP3A5 shows activity, it partly replaces the metabolic clearance of CYP3A4 in the network. Whether this holds true and a DDGI with clarithromycin or itraconazole and CYP3A5 only leads to a slightly increased SL exposure should be further investigated.

Apart from individual DDIs or DGIs, there is currently no recommendation for simvastatin DDGIs available.<sup>39</sup> Unfortunately, this is not only the case for simvastatin but reflects the situation for the majority of available drugs.<sup>4</sup> The standard to overcome this deficiency are clinical trials. However, due to the combinatorial explosion of possibilities for complex DDGIs exhaustive investigation via clinical studies is not feasible.<sup>4,9</sup> As shown in the performed cluster analysis (**Figure 4a**) for complicated DDGIs, no generally valid rule or therapy recommendation can be given, making it indeed necessary to investigate DDGIs on an individual level. With the rapid increase in efficiency and availability of computational resources (e.g., via cloud computing) the application of rich PBPK DDGI networks for MIPD, as shown in the presented study, seems feasible. Yet, clinical studies evaluating more complex situations like DDGIs are urgently needed to challenge, refine, and validate MIPD predictions.<sup>12</sup>

Even though the presented work exceeds the number of currently available dose recommendations by far, it still only applies to a small fraction of possible simvastatin DDGIs. Furthermore, it should be noted that dose optimization was only performed for matching exposure and not linked with a pharmacodynamic (PD) model connecting SL and SA exposure with drug efficacy like change in LDL levels or drug toxicity.<sup>43,45,46</sup> Such a PBPK/PD MIPD decision support system could enable clinicians to individually balance therapy risks and chances.<sup>47,48</sup> However, as recent investigations have shown, those models should also regard the exposure of SL, which had not been recognized for a long time.<sup>49</sup> Results from Tahaa *et al.* indicate that SL could be more relevant for drug's toxicity, whereas SA could be more important

for efficacy.<sup>49</sup> For this reason, the exposure marker cost function used for dose optimization was derived from both exposure deviations in order to account for SL exposure deviations as well. By further implementing a weighting factor, the clinician is still free to set the influence for both species individually. Nevertheless, this highlights that further models and model extensions are required to enlarge the current network. Fortunately, the established PBPK network shows enough flexibility to be extended as soon as more models for PD effects, perpetrator, or victim compounds are available.<sup>10–12,23</sup> Such models can then easily be linked with the current network and subsequently be used for further optimizations.<sup>10–12,23</sup>

The simulation analyses for DDGI scenarios were simulated for 7 days + 24 hours postdose. Although, for single drug treatment, this simulation time should be sufficient to reach PK steady-state conditions for all compounds investigated,<sup>50–53</sup> this assumption might not hold true for complex DDGI scenarios. However, as *a priori* effect estimations of complex DDGI scenarios on drug half-lives is not feasible, this should be considered for any follow-up simulation analysis.

As stated by Gonzalez and coworkers, a precision dosing strategy for clinical practice does not only rely on the development of predictive dosing models, but also on the integration into a decision support system accessible by the physician.<sup>8</sup> Thus, we provide an exemplary implementation of such a system for simvastatin to demonstrate ease of use for modeling nonexperts via a web-based solution.

In conclusion, a novel physiologically based precision dosing approach was successfully developed to study complex DDGI network scenarios for the model drug simvastatin. Findings from extensive cluster analysis of various DDGIs showed no generalized pattern for dose adjustments suggesting the need for individualized MIPD approaches to ensure effectiveness of therapy and prevention of severe ADRs. It could be demonstrated that adaption of whole-body PBPK modeling for MIPD allows the flexible extension and requalification of already established interaction networks more easily and with greater confidence for unknown scenarios than already established tooling for MIPD. Future developments should focus on enhancing the capabilities of PBPK modeling by integration of Bayesian adaptive control mechanisms like maximum *a posteriori* estimation allowing more fine-grained personalized readjustment for DDI-sensible and DGI-sensible drugs. Efforts for open access model deployment should be promoted for more widespread utilization. Besides open access to models, the integration with easy to use decision support systems is crucial to allow the adaption into clinical practice. Thus, for further use, all simvastatin DDGI network model files are publicly available (<https://github.com/Clinical-Pharmacy-Saarland-University>) and the physiologically based precision dosing decision support system is deployed for open access at [simvastatin.precisiondosing.de](http://simvastatin.precisiondosing.de).

#### SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website ([www.cpt-journal.com](http://www.cpt-journal.com)).

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

## AUTHOR CONTRIBUTIONS

J.-G.W., D.S., M.S., and T.L. wrote the manuscript. J.-G.W., M.S., and T.L. designed the research. J.-G.W. performed the research. J.-G.W., D.S., and T.L. analyzed the data.

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## Part III

### DISCUSSION AND CONCLUSIONS

The chapter provides a comprehensive and concluding discussion of the results presented in this thesis.



## DISCUSSION

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Our modern healthcare and economic system is heavily burdened by ADRs and ADEs [7, 8]. Significant drivers of ADRs and ADEs are DDIs, DGIs, and DDGIs [17, 19, 82]. A promising solution to overcome them, is to improve the drug development process by MID3 as well as to optimize post-marketing drug therapy regimen by MIPD techniques [3, 50, 51]. PBPK modeling, which belongs to the MID3 and MIPD portfolio, is particularly useful for this purpose as it can also reduce the number of expensive and complex clinical trials that would otherwise have had to be carried out to investigate the DDGIs [52, 83–85].

Thus, the presented work had three objectives. Namely, to improve the PBPK modeling workflow, to support the drug development process of the NTE zoptarelin doxorubicin and to propose therapy recommendations for simvastatin DDGIs including the development of a DSS.

### 4.1 DATA DIGITIZING FOR MODEL DEVELOPMENT

The first publication presented in this thesis focused on data digitizing as an important cornerstone of PBPK and QSP modeling (see Section 3.1). Commonly, PBPK models rely on *a priori* information for estimating unknown system and drug dependent parameters [1, 74–79]. Hence, the quality and accuracy of a PBPK model is correlated with the amount of data and information available for parameter estimation [86]. Moreover, like for other predictive models, training and test data are necessary to evaluate the predictive performance of PBPK models [1, 78, 87]. Unfortunately, if published, the necessary information and in particular, clinical time-dependent data like plasma-concentration time profiles are rarely available as tabulated numerical values as described in Section 3.1. They are often presented in a condensed way as mean profiles and in the form of graphs (see Section 3.1). This is where data digitizing is a useful and increasingly used technique to translate the graphical presentation into numerical values to overcome potential knowledge gaps [1]. As shown in Section 3.1, a 16 % increase per year in publications citing digitizing software together with PBPK or QSP was found. It was also observed that the investigated digitizing software showed an overwhelming precision with a mean median symmetric accuracy (MSA) of 0.99 % [1]. In contrast, it was noticed that digitized data errors are rather related to preexisting inconsistencies in the published manuscripts.

These findings are of high importance also for the established simvastatin PBPK DDGI network, as presented in Section 3.3 and Section A.3, because a PBPK model developed with biased data, could lead to false predictions [1, 52, 88]. Following, this can result in unsafe dose recommendations which pose potentially harm for patients [1, 52, 88]. For this reason, it is important to follow the workflow presented in Section 3.1, which includes some quality control (QC) approaches to verify data quality and integrity. If identified inconsistencies cannot be resolved, the digitized data should be used very carefully and only if they are critical for model development. To mitigate the risk of "outlier data" that subsequently bias the final PBPK model, one could follow the law of large numbers and increase the number of digitized studies [89]. For example, in the model presented in Section 3.3 and Section A.3, in total 132 simvastatin lactone (SL) and SA profiles were digitized to reduce the impact of single, potentially biased profiles.

Another approach for identifying model misspecifications is the aforementioned use of training and test datasets as shown in Figure 1.5. However, for PBPK modeling, currently, no best practices how to separate the available data in test and training datasets are established. In contrast, for other predictive modeling strategies, recommendations are on hand [87, 90]. For example, one regular approach is to follow the Pareto principle, which would mean a random distribution of all data available at a ratio of 80 to 20 in training and test dataset [87, 90–92]. Nevertheless, this would only make limited sense for developing a PBPK model, since, as shown in Section 3.3 and Section A.3, literature data are only sparsely available for many investigated effects. For example, for most of the investigated DDIs only one profile and sometimes only a single peak plasma concentration ( $C_{\max}$ ) or AUC value were on hand (see Section 3.3 and Section A.3).

Another problem is that many sampling points have a dependence on the study in which they were generated. However, especially time-dependent measurements differ in the number and distribution of sampling points per study (see Section A.3). Thus, not every profile is suitable for informing a model regarding, for instance, terminal elimination or  $C_{\max}$ . For the two reasons mentioned above, a purely random split could easily lead to uneven distribution, making either the implementation of an effect or its evaluation impossible [92]. Therefore, for most published PBPK models, a subjective, manual division of the data into test and training was performed [74–79]. The influence of this procedure on model quality, as well as rational, alternative best practices should be part of further investigations.

#### 4.2 ZOPTARELIN DOXORUBICIN DRUG-DRUG INTERACTION POTENTIAL

The second publication aimed to assess the DDI potential of zoptarelin doxorubicin concerning OATP1B3 and OCT2 (see Section 3.2 and

Section A.2). The detection and characterization of DDIs is an integral part of drug development, and detailed regulatory guidance on this topic is available [27, 93, 94]. Experiments for DDI characterization are commonly carried out in the preclinical development phase to identify drug interaction potentials [94]. Unfortunately, the translation of these preclinical results into clinical effects proves to be difficult [3, 94]. For this reason, extensive clinical studies have to be carried out regularly [27]. However, this is problematic in particular for oncological substances [24, 25]. As cancer patients receive many drugs at once that cannot be discontinued during a clinical study, they are very susceptible to DDIs [24, 25]. This circumstance also makes the interpretation of clinical study results difficult [24, 25]. In addition, the performance of such studies with NTEs whose efficacy and toxicity have not yet been conclusively investigated can be ethically challenging [2]. All in all, new techniques are needed to better translate the preclinical data and possibly avoid clinical studies altogether [2].

Two such approaches were presented in detail in Section 3.2. The first promising technique utilized PBPK modeling to predict unbound tissue concentration of zoletarelin doxorubicin in organs where OATP1B3 and OCT2 are expressed. Subsequently, a relative change of apparent Michaelis-Menten constant ( $K_M$ ) and transportation velocity of OATP1B3 and OCT2 could be calculated, indicating a limited risk for a clinical relevant DDI. Going even further; by developing additional PBPK models for the OATP1B3 and OCT2 substrates simvastatin and metformin, clinical worst-case scenarios were predicted further confirming this assumption (see Section 3.2). Besides, these precursor models of simvastatin and metformin laid the foundation for further DDGI networks as shown in Section 3.3 and by Hanke et al. [80].

#### 4.3 SIMVASTATIN DRUG-DRUG-GENE INTERACTION NETWORK

The third publication dealt with the development of a simvastatin PBPK DDGI network, the derivation of dose recommendations, and the establishment of a DSS (see Section 3.3).

Simvastatin shows a complex PK, which is influenced by several DDIs and DGIs (see Figure 1.3). Despite several available PK models for simvastatin, no recommendations for potential DDGIs are on hand so far [3, 39, 95–98]. This could be because previously developed models were only empirical or semi-mechanistical simvastatin PK models [3, 39, 95–98]. Although they are well suited to study single influencing factors, they are limited in their extensibility [59]. For instance, for each feature added to such a model, additional study data are required to quantify the observed effects [59]. In contrast, whole-body PBPK models are capable of also predicting unobserved DDIs by coupling individually developed models together as depicted in Figure 1.7 [74–79]. Thus, even if the presented simvastatin model (see Section 3.3)

does currently not cover every conceivable simvastatin DDGIs, it can be easily extended to do so. Fortunately, due to the active scientific community, likely, further perpetrator and victim models will soon be available, which then can be coupled with the established simvastatin model to derive further recommendations for DDGIs [99].

Besides, a particular focus was placed on the correct implementation of cytochrome P450 3A4 (CYP3A4). Cytochrome P450 (CYP) enzymes account for roundabout 75 % of the total drug metabolism [100] and CYP3A4 in particular, has a leading role within the CYP family [101], as shown in Figure 4.1. This also applies to simvastatin whereas CYP3A4 is the most influencing metabolic enzyme of simvastatin's PK and also responsible for many of simvastatin related DDIs (see Section 3.3 and Section A.3).

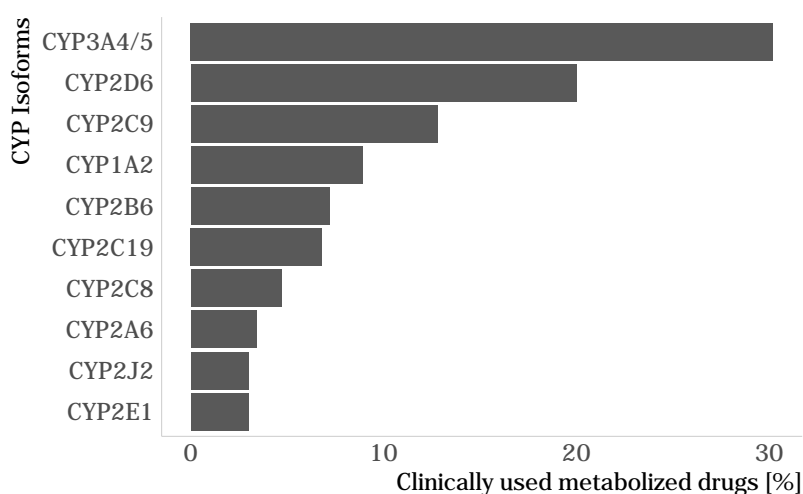


Figure 4.1: Fraction of clinically used drugs metabolized by various CYP isoforms [101].

To validate the correct implementation of CYP3A4 into the model, it was extensively evaluated as shown in Section A.3. This includes, as recommended by the recently released EMA guideline on how to report a PBPK model, sensitivity analyses [102]. Thereby, the final sensitivity analysis, as presented in Section A.3 nicely showed the importance of CYP3A4 on SL and SA exposure in the model.

Although several different DDIs and DGIs could be predicted accurately by the presented work, it has to be noted that some of the data available may only be of limited credibility for model evaluation. By performing QCs measures as described in Section 3.1, several studies revealed unexpected heterogeneity regarding their presented data. For example, Choi et al. [103] listed AUC and  $C_{max}$  values for many different DGIs. However, QCs revealed that presented dose-normalized values shown by Choi et al. [103] for SA were significant and consis-

tently larger than to most of the studies available (see Section A.3). A possible explanation could have been the ethnicity of the study populations. While most of the available studies recruited predominantly Caucasians, it can be assumed that Choi et al. [103], because their study was conducted in Korea, had mainly involved Koreans. The assumption of PK differences for simvastatin among various ethnic groups was partly confirmed by another recent study focusing on simvastatin PK changes between the three East Asian populations and Caucasian's [104].

Another problem concerning the NCA values' quality was encountered in the DDI study with itraconazole [105]. In this study for quantification of SL and SA exposure, different analysis techniques (high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS)) were used. As a result the lower limit of detection (LLOD) for SL was high ( $10 \text{ ng ml}^{-1}$ ), which led to many SL samples below the LLOD in the placebo phase. Moreover, in the same study, SA was quantified as total SA after hydrolysis of SL and other SL metabolites in the probes. All in all, this meant that the plasma concentration-time profiles given by Neuvonen, Kantola, and Kivistö [105] could not directly be used for model evaluation. Similar conclusions were drawn by Tsamandouras et al. [95] in his attempt of reusing the data for model evaluation. Luckily, Neuvonen, Kantola, and Kivistö [105] stated that for SL an at least 10-fold increase in exposure under itraconazole co-treatment was observed compared to placebo. This value was subsequently used for model evaluation. In contrast, a published increase of SA exposure was not be used for evaluation since, due to assay limitations mentioned earlier, it could not be compared to any model output.

Both examples, the data from Choi et al. [103] and Neuvonen, Kantola, and Kivistö [105], highlight the necessity of additional DDI and DGI or even DDGI intervention studies to support further PBPK model development and evaluation. Also, the same conclusion as drawn in Section 3.1 applies. Instead of condensed information, the complete study protocol and observed raw values for such interaction studies should be available. This way, for example, the question of which ethnicity the participants in the study performed by Choi et al. [103] belong to could have been answered.

After model development and evaluation, the final model was used to optimize several DDGI scenarios using matching exposure. For this purpose, a combined exposure marker for SL and SA was derived as described in Section 3.3. An alternative approach would have been to couple the model with a suitable PD model extension. However, this was waived since neither an appropriate extension was available nor the necessary data were on hand. For example, in a study by Lippert et al. [96] a toxicodynamic marker for simvastatin acid was derived as a

function of solute carrier organic anion transporter family member 1B1 (OATP1B1) transporter activity. However, this toxicodynamic marker would not have been suitable for the presented work since the aim was to cover several DDGIs, some of them not focusing on OATP1B1. Moreover, as described by Taha et al. [106] there is evidence that the toxicokinetic of simvastatin is more dependent on exposure of SL compared to SA. Thus, a suitable PD model extension should differentiate between the effects of SL and SA exposure. Moreover, it should not only focus on simvastatin toxicokinetics but also cover therapy relevant efficacy biomarkers like low-density lipoprotein cholesterol (LDL-C) [107]. If both components, efficacy and toxicity of simvastatin, would be covered by a PD model extension, it could be coupled with the presented simvastatin PK model to calculate a therapy related net clinical benefit (NCB) [108]. The NCB could subsequently be used to compare different therapy regimens and optimize them under DDGIs conditions based on hard endpoints like probabilities for ADEs or therapy success rates. Similar approaches can already be found in the literature [109]. However, until then, matching exposure is a suitable alternative as this approach is, for instance, recommended for other applications like pediatric extrapolation [110].

The results from the dose optimization process were afterward transferred into an interactive DSS that allows deriving MIPD recommendations tailored to each DDGI of interest (see Section 3.3). However, it has to be noted that although PBPK modeling is emphasized for the prediction of DDIs and DGIs [51, 66, 70–72, 76] further evaluation whether also complicated DDGIs can be predicted using PBPK modeling, is still pending.

Apart from that, the developed DSS has yet to prove its acceptance and usefulness in clinical practice. For this purpose, further studies and user surveys should be conducted. Those could focus on the perceived usefulness and the perceived ease-of-use of the tool as parameters related to the probability that a technology will be accepted by potential users [111, 112]. Nevertheless, even if further evaluations of the presented DSS are needed, it shows exemplarily how MIPD can be truly brought from pure theory to the patient's bedside [48].



## CONCLUSIONS

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The presented work added valuable knowledge on improving digitized data quality, necessary for PBPK model development and evaluation. This will most likely also affect the accuracy of the developed PBPK models and thus their quality for use in MID3 and MIPD. Further, it was shown how to assess the DDI potential of NTEs based on the example of zoptarelin doxorubicin using PBPK MID3 techniques instead of ethically challenging clinical DDI studies. Moreover, complex DDGIs scenarios for simvastatin were addressed with a PBPK MIPD approach. Finally, the derived simvastatin therapy recommendations were made available online in the form of a newly developed DSS. Since PBPK MIPD is a novel approach in the management of DDGIs, additional studies will be necessary to conclusively evaluate this technique, especially in the prevention of ADEs.



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Part IV

APPENDIX







## SUPPORTING INFORMATION

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A.1 SUPPORTING INFORMATION PUBLICATION I - DATA DIGITIZING:  
ACCURATE AND PRECISE DATA EXTRACTION FOR QUANTITATIVE  
SYSTEMS PHARMACOLOGY AND PHYSIOLOGICALLY-BASED PHAR-  
MACOKINETIC MODELING

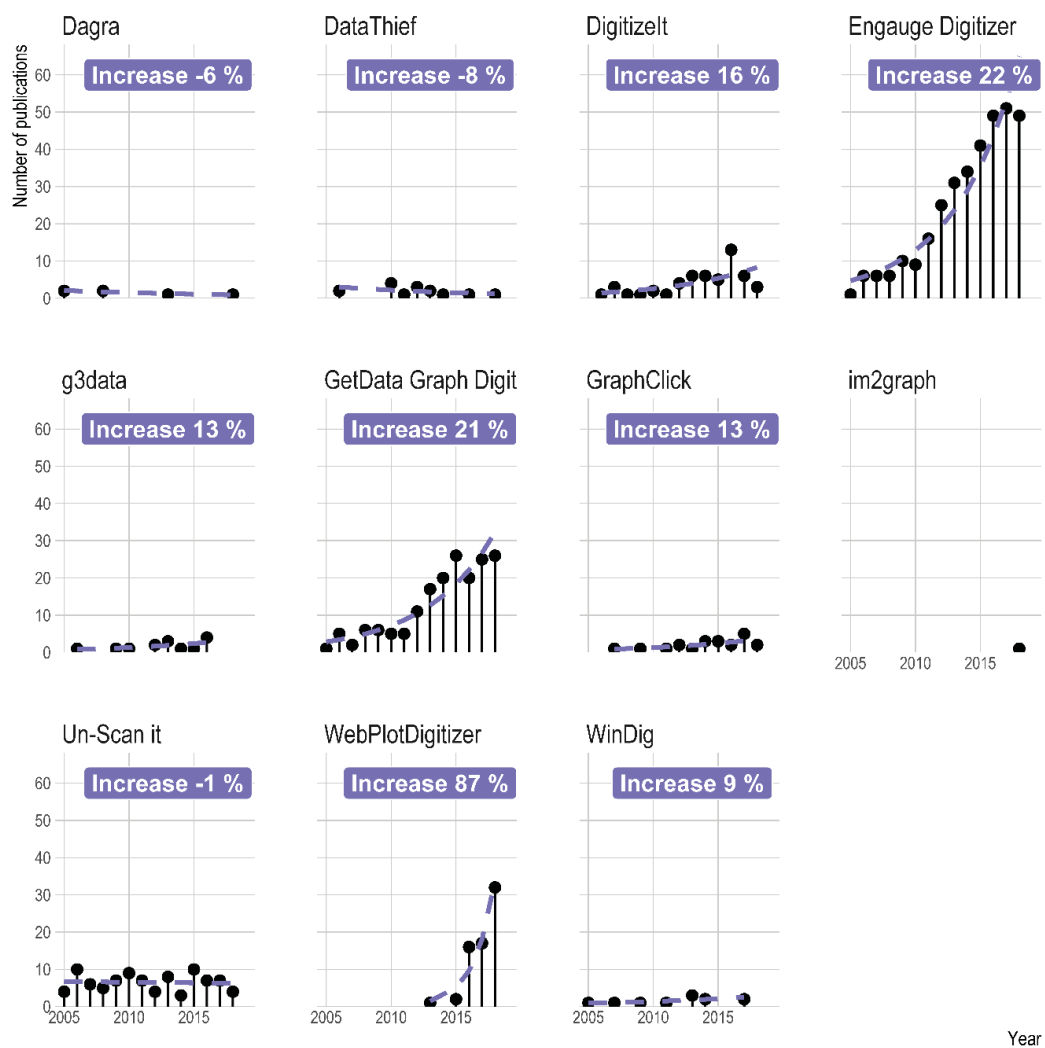


Figure S1 Number of publications containing the terms “systems pharmacology” or “physiologically based pharmacokinetic” and the names of the digitization software packages investigated over the last few years. Labels and dashed purple line show model estimated values and increase per year using Poisson regression. For all subplots, solid lollipops represent the observed values.

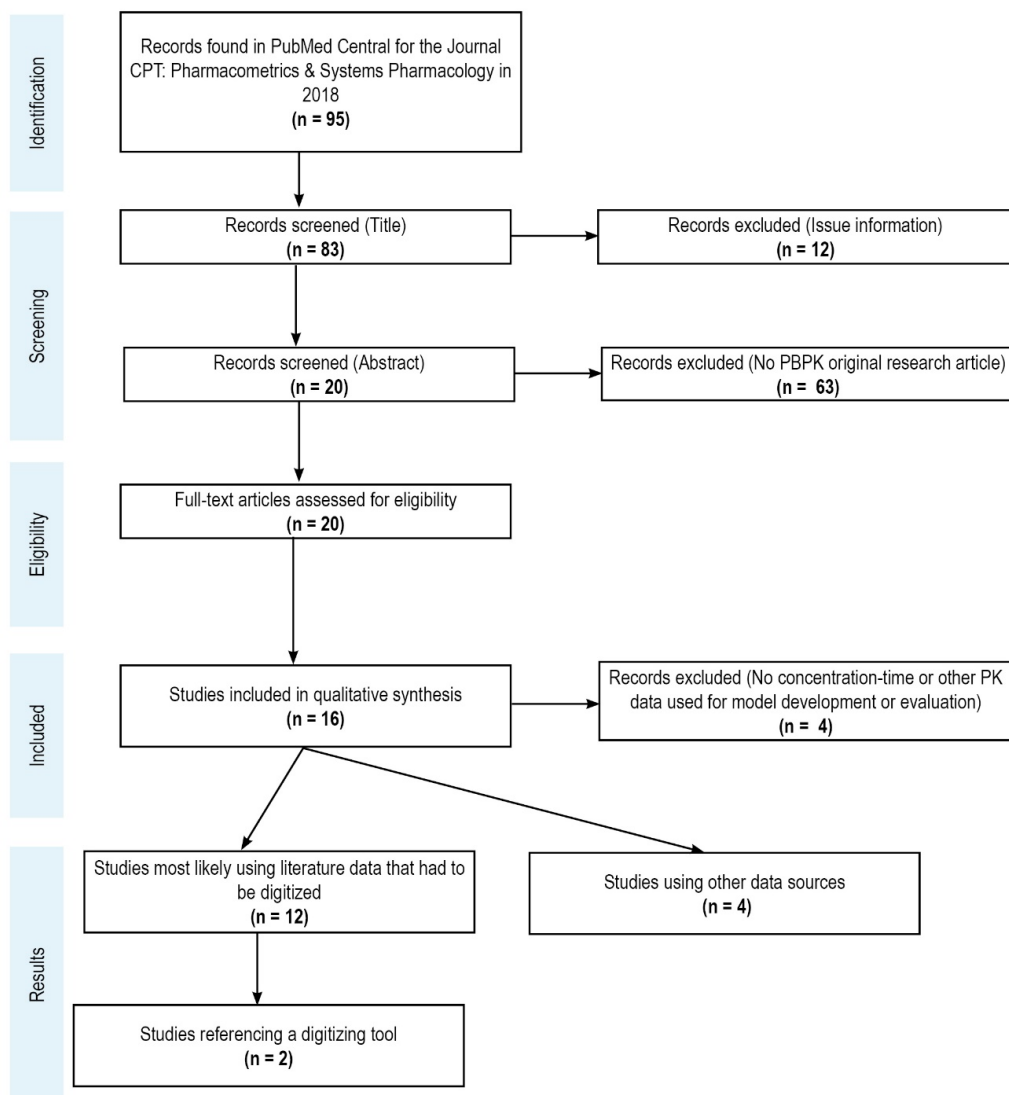


Figure S2 Flow-chart of the performed literature search in CPT CPT: PSP (Online ISSN: 2163-8306) from 2018. Records were reviewed manually in order to identify articles related to PBPK that referenced a digitizing software and most likely had used literature data.

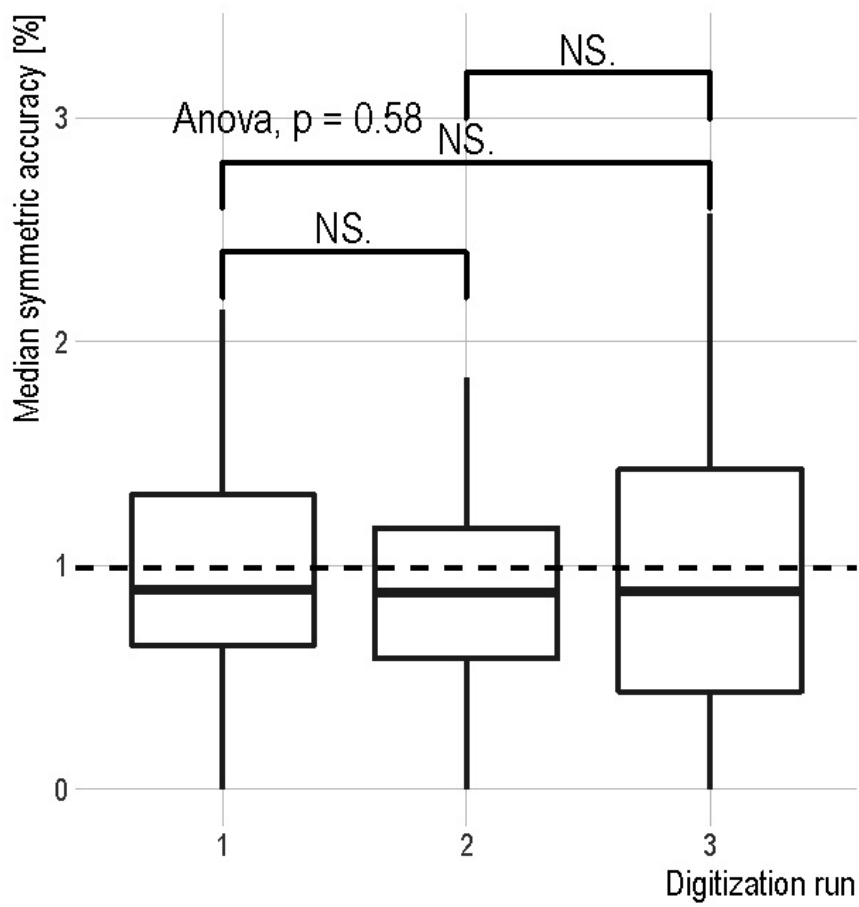


Figure S3 Boxplot of the median symmetric accuracy for each digitization run performed in the study. In addition, arithmetic mean of all groups is shown as dashed line.

Software Name	Platform	Cost [\$]	Total number of publication frequencies	Features
Dagra	Windows	49.95	18	<b>Import:</b> From clipboard, via built-in screen-shot tool. <b>Supported formats:</b> .bmp, .emf, .gif, .jpg, .jpeg, .png, .tif, .tiff, .wmf. <b>Digitizing features:</b> One graph at once, point digitization (1 point per click), curve digitization (curve tracing using Bezier curves), zoom panel, axis scaling: linear, log <sub>10</sub> or log <sub>e</sub> . <b>Export:</b> To clipboard, to text file, Dagra files can be opened in Matlab, Python or Excel. <b>Other:</b> Appearance rating from 0 (not appealing) to 10 (appealing): 3
DataThief	Windows, MacOS, Unix	25	20	<b>Supported formats:</b> .gif, .jpg, .jpeg, .png <b>Digitizing features:</b> One graph at once, point digitization (1 point per click), curve digitization (line tracing), can digitize polar coordinates, axis scaling: user defined <b>Export:</b> To text file <b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 6, written in Java (no installation required)
dcsDigitiser	Windows	423	0	Trial version could not be tested since the software interfere with windows defender.
DigitizeIt	Windows, MacOS, Unix	49	58	<b>Import:</b> From clipboard. <b>Supported formats:</b> .bmp, .jfif, .gif, .jpg, .jpeg, .png, .tif, .tiff, .ico <b>Digitizing features:</b> One graph at once, point digitization (1 point per click or automatic), curve digitization (line tracing), axis scaling: linear, logarithmic, 1/x. <b>Export:</b> To csv, to clipboard <b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 7, graphs can be zoomed, mirrored and rotated, can handle tilted or distorted graphs, axes do not need to be orthogonal
Engauge	Windows, MacOS, Unix	Free	418	<b>Supported formats:</b> .bmp, .cur, .gif, .icns, .ico, .jpeg, .jpg, .pbm, .pdf, .pgm, .ppm, .png, .pgm, .tga, .tif, .tiff, .wbmp, .webp, .xbm, .xpm <b>Digitizing features:</b> Multiple graphs at once, point digitization (1 point per click or automatic), curve digitization (line tracing cubic spline interpolation), axis scaling: linear, logarithmic, date and

				time values, or as degrees, minutes and seconds, can digitize polar coordinates, image processing, grid lines <b>Export:</b> To csv/tsv, to clipboard <b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 8, various wizards (interactive tutorials), axes checker, axes with only one known coordinate (floating axes) can be digitized, geometry window displays geometric information about the selected curve, curve Fitting Window fits a polynomial function to the selected curve, various customization options.
g3data	Windows	Free	15	Could not be tested because the software needs separate compilation.
GetData	Windows	30	225	<b>Import:</b> From clipboard. <b>Supported formats:</b> .bmp, .jpg, .tif, .pcx <b>Digitizing features:</b> One graph at once, point digitization (1 point per click), curve digitization (curve tracing), zoom panel, axis scaling: linear, log <sub>10</sub> <b>Export:</b> To clipboard, to text file, to csv <b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 8
GraphClick	MacOS	Free	22	No longer under development.
im2graph	Windows, Linux	Free	1	For installation of the freeware version a download-link is required. However, we did not receive a link and thus, could not test the software.
Un-Scan it	Windows, MacOS	345	120	<b>Import:</b> From clipboard <b>Supported formats:</b> .bmp, .gif, .jpeg, .jpg, .tiff, .png, .tga, .pcx <b>Digitizing features:</b> One graph at once, point digitization (1 point per click), curve digitization (curve tracing – also for intersecting lines and dashed/dotted lines), bar-chart digitization, contour plot digitization, shape/Drawing digitization, polar coordinate digitization, zoom panel, separate “graph-screen-mode”, axis scaling: linear, log <sub>10</sub> <b>Export:</b> To clipboard, to text file, to csv file <b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 7, grid line filters, automatic line follow mode, raster scan mode, corrects for tilted graphs and variable line thickness, various post-processing features (area integration between cursors / data smoothing etc.).

WebPlotDigitizer	Web based	Free	103	<p><b>Supported formats:</b> Not directly stated. Probably the most important formats. <b>Digitizing features:</b> One graph at once, point digitization (1 point per click or automatic), curve digitization (curve tracing), bar-chart digitization, ternary diagram digitization, map with scale bar digitization, polar coordinate digitization, image digitization, zoom panel, axis scaling: linear, log<sub>10</sub>, measurement calculations (distance, area, angle), dataset cleaning functions (sorting/renaming), can remove gridlines <b>Export:</b> To csv file, save project as .json file, to plotly, to clipboard</p> <p><b>Other</b> Appearance rating from 0 (not appealing) to 10 (appealing): 8, as software or as browser-plugin available, can run javascripts</p>
WinDig	Windows	Free	15	Could not be tested since it is only available as 16bit version
xyExtract	Windows	45	2	Could not be tested since no windows 10 version is available.

**Table S2: Studies used for comparing published numeric and digitized  $C_{\max}$  values**

<b>Author</b>	<b>Mean <math>\zeta</math> [%]</b>	<b>Smallest <math>\zeta</math> [%]</b>	<b>Largest <math>\zeta</math> [%]</b>	<b>N <math>\zeta</math></b>	<b>Reference</b>
Alakhali 2013	1.3	1.3	1.3	1	<sup>1</sup>
Ayalasomayajula 2007	2.8	0.75	4.1	4	<sup>2</sup>
Ayalasomayajula 2016	12	6.8	17	2	<sup>3</sup>
Backman 2000	15	2.5	41	4	<sup>4</sup>
Bergman 2009	13	8.4	20	4	<sup>5</sup>
Boulenc 2016	15	9.4	23	3	<sup>6</sup>
Cermak 2009	19	9	36	4	<sup>7</sup>
Chin 1995	3.4	3.4	3.4	1	<sup>8</sup>
Dai 2013	13	0.92	25	4	<sup>9</sup>
Daneshmend 1984	16	4.6	37	8	<sup>10</sup>
Devineni 2015	16	13	23	4	<sup>11</sup>
Dingemans 2014	15	5.8	35	8	<sup>12</sup>
Eap 2004	17	7.9	27	2	<sup>13</sup>
Huang 1986	19	8.2	29	3	<sup>14</sup>
Jacobson 2004	13	0.35	42	6	<sup>15</sup>
Kang 2004	0.038	0.038	0.038	1	<sup>16</sup>
Kantola 1998	17	6.8	35	6	<sup>17</sup>
Keskitalo 2008	24	11	35	4	<sup>18</sup>
Keskitalo 2009	20	4.9	64	6	<sup>19</sup>
Kim 2007	32	25	40	3	<sup>20</sup>



Knupp 1993	15	15	15	1	21
Kosoglou 2011	22	7.2	33	4	22
Krishna 2012	8.9	1.3	16	8	23
Kyrklund 2000	25	12	35	4	24
Lam 2003	18	14	23	2	25
Lilja 1998	21	11	34	4	26
Lilja 2000	31	31	31	1	27
Lilja 2004	13	3.7	27	4	28
McKenney 2006	21	20	22	2	29
Neuvonen 1998	89	19	160	2	30
Obrien 2003	34	23	49	4	31
Pasanen 2006	18	5	34	6	32
Polk 1999	12	12	12	1	33
Polli 2013	16	2.9	29	2	34
Sekar 2008	4.3	4.3	4.3	1	35
Shanmugam 2011	6.7	0.3	13	2	36
Stephen 1991	9.8	9.8	9.8	1	37
Stoch 2009	7.9	3.9	12	4	38
Sugimoto 2001	9.9	5.7	14	2	39
Teng 2013	16	4.5	31	4	40
Tham 2006	170	0.67	330	2	41
Tubic-Grozdanis 2008	18	7	50	6	42

Tuteja 2014	32	13	54	4	43
Ucar 2004	32	2.2	63	4	44
Winsemius 2014	94	52	170	12	45
Xu 2014	440	0.69	1800	6	46
Zhao 2015	24	3.7	54	10	47

a: Number of values extracted from the study for which  $\zeta$  was calculated

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**Table S3: Studies used for comparing published numeric and digitized sample time point values**

<b>Author</b>	<b>Mean <math>\zeta</math> [%]</b>	<b>Smallest <math>\zeta</math> [%]</b>	<b>Largest <math>\zeta</math> [%]</b>	<b>N <math>\zeta^a</math></b>	<b>Reference</b>
Alakhali 2013	0.64	0	2.2	10	<sup>1</sup>
Ayalasomayajula 2007	0.01	0	0.057	71	<sup>2</sup>
Ayalasomayajula 2016	5.8	0	47	24	<sup>3</sup>
Backman 2000	0.29	3.40E-05	2.3	40	<sup>4</sup>
Bergman 2004	0.0051	0	0.045	76	<sup>5</sup>
Bergman 2009	0.54	5.00E-04	4.2	44	<sup>6</sup>
Cermak 2009	1	0	4.5	56	<sup>7</sup>
Chung 2006	1	6.80E-04	9.5	24	<sup>8</sup>
Dai 2013	0.0027	0	0.028	98	<sup>9</sup>
DeGorter 2012	0.83	0.031	3	11	<sup>10</sup>
Derks 2010	0.0081	0	0.071	44	<sup>11</sup>
Devineni 2015	0.64	0	4.7	45	<sup>12</sup>
Falcao 2013	0.68	0	9.4	43	<sup>13</sup>
Geboers 2016	0.24	0	2.9	99	<sup>14</sup>
Gehin 2015	0.86	3.10E-04	18	49	<sup>15</sup>
Hasunuma 2016	0.68	0.032	4	22	<sup>16</sup>
Hoch 2013	0.1	0	2.5	118	<sup>17</sup>
Hoch 2013	0.017	0	0.07	48	<sup>18</sup>
Hsyu 2001	0.001	0	0.0053	49	<sup>19</sup>
Itkonen 2015	0.28	0	2.6	65	<sup>20</sup>
Jacobson 2004	0	0	0	34	<sup>21</sup>

Kang 2004	1.6	0.0029	13	11	22
Kantola 1998	0.29	0.0042	5.4	46	23
Kasichayanula 2012	1.6	0	13	43	24
Keskitalo 2009	0.42	0	2.7	59	25
Kim 2007	1.7	0.0083	9.3	33	26
Kosoglou 2011	0.014	0	0.056	56	27
Krishna 2007	0	0	0	8	28
Krishna 2009	0	0	0	78	29
Krishna 2012	1.4	0	11	63	30
Kyrklund 2000	0.071	0	1.2	40	31
Lee 2017	1.1	0.0021	9.3	20	32
Lilja 1998	0.23	0	3.3	35	33
Lilja 2000	0.069	0	1.3	80	34
Lilja 2004	0.33	0	4.1	38	35
Marino 2000	32	0.21	140	18	36
Martin 2016	0.62	0	8.3	22	37
McKenney 2006	0.0031	0	0.019	88	38
Mousa 2000	0.82	0.013	4.5	20	39
NDA 206679	4.1	6.20E-04	74	72	40
NDA 22425	0.0053	0	0.024	94	41
Neuvonen 1998	0.14	5.20E-05	0.72	22	42
O'Brien 2003	0.82	0	5.6	38	43

Offman 2017	0.023	0	0.075	60	44
Park 2016	0.046	0	0.18	52	45
Pasanen 2006	0.43	0	1.3	60	46
Patel 2011	0.0064	0	0.026	44	47
Polli 2013	1.5	0.019	17	18	48
Prueksaritanont 2005	1.6	2.90E-04	15	49	49
Schmitt 2011	0.43	0	7.6	46	50
Shanmugam 2011	2.7	0.082	19	23	51
Simard 2001	0.013	0	0.089	89	52
Sugimoto 2001	42	0.16	180	29	53
Sunkara 2007	0.014	0	0.077	66	54
Teng 2013	1.3	0	23	44	55
Teng 2013	0.03	0	0.087	91	56
Tubic-Grozdanis 2008	1.1	0.005	16	84	57
Tuteja 2014	0.2	0	3.2	44	58
Ucar 2004	0.45	0	4.8	40	59
Vree 2001	2.4	0.058	15	34	60
Winsemius 2014	0.21	0	6.4	204	61
Xu 2014	1	0	9.3	92	62
Yu 2009	1.6	0.002	22	46	63
Zhao 2015	1.6	0	19	92	64
Zhi 2003	2.2	0	25	35	65

Ziviani 2001	0.0092	0	0.044	47	66
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a: Number of values extracted from the study for which  $\zeta$  was calculated

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**Table S3: Studies used for comparing published numeric and digitized sample time point values**

<b>Author</b>	<b>Mean <math>\zeta</math> [%]</b>	<b>Smallest <math>\zeta</math> [%]</b>	<b>Largest <math>\zeta</math> [%]</b>	<b>N <math>\zeta^a</math></b>	<b>Reference</b>
Alakhali 2013	0.64	0	2.2	10	<sup>1</sup>
Ayalasomayajula 2007	0.01	0	0.057	71	<sup>2</sup>
Ayalasomayajula 2016	5.8	0	47	24	<sup>3</sup>
Backman 2000	0.29	3.40E-05	2.3	40	<sup>4</sup>
Bergman 2004	0.0051	0	0.045	76	<sup>5</sup>
Bergman 2009	0.54	5.00E-04	4.2	44	<sup>6</sup>
Cermak 2009	1	0	4.5	56	<sup>7</sup>
Chung 2006	1	6.80E-04	9.5	24	<sup>8</sup>
Dai 2013	0.0027	0	0.028	98	<sup>9</sup>
DeGorter 2012	0.83	0.031	3	11	<sup>10</sup>
Derks 2010	0.0081	0	0.071	44	<sup>11</sup>
Devineni 2015	0.64	0	4.7	45	<sup>12</sup>
Falcao 2013	0.68	0	9.4	43	<sup>13</sup>
Geboers 2016	0.24	0	2.9	99	<sup>14</sup>
Gehin 2015	0.86	3.10E-04	18	49	<sup>15</sup>
Hasunuma 2016	0.68	0.032	4	22	<sup>16</sup>
Hoch 2013	0.1	0	2.5	118	<sup>17</sup>
Hoch 2013	0.017	0	0.07	48	<sup>18</sup>
Hsyu 2001	0.001	0	0.0053	49	<sup>19</sup>
Itkonen 2015	0.28	0	2.6	65	<sup>20</sup>
Jacobson 2004	0	0	0	34	<sup>21</sup>

Kang 2004	1.6	0.0029	13	11	22
Kantola 1998	0.29	0.0042	5.4	46	23
Kasichayanula 2012	1.6	0	13	43	24
Keskitalo 2009	0.42	0	2.7	59	25
Kim 2007	1.7	0.0083	9.3	33	26
Kosoglou 2011	0.014	0	0.056	56	27
Krishna 2007	0	0	0	8	28
Krishna 2009	0	0	0	78	29
Krishna 2012	1.4	0	11	63	30
Kyrklund 2000	0.071	0	1.2	40	31
Lee 2017	1.1	0.0021	9.3	20	32
Lilja 1998	0.23	0	3.3	35	33
Lilja 2000	0.069	0	1.3	80	34
Lilja 2004	0.33	0	4.1	38	35
Marino 2000	32	0.21	140	18	36
Martin 2016	0.62	0	8.3	22	37
McKenney 2006	0.0031	0	0.019	88	38
Mousa 2000	0.82	0.013	4.5	20	39
NDA 206679	4.1	6.20E-04	74	72	40
NDA 22425	0.0053	0	0.024	94	41
Neuvonen 1998	0.14	5.20E-05	0.72	22	42
O'Brien 2003	0.82	0	5.6	38	43

Offman 2017	0.023	0	0.075	60	44
Park 2016	0.046	0	0.18	52	45
Pasanen 2006	0.43	0	1.3	60	46
Patel 2011	0.0064	0	0.026	44	47
Polli 2013	1.5	0.019	17	18	48
Prueksaritanont 2005	1.6	2.90E-04	15	49	49
Schmitt 2011	0.43	0	7.6	46	50
Shanmugam 2011	2.7	0.082	19	23	51
Simard 2001	0.013	0	0.089	89	52
Sugimoto 2001	42	0.16	180	29	53
Sunkara 2007	0.014	0	0.077	66	54
Teng 2013	1.3	0	23	44	55
Teng 2013	0.03	0	0.087	91	56
Tubic-Grozdanis 2008	1.1	0.005	16	84	57
Tuteja 2014	0.2	0	3.2	44	58
Ucar 2004	0.45	0	4.8	40	59
Vree 2001	2.4	0.058	15	34	60
Winsemius 2014	0.21	0	6.4	204	61
Xu 2014	1	0	9.3	92	62
Yu 2009	1.6	0.002	22	46	63
Zhao 2015	1.6	0	19	92	64
Zhi 2003	2.2	0	25	35	65

Ziviani 2001	0.0092	0	0.044	47	66
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a: Number of values extracted from the study for which  $\zeta$  was calculated

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**Table S4: Study records identified in “CPT: Pharmacometrics & Systems Pharmacology” in 2018 as original PBPK research articles**

Year	Title	PBPK original research article	Data digitized	Data digitization mentioned	Digitizing tool cited	Reference
2018	Applied Concepts in PBPK Modeling: How to Extend an Open Systems Pharmacology Model to the Special Population of Pregnant Women	Yes	Most likely	No	No	1
2018	Quantitative Systems Pharmacology Modeling of Acid Sphingomyelinase Deficiency and the Enzyme Replacement Therapy Olipudase Alfa Is an Innovative Tool for Linking Pathophysiology and Pharmacology	Yes	Unlikely (access to clinical study data)	No	No	2
2018	Drug Dosing in Pregnant Women: Challenges and Opportunities in Using Physiologically Based Pharmacokinetic Modeling and Simulations	Yes	Unlikely (no concentration-time or other PK data used)	No	No	3
2018	A Strategy to Refine the Phenotyping Approach and Its Implementation to Predict Drug Clearance: A Physiologically Based Pharmacokinetic Simulation Study	Yes	Most likely	No	No	4
2018	Physiologically Based Pharmacokinetic Approach to Determine Dosing on Extracorporeal Life Support: Fluconazole in Children on ECMO	Yes	Most likely	No	No	5
2018	PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin	Yes	Yes	No	No	6
2018	Using a Vancomycin PBPK Model in Special Populations to Elucidate Case-Based Clinical PK Observations	Yes	Most likely	Yes	GetData Graph Digitizer	7
2018	A Partial Differential Equation Approach to Inhalation Physiologically Based Pharmacokinetic Modeling	Yes	Unlikely (no concentration-time or other PK data used)	No	No	8
2018	Quantitative Prediction of OATP-Mediated Drug-Drug Interactions With Model-Based Analysis of Endogenous Biomarker Kinetics	Yes	Most likely	No	No	9
2018	Drugs Being Eliminated via the Same Pathway Will Not Always Require Similar Pediatric Dose Adjustments	Yes	Unlikely (no concentration-time or other PK data used)	No	No	10
2018	Comprehensive PBPK Model of Rifampicin for Quantitative Prediction of Complex Drug-Drug Interactions: CYP3A/2C9 Induction and OATP Inhibition Effects	Yes	Most likely	No	No	11
2018	PBPK Modeling of Coproporphyrin I as an Endogenous Biomarker for Drug Interactions Involving Inhibition of Hepatic OATP1B1 and OATP1B3	Yes	Most likely	No	No	12
2018	PBPK Model of Morphine Incorporating Developmental Changes in Hepatic OCT1 and UGT2B7 Proteins to Explain the Variability in Clearances in Neonates and Small Infants	Yes	Most likely	Yes	GetData Graph Digitizer	13
2018	A Quantitative Systems Pharmacology Kidney Model of Diabetes Associated Renal Hyperfiltration and the Effects of SGLT Inhibitors	Yes	Yes	No	No	14

2018	Application of PBPK Modeling and Virtual Clinical Study Approaches to Predict the Outcomes of CYP2D6 Genotype-Guided Dosing of Tamoxifen	Yes	Most likely	No	No	15
2018	Prediction of the Pharmacokinetics of Pravastatin as an OATP Substrate Using Plateable Human Hepatocytes With Human Plasma Data and PBPK Modeling	Yes	Most likely	No	No	16
2018	Development, Verification, and Prediction of Osimertinib Drug-Drug Interactions Using PBPK Modeling Approach to Inform Drug Label	Yes	Unlikely (access to clinical study data)	No	No	17
2018	Pediatric Dosing of Ganciclovir and Valganciclovir: How Model-Based Simulations Can Prevent Underexposure and Potential Treatment Failure	Yes	Unlikely (access to database)	No	No	18
2018	A Novel PBPK Modeling Approach to Assess Cytochrome P450 Mediated Drug-Drug Interaction Potential of the Cytotoxic Prodrug Evofosfamide	Yes	Unlikely (access to clinical study data)	No	No	19
2018	Modulation of Cell State to Improve Drug Therapy	Yes	Unlikely (no concentration-time or other PK data used)	No	No	20

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A.2 SUPPORTING INFORMATION PUBLICATION II - A PHYSIOLOGI-  
CALLY BASED PHARMACOKINETIC (PBPK) PARENT-METABOLITE  
MODEL OF THE CHEMOTHERAPEUTIC ZOPTARELIN DOXORUBICIN  
— INTEGRATION OF IN VITRO RESULTS, PHASE I AND PHASE II  
DATA AND MODEL APPLICATION FOR DRUG-DRUG INTERACTION  
POTENTIAL ANALYSIS

**A physiologically-based pharmacokinetic (PBPK) parent-metabolite model of the chemotherapeutic zoptarelin doxorubicin - integration of in vitro results, Phase I and Phase II data and model application for drug-drug interaction potential analysis**

**Metformin Supplementary**

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## PBPK model of metformin

### 1. Introduction

The biguanide metformin is the first-line therapeutic agent for the treatment of type 2 diabetes mellitus. Metformin reduces hepatic gluconeogenesis, reduces intestinal absorption of glucose and increases insulin sensitivity and glucose uptake into peripheral tissues [1].

Because of its hydrophilic structure, metformin shows an exceptionally low lipophilicity ( $\log P = -1.43$ ) and is not bound to plasma proteins [2]. Following oral administration, 50 - 60% of a dose are absorbed and peak plasma concentrations are reached within 2 h. The plasma half-life of metformin is 2.5 - 7 h after intravenous infusion and 4 - 8 h following oral administration [3]. Metformin is not subject to hepatic metabolism and mainly renally excreted. After intravenous administration 80 - 100% of the dose are recovered unchanged in the urine; following oral administration the fraction excreted unchanged alternates between 50 and 75%. The observed renal clearance of metformin is much higher than the glomerular filtration rate (GFR), suggesting active renal secretion [2–4]. Metformin is reported to be a substrate of the organic cation transporter (OCT) 1, the kidney specific OCT2 and of OCT3 [5, 6]. These transporters are localized at the basolateral membranes of renal cells, hepatocytes, enterocytes and cells of many other organs. Metformin is also transported by the  $H^+$  organic cation antiporters "multidrug and toxin extrusion protein" (MATE) 1 and MATE2-K [7]. These efflux transporters are primarily expressed in the liver (MATE1) and in the kidney (MATE1, MATE2-K) at the apical (luminal) membranes. In vivo, OCT and MATE transporters form a functional unit to transport organic cations from the blood through hepatocytes and renal tubule cells into the bile and urine, resulting in effective biliary and renal secretion.

### 2. Materials and Methods

#### Software

PBPK modeling was performed with PK-Sim 7.0.0. Parameter optimization was accomplished using the Monte Carlo algorithm implemented in PK-Sim. Digitization of published plasma concentration-time curves was performed with GetData Graph Digitizer (V 2.26). Graphics and further statistical analyses were generated with R (V 3.3.2) using the graphical interface RStudio (V 1.0.136).

#### Model development

For model development, physicochemical parameters as well as individual and mean plasma concentration-time profiles of metformin after intravenous single dose (250 - 1000 mg), oral single dose (250 - 2550 mg) and oral multiple dose (250 - 1000 mg) administration were obtained from literature. Data was separated into training and test datasets for model development and evaluation, respectively (for a detailed study summary see Suppl. Tab. 1). The training dataset contained a study describing the extent of metformin distribution into erythrocytes [8]. Furthermore, fraction excreted to urine data following intravenous (250 and 500 mg) and oral administration (500 mg) of metformin was used in the training dataset to inform the renal secretion process.

For population simulations, a virtual Caucasian population was generated containing 50 male and 50 female individuals, 20 - 50 years of age, with body weights of 40 - 120 kg. The ICRP (International Commission on Radiological Protection) database in PK-Sim was used for generation of this population [9]. For model evaluation, the medians and 90% prediction intervals of population simulation plasma concentration-time profiles were calculated and used to generate visual predictive checks (predicted versus observed plasma concentrations) for the training and test datasets.

### 3. Results

#### Metformin modeling

To limit the number of parameters to be optimized, only the most important processes were implemented into the final metformin model. These are (1) passive distribution into blood cells and the cells of all organs except renal cells, (2) active uptake from blood into renal cells by OCT2 and (3) renal secretion into urine by MATE2-K. Glomerular filtration and enterohepatic cycling were enabled, as these processes are active under physiological conditions.

#### Drug-dependent parameters

All drug-dependent parameters taken from literature with their references as well as all optimized parameter values are given in Suppl. Tab. 2.

#### System-dependent parameters

Expression of the implemented transporters and the geometric standard deviation of their log-normal distribution in virtual populations are given in Suppl. Tab. 3. No other system-dependent parameters were changed or adjusted.

#### Model performance

**Training dataset:** The training dataset performance of the final metformin model, predicting plasma concentrations following intravenous (250 mg and 500 mg) and oral (500 - 1500 mg) administration of metformin, is presented in Suppl. Fig. 1, 3 and 5 - 8.

Predicted compared to observed fraction excreted to urine following intravenous (250 mg and 500 mg) and oral (500 mg) administration is presented in Suppl. Fig. 2 and 4.

Suppl. Fig. 6 shows predicted and observed plasma and erythrocyte concentrations following oral administration of 850 mg metformin.

**Test dataset:** The test dataset performance of the final metformin model, predicting plasma concentrations following intravenous (1000 mg) and oral (250 - 2550 mg) administration of metformin, is presented in Suppl. Fig. 9 - 20.

### 4. Discussion

#### Model performance

Metformin pharmacokinetics show high inter-individual variability in absorption, apparent volume of distribution (654 +/- 358 L) and renal clearance (335 - 615 mL/min) [1, 10]. The slow absorption of metformin rate-limits its disposition [2, 3] so that variability in absorption causes additional variation during the elimination phase of metformin plasma concentration-time profiles. Evaluation of predicted compared to observed clinical data following intravenous application suggests that the current model overpredicts the velocity of distribution into tissues and underpredicts the rate of excretion of metformin (Suppl. Fig. 1, 2, 9). The model accurately describes the plasma and urine concentrations after single oral administration of 500 mg (Suppl. Fig. 3, 4). The 500 mg multiple dose simulations show a good prediction of the trough concentrations with too rapid absorption and an overprediction of  $C_{max}$  (Suppl. Fig. 5, 12, 13). The same phenomenon can be observed for some of the other studies, especially with administration of higher doses of metformin in the fasted state (Suppl. Fig. 14, 16 - 18), but there are also simulations that underpredict  $C_{max}$  (Suppl. Fig. 8, 10, 15), due to the documented inter-individual variability. The model simulations of metformin administration together with food nicely

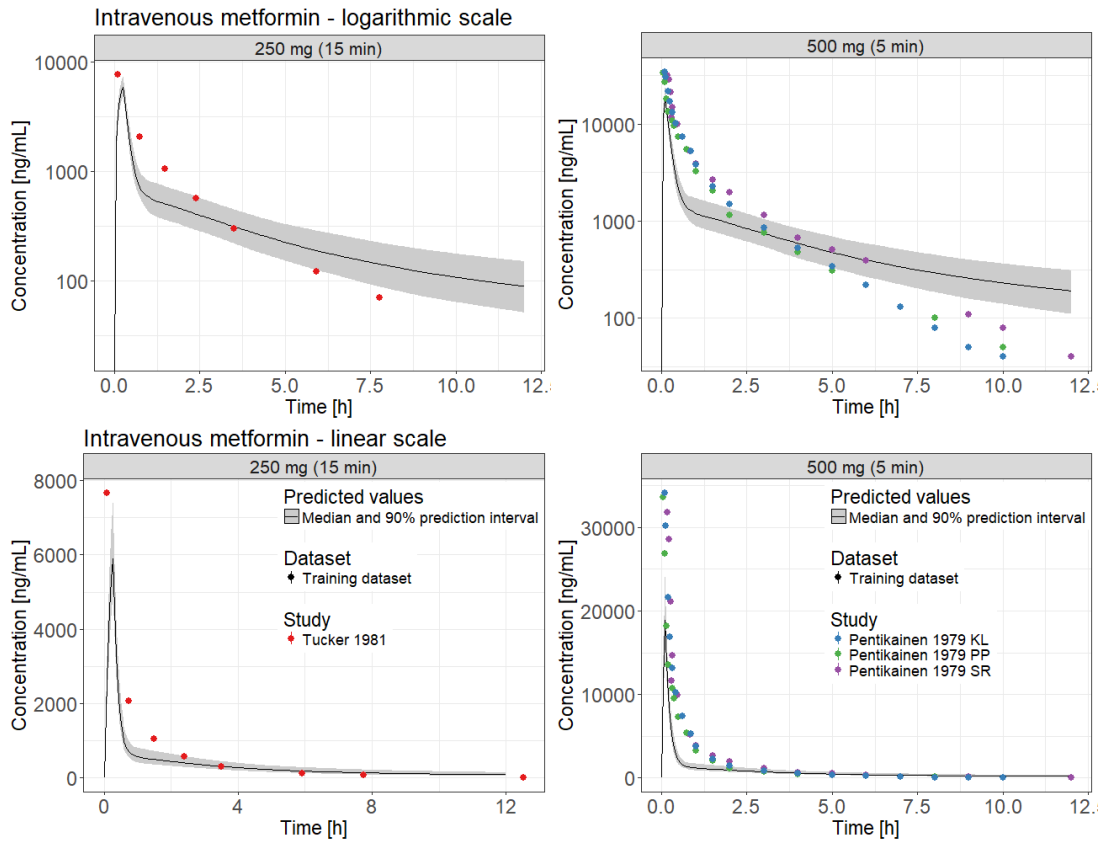
predict the plateau-like shape of the measured plasma concentration-time curves around  $C_{\max}$  (Suppl. Fig. 6, 11). Plasma and erythrocyte concentrations of the study of Robert et al. [8] are also very well described (Suppl. Fig. 6).

#### **Model limitations**

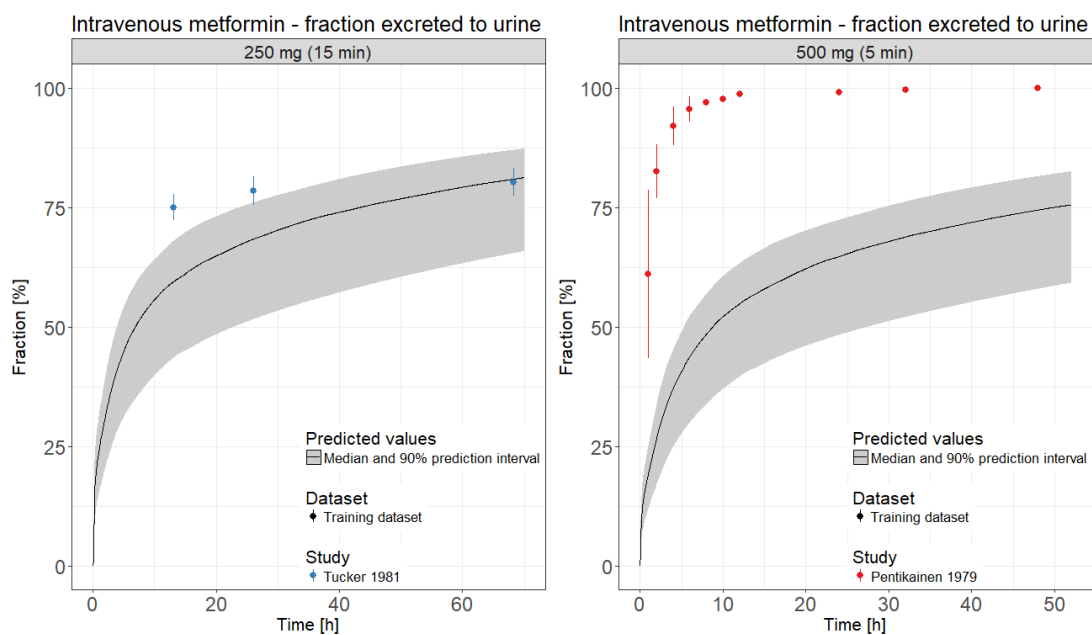
Metformin is recommended by the FDA as OCT2/MATE transport victim drug for the use in clinical DDI studies [11]. The purpose of the presented metformin PBPK model is to accurately incorporate these processes so that this model is fit to be coupled to models of OCT2 and MATE2-K perpetrator drugs and applied for DDI prediction.

Metformin is positively charged at physiological pH ( $pK_a = 11.5$  (base)) and highly hydrophilic ( $\log P = -1.43$ ). Therefore, passive diffusion of metformin through lipid bilayers is very slow. Nevertheless, distribution and accumulation into red blood cells has been described, with a much longer elimination half-life from erythrocytes (23 h), than from plasma (3 h) [8]. Furthermore, the apparent volume of distribution of metformin is high, in spite of its exceptionally low lipophilicity. The mechanism of this partitioning into red blood cells is currently not understood. Transport in combination with target-binding, binding to other intracellular components, some kind of trapping within organelles or sticking to the cellular membranes of red blood cells are possible explanations. As the mechanism of this accumulation is unclear, an asymmetric permeability from plasma into red blood cells was incorporated. The wide distribution into body tissues is most probably mediated by active transport processes and was modeled by an overall asymmetric permeability from the interstitial space into the cells of all organs except kidney, as so far, only the kidney specific isoforms of OCT and MATE transporters have been incorporated into this model.

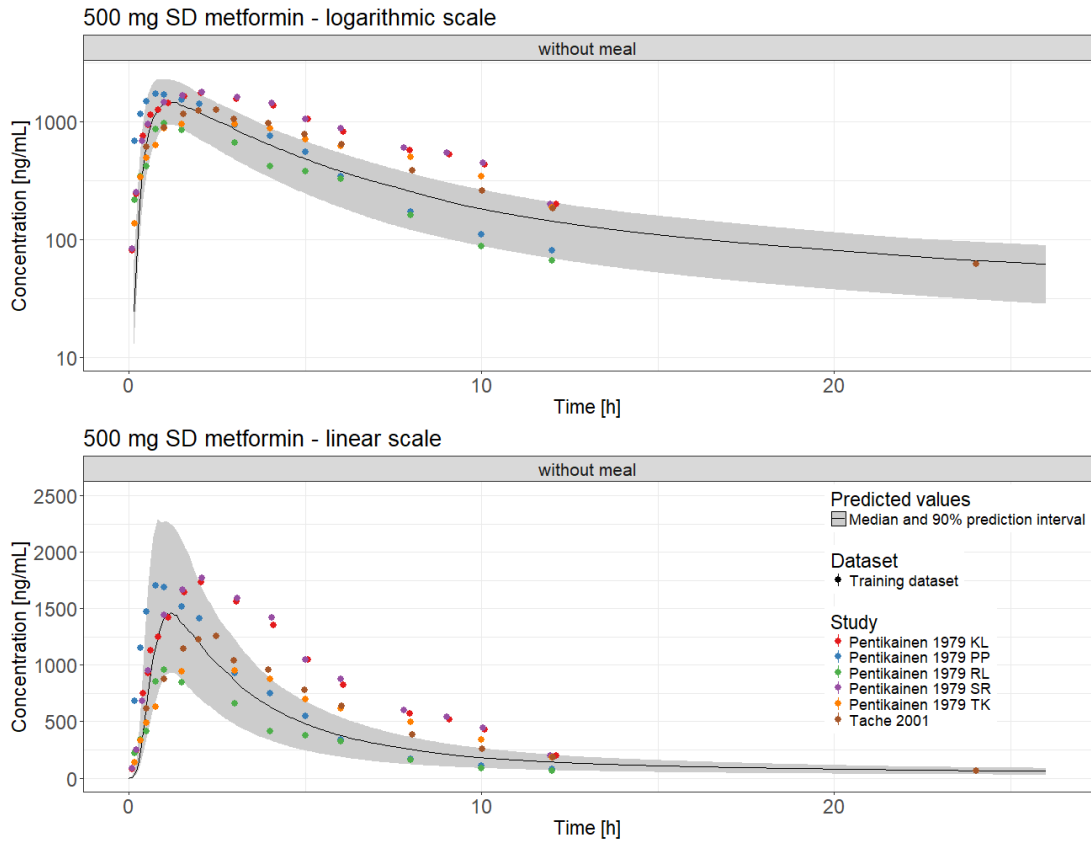
To define the contribution of the two implemented transport processes by OCT2 and MATE2-K, fraction excreted to urine data after intravenous and oral administration of metformin have been included into the training dataset. In addition to an accurate description of unchanged drug recovered in urine, evaluation of this model with measured metformin concentrations in the kidney and by prediction of OCT2 and MATE2-K mediated DDIs is needed.



**Suppl. Fig. 1** Training dataset: Population simulations compared to observed data of metformin plasma concentrations following intravenous administration of 250 mg (left panel) and 500 mg (right panel). Clinical data are shown as dots (Pentikainen KL, PP and SR are individual data) [2, 3]. Population simulation medians are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals



**Suppl. Fig. 2** Training dataset: Population simulations compared to observed data of metformin fraction excreted to urine following intravenous administration of 250 mg (left panel) and 500 mg (right panel). Clinical data are shown as dots (+/- standard deviation) [2, 3]. Population simulation medians are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals



**Suppl. Fig. 3** Training dataset: Population simulations compared to observed data of metformin plasma concentrations following single oral administration of 500 mg. Clinical data are shown as dots (Pentikainen KL, PP, RL, SR and TK are individual data) [2, 12]. Population simulation medians are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals

**A physiologically-based pharmacokinetic (PBPK) parent-metabolite model of the chemotherapeutic zoptarelin doxorubicin - integration of in vitro results, Phase I and Phase II data and model application for drug-drug interaction potential analysis**

**Simvastatin Supplementary**

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## PBPK model of simvastatin lactone and simvastatin acid

### 1. Introduction

Simvastatin is a 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase inhibitor. It is widely used in the treatment of hypercholesterolemia and belongs to the ten most prescribed drugs in industrial nations [1]. Simvastatin is administered orally as a prodrug in its lactone form (dosing range 5 - 80 mg/day [2]) and is converted to the active acid by a combination of enzyme-mediated hydrolysis and spontaneous chemical conversion [3]. The enzyme predominantly responsible for the hydrolysis of simvastatin lactone to simvastatin acid is paraoxonase 3 (PON3) [4, 5]. Simvastatin lactone is highly lipophilic, resulting in good absorption of approximately 60% of an administered dose, but shows extensive first pass metabolism reducing its oral bioavailability to 5% [3]. Simvastatin lactone is mainly metabolized by CYP3A4 [6], while simvastatin acid is metabolized by CYP3A4 (>80%) and CYP2C8 (<20%) as well as transported by organic anion-transporting polypeptide 1B (OATP1B) [7]. A further process discussed for the pharmacokinetics of simvastatin acid is recyclization to the lactone form, either spontaneously, or via enzymatic formation of an intermediate glucuronide. Suppl. Fig. 1 depicts the metabolization pathways of statins in general.

### Objectives

The purpose of this work was to establish a whole body parent-metabolite PBPK model of simvastatin lactone (prodrug) and acid (pharmacologically active metabolite) as a CYP3A and OATP1B victim drug model for drug-drug interaction studies

- that accurately predicts plasma concentrations of simvastatin lactone and acid over a broad dosing range
- that has been evaluated by showing good prediction of simvastatin lactone and acid plasma concentrations in drug-drug interaction (DDI) studies with rifampicin and clarithromycin as CYP3A4 perpetrator drugs
- that has been evaluated by showing good prediction of simvastatin lactone and acid plasma concentrations in individuals with different OATP1B1 genotypes

### 2. Materials and Methods

#### Software

PBPK modeling was performed with PK-Sim 7.0.0. Parameter optimization was accomplished using the Monte Carlo algorithm implemented in PK-Sim. Digitization of published plasma concentration-time curves was performed with GetData Graph Digitizer (V 2.26). Graphics and further statistical analyses were generated with R (V 3.3.2) and the graphical user interface RStudio (V 1.0.136).

#### Model development

For model development, physicochemical parameters as well as plasma concentration-time profiles of simvastatin lactone and simvastatin acid after oral single dose (SD) and multiple dose (MD) administration (range 20 - 80 mg) were obtained from the literature. Data was separated into training and test datasets for model development and evaluation, respectively (for a detailed study summary see Suppl. Tab. 1). The training dataset contained a study showing the impact of different OATP1B1 genotypes on the plasma concentrations of simvastatin acid [8]. This study was included to define the contribution of this transporter to simvastatin acid pharmacokinetics. For studies that did not specify



the OATP1B1 genotype, the wild type variant was assumed. Due to the lack of clinical trials of direct administration of simvastatin acid, simvastatin lactone and simvastatin acid model development was performed in parallel.

For population simulations, a virtual Caucasian population was generated containing 50 male and 50 female individuals, 20 - 50 years of age, with body weights of 40 - 120 kg. The ICRP (International Commission on Radiological Protection) database in PK-Sim was used for generation of this population [13]. For model evaluation, the arithmetic means and 90% prediction intervals of population simulation plasma concentration-time profiles were calculated and used to generate visual predictive checks (predicted versus observed plasma concentrations) for the training and test datasets.

To test the contribution of the implemented CYP3A4 metabolism, the final simvastatin model was coupled to PBPK models of the CYP3A4 perpetrators rifampicin (CYP3A4 inducer, [9]) and clarithromycin (CYP3A4 inhibitor, [10]). Plasma concentrations of simvastatin lactone and simvastatin acid during co-administration with these perpetrator drugs were predicted and compared to observed data.

### 3. Results

#### Simvastatin modeling

To limit the number of parameters to be optimized, only the most important processes were implemented into the final simvastatin parent-metabolite model. For simvastatin lactone these are (1) PON3 mediated hydrolysis to generate simvastatin acid and (2) CYP3A4 mediated clearance. For simvastatin acid these are (3) hepatic uptake by OATP1B1 and (4) CYP3A4 mediated clearance. The OATP1B1 transport was implemented with two different  $K_M$  values and two different transport rates, to describe the impact of the investigated OATP1B1 polymorphism on simvastatin acid plasma concentrations. For both, parent and metabolite, glomerular filtration and enterohepatic cycling were enabled.

#### Drug-dependent parameters

All drug-dependent parameters taken from the literature with their references as well as all optimized parameter values are given in Suppl. Tab. 2.

#### System-dependent parameters

Expression of the implemented enzymes and transporters as well as the geometric standard deviation of their log-normal distribution in virtual populations are given in Suppl. Tab. 3. No other system-dependent parameters were changed or adjusted.

#### Model performance

**Training dataset:** The training dataset performance of the final model, predicting simvastatin lactone and simvastatin acid plasma concentrations following oral administration of 20, 40, 60 or 80 mg simvastatin lactone, is presented in Suppl. Fig. 2 - 6.

Suppl. Fig. 7 shows the predicted compared to observed plasma concentrations following oral administration of 40 mg simvastatin lactone to individuals with different OATP1B1 genotypes [8]. The transport rates of the two homozygous OATP1B1 isoforms (c.521TT wild type and c.521CC) were optimized, the transport rate of the heterozygous isoform (c.521TC) has been predicted.

**Test dataset:** The test dataset performance of the final model, predicting simvastatin lactone and simvastatin acid plasma concentrations following oral administration of 20, 40 or 80 mg simvastatin lactone, is presented in Suppl. Fig. 8 - 10.

### Model application

As a further means of model evaluation, the final simvastatin model was applied to predict clinical DDI studies. Simvastatin plasma concentrations during two different trials studying co-administration of simvastatin lactone and the CYP3A4 perpetrator drugs rifampicin [11] and clarithromycin [12] were predicted and compared to observed data.

In the rifampicin DDI study, a single dose of 40 mg simvastatin lactone was administered 17 h after the last dose of a 600 mg QD, 5 day rifampicin regimen. Thus, no inhibitory effects of rifampicin on CYP3A4 or OATP1B1 are expected, solely pure CYP3A4 induction. In the clarithromycin DDI study, once daily doses of 40 mg simvastatin lactone were administered together with the morning doses of a 500 mg BID, 7 day clarithromycin regimen. Unfortunately, only simvastatin lactone plasma concentrations have been reported from this study, allowing no interpretation of the effect of clarithromycin on OATP1B1 and simvastatin acid. Predicted and observed plasma concentrations are shown in shown in Suppl. Fig. 11 and 12. Predicted and observed AUC ratios (AUC during perpetrator treatment / AUC without co-administration of DDI perpetrator) of these DDIs are compared in Suppl. Tab. 4 and 5.

## 4. Discussion

### Model performance

The final parent-metabolite PBPK model accurately describes the plasma concentration-time profiles of simvastatin lactone and simvastatin acid after oral administration of 20 - 80 mg simvastatin lactone. There is a slight terminal overprediction of the lactone, but not of the acid concentrations, following single dose administration of 40 mg simvastatin lactone. Nevertheless, the studies of multiple dose administration of 40 mg simvastatin lactone are well predicted. This phenomenon might be caused by variability in body weight or genetic polymorphisms of involved metabolizing enzymes or transporters of these relatively small study populations that have not been taken into account for the model predictions.

To define the contribution of OATP1B1 to the disposition of simvastatin acid, information of a clinical trial studying the impact of the OATP1B1 c.521 polymorphism on simvastatin acid pharmacokinetics has been included into the training dataset. The final simvastatin model accurately predicts the simvastatin acid plasma concentrations of individuals of the three possible OATP1B1 genotypes.

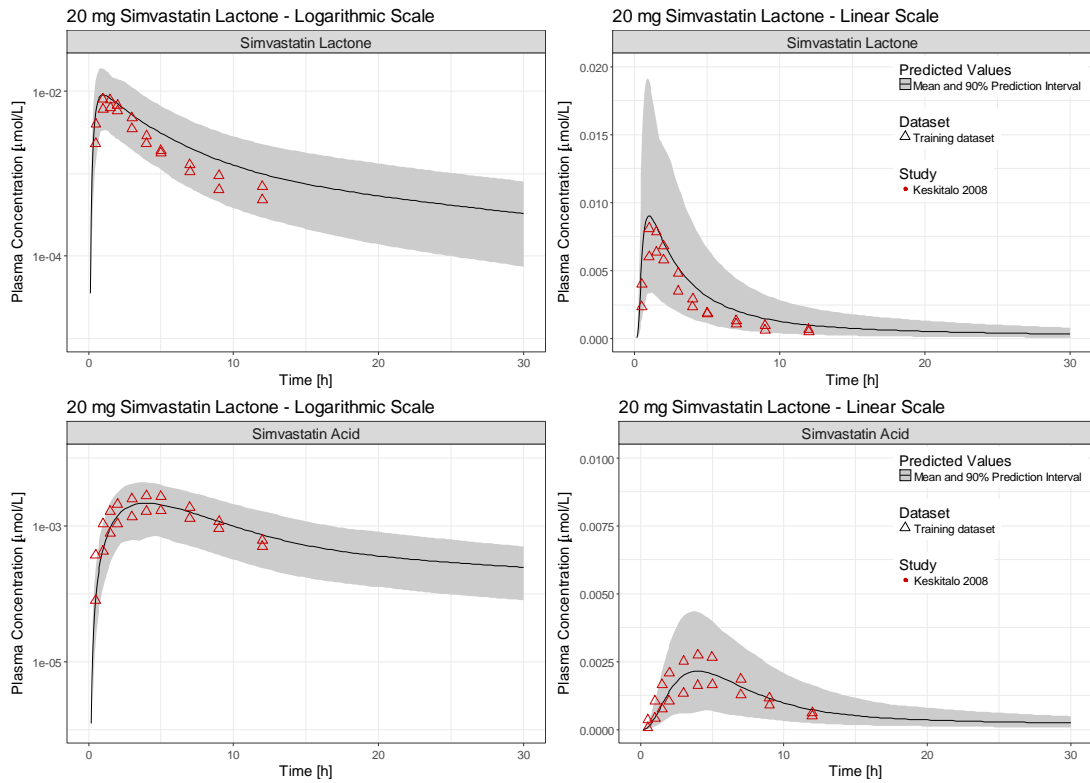
To evaluate the contribution of CYP3A4 to the metabolism of simvastatin lactone and simvastatin acid, clinical DDI studies with the CYP3A4 perpetrators rifampicin and clarithromycin have been predicted and compared to observed data. During the DDI with rifampicin, simvastatin lactone and simvastatin acid plasma concentrations are adequately predicted. In the DDI study with clarithromycin only simvastatin lactone concentrations have been reported and the effect of clarithromycin on simvastatin lactone peak plasma concentrations is underpredicted. Nevertheless, the predicted AUC ratios of simvastatin lactone and simvastatin acid are within twofold of the observed AUC ratios for both of the tested DDIs (see Suppl. Tab. 4 and 5).

### Model limitations

Simvastatin is listed by the FDA as a sensitive CYP3A substrate for the use in clinical DDI studies, and simvastatin acid is an approved OATP1B victim drug [13]. The purpose of the presented simvastatin PBPK model is to accurately incorporate these processes so that this model is fit to be coupled to models of CYP3A and OATP1B perpetrator drugs and applied for DDI prediction.

Mechanisms not implemented into the final simvastatin model include transport by breast cancer resistance protein (BCRP) or p-glycoprotein (P-gp). Genetic polymorphism in the ABCG2 gene encoding for BCRP has been described to influence the plasma concentrations of simvastatin lactone [14], while genetic polymorphisms in ABCB1 (P-gp) have been described to affect the plasma concentrations of simvastatin acid [15]. Implementation of these transport processes would further diminish the contribution of CYP3A4 and was therefore not retained in the final model. Another possible mechanism involved in simvastatin pharmacokinetics is reabsorption (enterohepatic cycling) following BCRP-mediated transport of simvastatin lactone into the bile, or following P-gp-mediated transport of simvastatin acid into bile with subsequent recyclization to the lactone. Information on the pharmacokinetics of simvastatin lactone and acid after intravenous administration, on bioavailability and on enterohepatic cycling would greatly help to improve our current understanding of the mechanisms affecting the plasma concentrations and DDI behaviour of this widely used lipid-lowering drug.





**Suppl. Fig. 2** Training dataset: Population simulations compared to observed data of simvastatin lactone (upper panel) and simvastatin acid (lower panel) plasma concentrations following single oral administration of 20 mg simvastatin lactone. Clinical data [15] are shown as triangles. Population simulation means are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals

**A physiologically-based pharmacokinetic (PBPK) parent-metabolite model of the chemotherapeutic zoptarelin doxorubicin - integration of in vitro results, Phase I and Phase II data and model application for drug-drug interaction potential analysis**

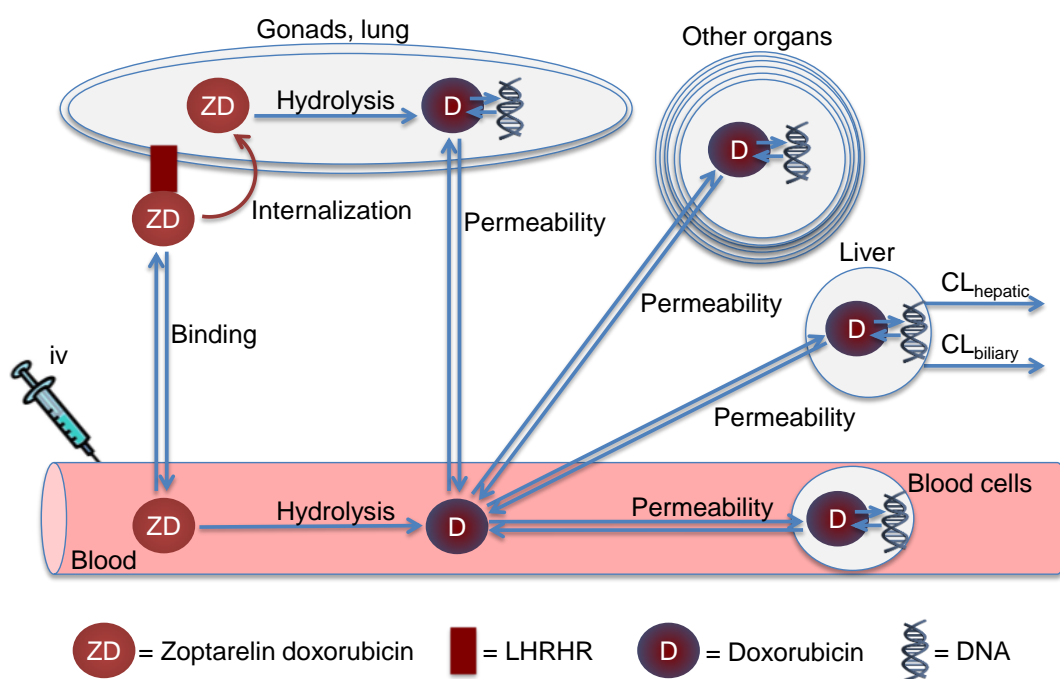
**Zoptarelin Doxorubicin Supplementary**

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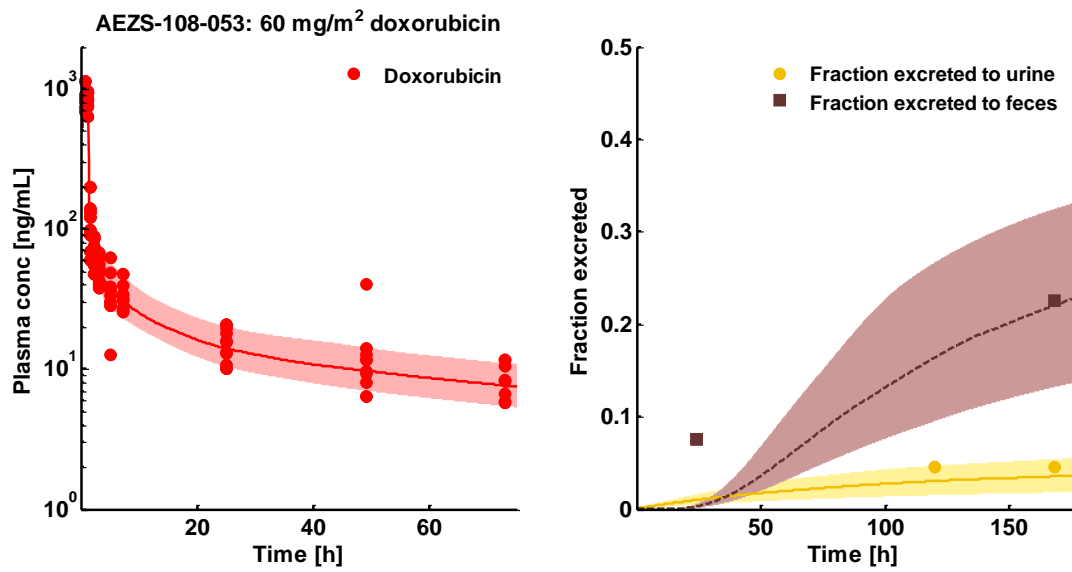
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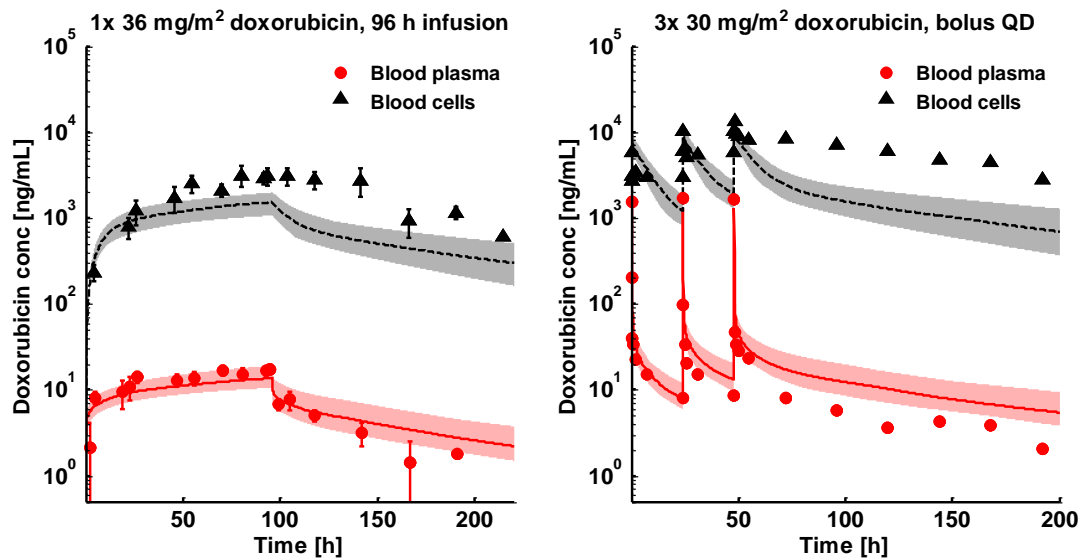


**Suppl. Fig. 1** Zoptarelin doxorubicin parent-metabolite PBPK model structure.  $CL_{biliary}$ : biliary plasma clearance,  $CL_{hepatic}$ : hepatic metabolic plasma clearance, iv: intravenous administration, LHRHR: luteinizing hormone-releasing hormone receptor

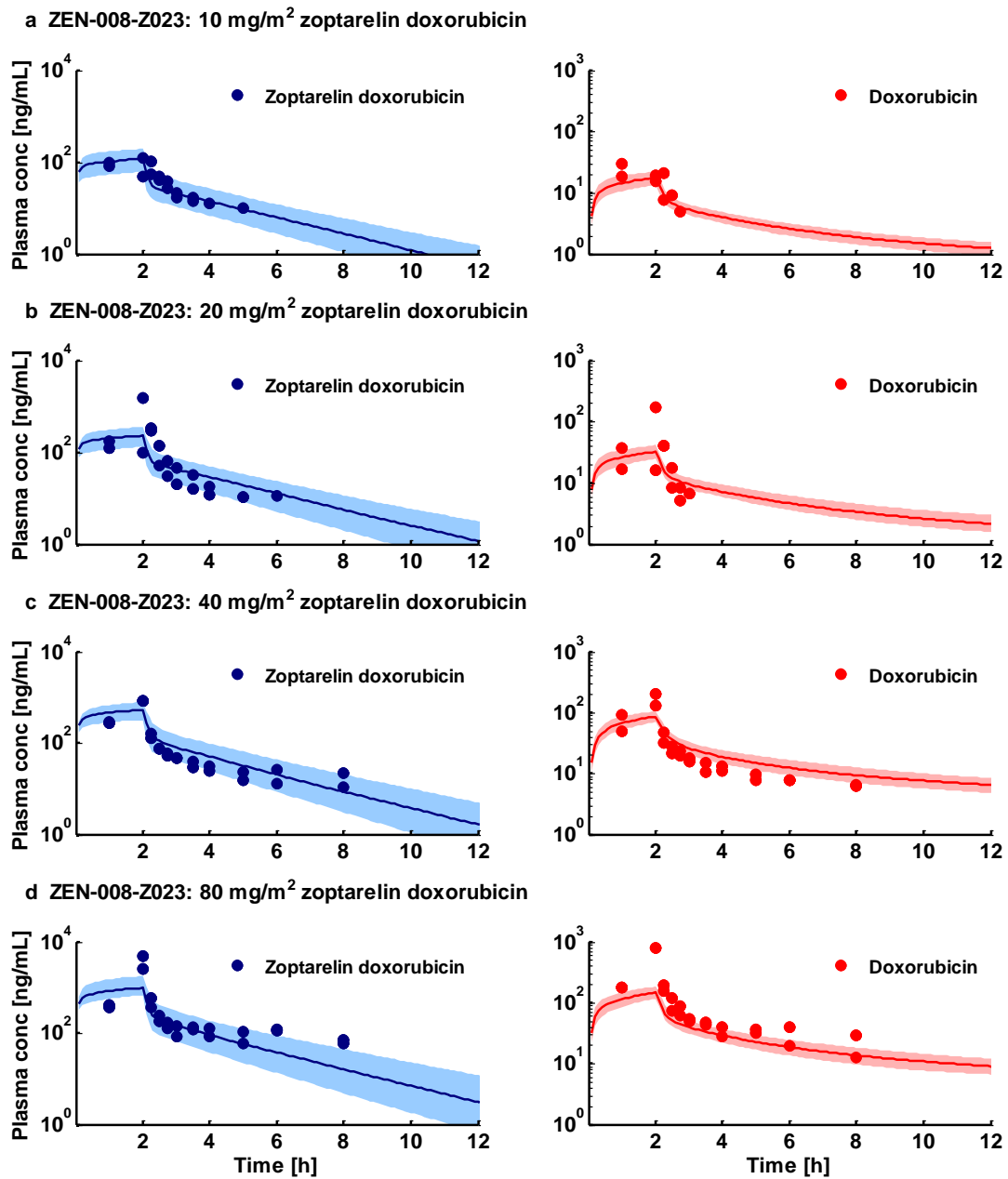


**Suppl. Fig. 2** Training dataset: Population simulations compared to observed data of doxorubicin plasma concentrations (red, semilog scale) and fractions excreted to urine and feces (yellow and brown, linear scale) following intravenous administration of 60 mg/m<sup>2</sup> doxorubicin. Clinical data (Study 3, doxorubicin arm, n = 9 and [1]) are shown as dots and squares. Population simulation medians are shown as lines or dashed lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals

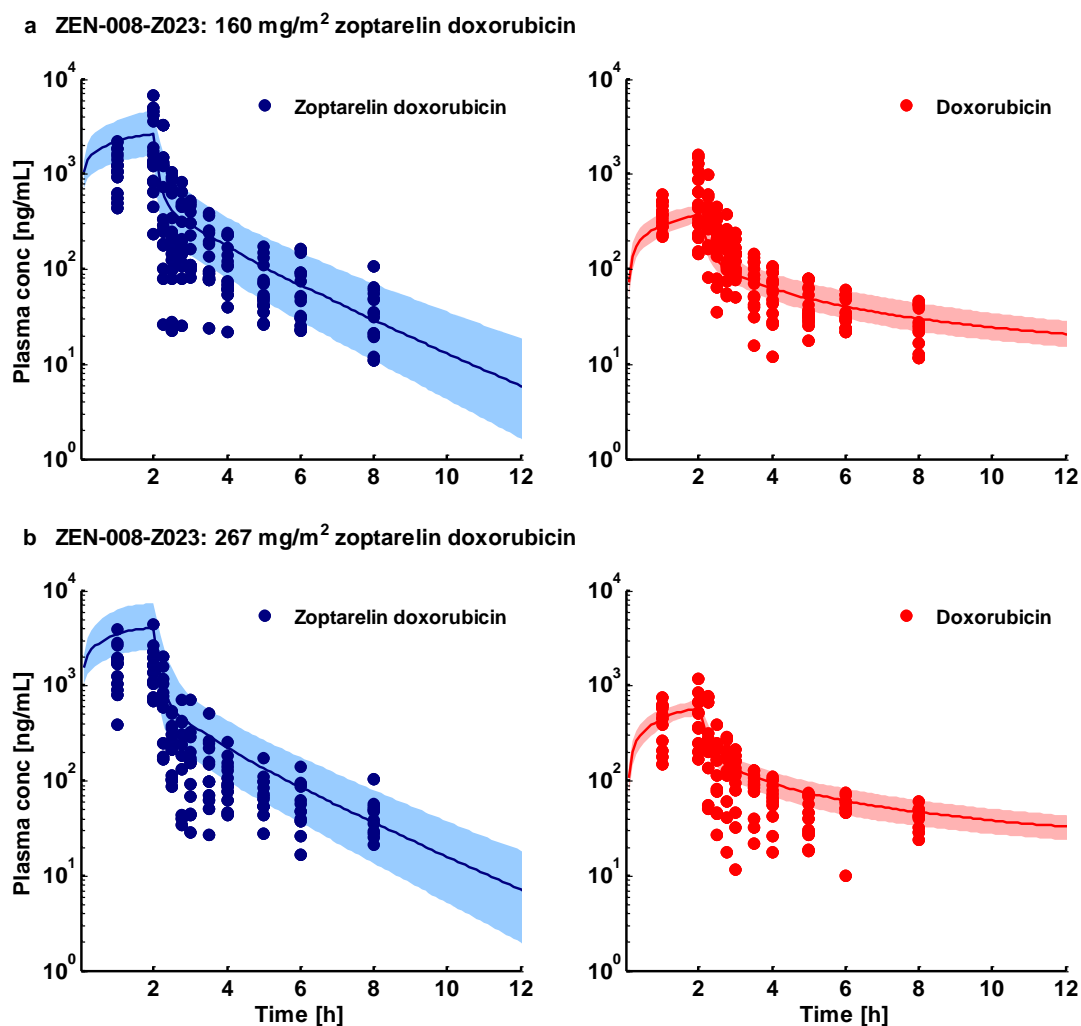




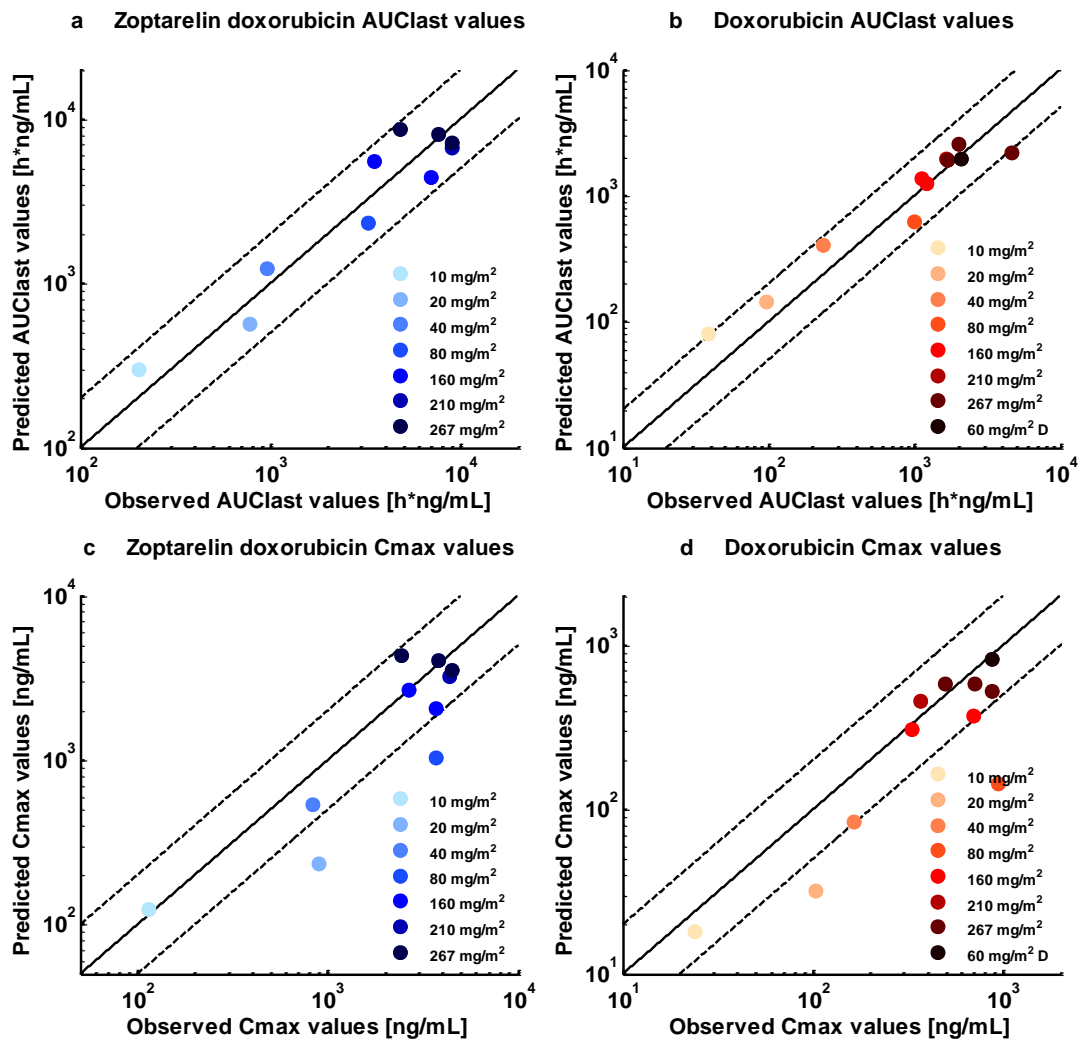
**Suppl. Fig. 3** Training dataset: Population simulations (semilog scale) compared to observed data of doxorubicin concentrations in blood plasma (red) and in nucleated blood cells (black) following intravenous administration of 1x 36 mg/m<sup>2</sup> doxorubicin as 96 h long-term infusion (left) or 3x 30 mg/m<sup>2</sup> doxorubicin as daily bolus infusions (right). Simulated blood cell concentrations represent free plus DNA-bound doxorubicin in the blood cell compartment. Clinical data ([2], n = 7 and [3], n = 7) are shown as dots and triangles ( $\pm$  SD values). Population simulation medians are shown as lines or dashed lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals



**Suppl. Fig. 4** Training dataset: Population simulations (semilog scale) compared to observed data of zoptarelin doxorubicin (blue) and doxorubicin plasma concentrations (red) following intravenous administration of 10, 20, 40 or 80 mg/m<sup>2</sup> (a, b, c, d) zoptarelin doxorubicin. Clinical data (Study 1, n = 1 for each dose, 2 cycles per patient, one dose every 3 weeks) are shown as dots. Population simulation medians are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals



**Suppl. Fig. 5** Test dataset: Population simulations (semilog scale) compared to observed data of zoptarelin doxorubicin (blue) and doxorubicin plasma concentrations (red) following intravenous administration of 160 or 267 mg/m<sup>2</sup> (a, b) zoptarelin doxorubicin. Clinical data (Study 1, n = 6 and n = 5, multiple cycles per patient, one dose every 3 weeks) are shown as dots. Population simulation medians are shown as lines; the shaded areas depict the 5<sup>th</sup> - 95<sup>th</sup> percentile population prediction intervals



**Suppl. Fig. 6** Model performance: Mean  $AUC_{last}$  (a, b) and  $C_{max}$  (c, d) values of population predictions compared to observed data (log scale) of zoptarelin doxorubicin (blue) and doxorubicin (red) plasma concentrations following intravenous administration of 10 to 267  $mg/m^2$  zoptarelin doxorubicin or of 60  $mg/m^2$  doxorubicin. Each dot represents a dosing group of one clinical study. Number of patients per dosing group and further details are listed in Zoptarelin Doxorubicin Supplementary Tables 1 and 2. The solid line marks the line of identity, the dashed lines show the 0.5 to 2.0-fold prediction success limits.  $AUC_{last}$ : area under the plasma concentration-time curve from time 0 to the last measurement,  $C_{max}$ : peak plasma concentration, D: intravenous administration of doxorubicin

A.3 SUPPORTING INFORMATION PUBLICATION III - PHYSIOLOGICALLY  
BASED PRECISION DOSING APPROACH FOR DRUG-DRUG-GENE  
INTERACTIONS: A SIMVASTATIN NETWORK ANALYSIS

Clinical Pharmacology & Therapeutics

## Physiologically Based Precision Dosing Approach for Drug-Drug-Gene Interactions: A Simvastatin Network Analysis

### Electronic Supplementary Material (ESM)

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# 1 General information

This chapter provides short introduction about all processes and mechanisms relevant for the simvastatin physiologically based pharmacokinetic (PBPK) drug-drug-gene interaction (DDGI) network model. In addition, it provides general information regarding the system-dependent parameters, which were the same for all model simulations to ensure a comparable model environment. Moreover, this chapter describes the methods used for model development as well as for model evaluation.

## 1.1 Mathematical implementation of DDIs and DGIs

### 1.1.1 DDIs

Required equations for the mathematical implementation of drug-drug interactions (DDIs) are predefined in the Open Systems Pharmacology Suite (OSPS) [1]. For a detailed description the reader is kindly referred to the OSP OPS documentation [1].

### 1.1.2 DGIs

Genetic polymorphisms in drug relevant target structures, metabolic enzymes or transporters can affect the pharmacokinetic (PK) or pharmacodynamic (PD) of an active compound [2]. For example, a different genotype might lead to changed metabolic activity of the phenotype and hence, result in poor, intermediate, extensive or ultra rapid metabolic activity states [2]. The main cause for genetic polymorphisms are single nucleotide polymorphisms (SNPs) [3].

For all drug-gene interactions (DGIs) implemented a change in the transporter or enzyme  $k_{\text{cat}}$  was assumed compared to wildtype. Although, also a change in  $K_M$  would be conceivable for the polymorphisms included in this network, no evidence for this mechanism could be found in literature. Besides, DGIs were implemented in a stepwise procedure. First, the homozygous wildtype  $k_{\text{cat}}$  as well as  $k_{\text{cat}}$  for homozygous polymorphic individuals were estimated. Afterwards, the implementation was evaluated using heterozygous individuals. For this purpose the  $k_{\text{cat}}$  for individuals with heterozygous genotypes were estimated according

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to Eq. (S1.1) assuming an additive relationship between the wildtype and the deficient pharmacogene. Besides, for all studies where no information about the genotype was provided, homozygous wildtype genotypes were assumed.

Equation : Drug-gene interaction enzyme / transporter activity for heterozygous individuals

$$k_{cat,heterozygous} = 0.5 * k_{cat,wildtype} + 0.5 * k_{cat,deficient} \quad (S1.1)$$

$k_{cat,heterozygous}$	=	heterozygous catalytic rate constant number
$k_{cat,wildtype}$	=	wildtype catalytic rate constant number
$k_{cat,deficient}$	=	homozygous deficient catalytic rate constant number

## 1.2 Network relevant metabolic enzymes and transporters

This chapter introduces network relevant metabolic enzymes and transporters. Table S1.1 lists the compounds included in the model network and their relationship as substrates, inhibitors or inducers with regard to the metabolic enzymes and transporters. Figures S1.1, S1.2, S1.3 visualize the relationships.

Table S1.1: Substrates, inhibitors and inducers used in the presented model network

	Enzyme	Substrate	Inhibitor	Inducer
<b>Process</b>	Chemical hydrolysis (simvastatin lactone)	Simvastatin Lactone	-	-
	<b>Metabolic enzyme</b>			
	AADAC	Rifampicin	-	Rifampicin
	CYP2C8	Simvastatin Acid	Simvastatin Acid	-
		-	Gemfibrozil	-
		-	Gemfibrozil glucuronide	-
		-	Rifampicin	Rifampicin
	CYP3A4	Simvastatin Lactone	Simvastatin Lactone	-
		Simvastatin Acid	Simvastatin Acid	-
		N-desalkyl-itraconazole	N-desalkyl-itraconazole	-
		Midazolam	-	-
		Keto-itraconazole	Keto-itraconazole	-
		Itraconazole	Itraconazole	-

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Table S1.1: Substrates, inhibitors and inducers used in the presented model network (*continued*)

Type	Enzyme	Substrate	Inhibitor	Inducer
		Hydroxy-itraconazole	Hydroxy-itraconazole	-
		Clarithromycin	Clarithromycin	-
		-	Rifampicin	Rifampicin
	CYP3A5	Simvastatin Lactone	-	-
	PON3	Simvastatin Lactone	-	-
		-	-	Rifampicin
		-	-	Gemfibrozil
		-	-	Gemfibrozil glucuronide
	UGT1A1	Simvastatin Acid	-	-
		-	Gemfibrozil	-
		-	Rifampicin	Rifampicin
		-	Gemfibrozil glucuronide	-
	UGT1A3	Simvastatin Acid	-	-
		-	Gemfibrozil	-
		-	Rifampicin	Rifampicin
	UGT2B7	Gemfibrozil	-	-
	Unspecific liver lactonization (simvastatin acid)	Simvastatin Acid	-	-
	Unspecific plasma hydrolysis (simvastatin lactone)	Simvastatin Lactone	-	-
<b>Influx transporter</b>				
	OATP1B1 (SLCO1B1)	Simvastatin Acid	Simvastatin Acid	-
		-	Clarithromycin	-
		-	Rifampicin	Rifampicin
		-	Gemfibrozil	-
		-	Gemfibrozil glucuronide	-
		-	Hydroxy-itraconazole	-
		-	Keto-itraconazole	-
		-	Simvastatin Lactone	-
	OATP1B3 (SLCO1B3)	-	Clarithromycin	-
		-	Rifampicin	Rifampicin

## 1 General information

Table S1.1: Substrates, inhibitors and inducers used in the presented model network (*continued*)

Type	Enzyme	Substrate	Inhibitor	Inducer
		-	Gemfibrozil	-
		-	Gemfibrozil glucuronide	-
		-	Hydroxy-itraconazole	-
		-	Keto-itraconazole	-
	Unspecific liver influx (gemfibrozil)	Gemfibrozil	-	-
<b>Efflux transporter</b>				
	BCRP (ABCG2)	Simvastatin Lactone	-	-
		-	Rifampicin	Rifampicin
		-	Itraconazole	-
		-	Keto-itraconazole	-
		-	Simvastatin Acid	-
	MRP2 (ABCC2)	Gemfibrozil glucuronide	-	-
		-	Gemfibrozil	-
		-	Rifampicin	-
	P-gp (ABCB1)	Simvastatin Acid	-	-
		-	Clarithromycin	-
		-	Rifampicin	Rifampicin
		-	Itraconazole	-
		-	N-desalkyl- itraconazole	-
		-	Hydroxy-itraconazole	-
		-	Keto-itraconazole	-
		-	Simvastatin Lactone	-

## 1 General information

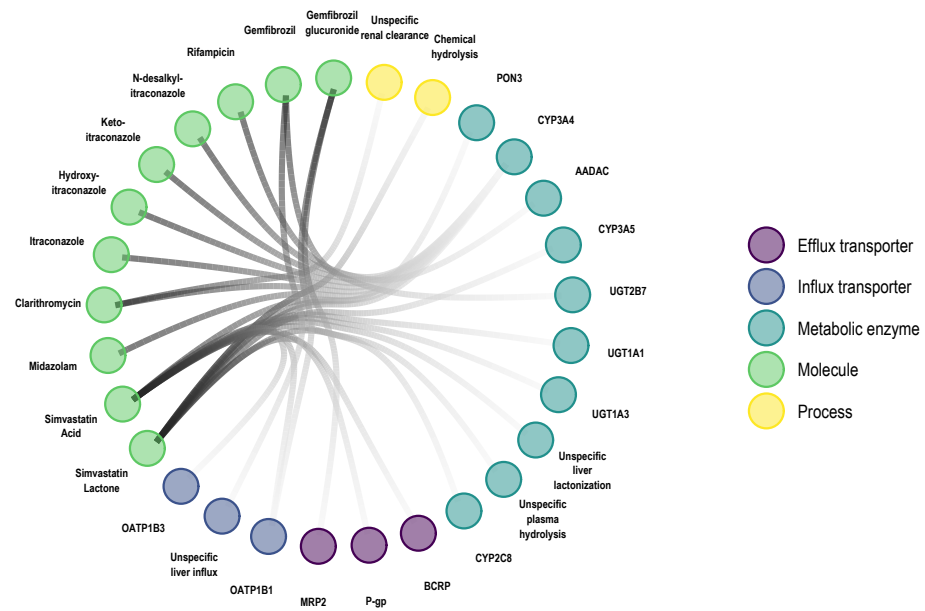


Figure S1.1: Network included compounds and their roles as substrates, inhibitors or inducers: Substrates

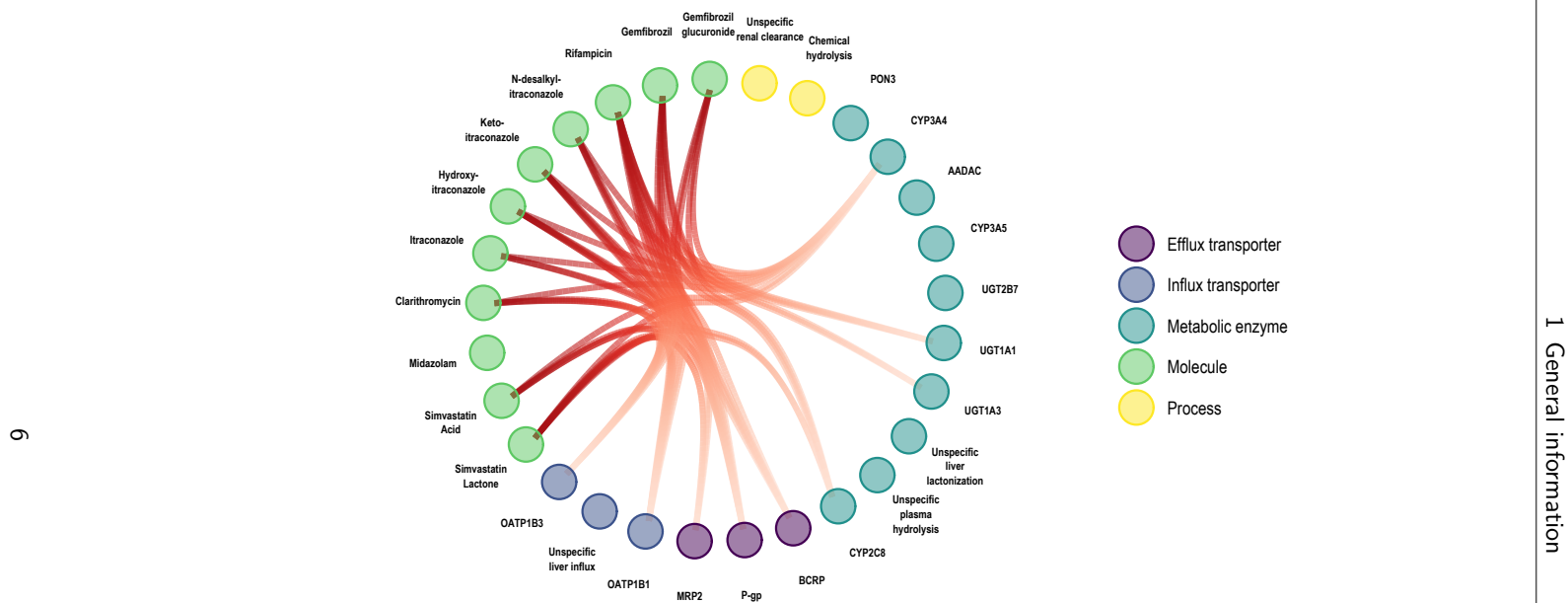


Figure S1.2: Network included compounds and their roles as substrates, inhibitors or inducers: Inhibitors

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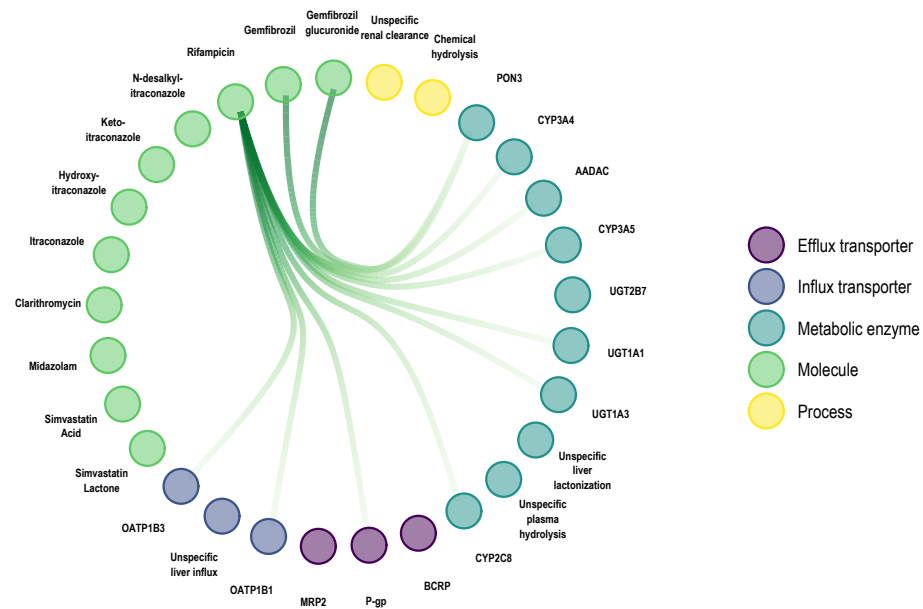


Figure S1.3: Network included compounds and their roles as substrates, inhibitors or inducers: Inducers

## 1.3 Model development and system-dependent specifications

### 1.3.1 General modeling workflow

For model development, concentration-time profiles of published clinical studies covering the full reported dosing range were used. If available this included data of intravenous and oral administration after single or multiple doses. If information about the amount of unchanged drug excreted to urine or feces was available, they were also utilized to inform the model optimization. If parameters had to be identified the Monte-Carlo algorithm implemented in PK-Sim<sup>®</sup> was used, utilizing the root mean square error (RMSE) as described in Eq. (S1.2) for parameter optimization.

Equation : Root-mean-square error

$$RMSE = \sqrt{\sum (c_{obs} - c_{pred})^2} \quad (S1.2)$$

RMSE = root mean square error  
 $C_{obs}$  = observed Concentration  
 $C_{pred}$  = predicted concentration

Parameter identifications were performed if either no literature values were on hand or multiple values with a broad range were available. Besides, model relevant information regarding all clinical studies available are listed in the corresponding Chapters 2.2.1 and 3.1 of the electronic supplementary material (ESM) including information about the assignment of each study to the training (model building) or test data set (model evaluation). For model development a mean prediction was used based on the mean demographic properties (gender, age, weight, height, race) of the respective study for each study in the training dataset. If values were missing, a mean value for age (30 years), weight (73 kg) and height (176 cm) was used. Quality of each parameter optimization was evaluated based on the graphical and statistical evaluation techniques as described in Chapter 1.4 of the ESM. Parameters of the final simvastatin model are given in Tables S2.3 and S2.5. Previously developed PBPK models used for the DDI network development were extended as described in the Chapter 3.1 of the ESM.

### 1.3.2 System-dependent parameters

All system-dependent parameters like reference concentrations, protein half-lives as well as tissue expression profiles of transporters and relevant metabolizing enzymes are listed in Table S1.2.



Table S1.2: System-dependent parameters and expression of relevant enzymes, transporters and other ADME processes

Enzyme / Transporter / Process (Gene)	Mean reference concentration <sup>a</sup>	Geometric standard deviation of the reference concentration <sup>b</sup>	Relative expression in the different organs	Half-life liver [hours]	Half-life intestine [hours]
<b>Enzymes</b>					
AADAC	1 [4]	1.4	RT-PCR [5]	36	23
CYP2C8	2.56 [6]	2.05 [1]	RT-PCR [7]	23	23
CYP3A4	4.32 [6]	1.18 liver [1], 1.46 intestine [1]	RT-PCR [7]	36 [8]	23 [9]
CYP3A5	0.04 [6]	1.4	RT-PCR [7]	36	23
PON3	1	1.4	Array [10]	36	23
UGT1A1	1	1.4	RT-PCR [5]	36	23
UGT1A3	1	1.4	RT-PCR [5]	36	23
UGT2B7	1 [4]	1.6 [1]	EST [1]	36	23
Unspecific liver lactonization (simvastatin acid)	1	1.4	Liver only	36	23
Unspecific plasma hydrolysis (simvastatin lactone)	1	1.4	Plasma only	36	23
<b>Processes</b>					
Chemical hydrolysis (simvastatin lactone)	1	-	Ubiquitous	36	23
<b>Transporters</b>					
BCRP ( <i>ABCG2</i> )	1	1.35 [11]	RT-PCR [12], with relative expression in blood cells set to 0.3046 [13]	36	23
MRP2 ( <i>ABCC2</i> )	1	1.49 [14]	Array [10]	36	23
OATP1B1 ( <i>SLCO1B1</i> )	1 [4]	1.54 (assumed) [15]	RT-PCR [12]	36	23
OATP1B3 ( <i>SLCO1B3</i> )	1 [4]	1.54 [15]	Array [10]	36	23

Table S1.2: System-dependent parameters and expression of relevant enzymes, transporters and other ADME processes (*continued*)

Enzyme / Transporter / Process (Gene)	Mean reference concentration <sup>a</sup>	Geometric standard deviation of the reference concentration <sup>b</sup>	Relative expression in the different organs	Half-life liver [hours]	Half-life intestine [hours]
P-gp ( <i>ABCB1</i> )	1.41 [16]	1.6 [15]	RT-PCR [12], with the relative expression in intestinal mucosa increased by factor 3.57 [16]	36	23
Unspecific liver influx (gemfibrozil)	1	1.4	Liver only	36	23
Unspecific liver influx (simastatin acid)	1	1.4	Liver only	36	23

<sup>a</sup>  $\mu\text{mol protein}^{-1}$  in the tissue of the highest expression. If no information on reference concentration was available it was set to  $1 \mu\text{mol protein}^{-1}$  and the catalytic rate constant ( $k_{cat}$ ) was optimized

<sup>b</sup> PK-Sim expression database profile

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In all individuals and populations, the enterohepatic circulation (EHC) was enabled (EHC continuous fraction set to one) by assuming a continuous flow from bile to duodenum. Furthermore, if no information about the renal state was specified a glomerular filtration rate of one was assumed which is equivalent to passive filtration without active secretion or reabsorption.

## 1.4 Model evaluation

For all observed concentration-time profiles population simulations using the virtual populations as described in Chapter 1.5 of the ESM for model evaluation were generated. The predicted profiles were subsequently used for the statistical as well as the graphical model evaluation. For this purpose, simulated median and 90 % confidence interval (CI) were calculated. Following, non-compartmental analysis (NCA) parameters like the area under the curve from first to last observation (AUC) and  $C_{\max}$  were calculated and used for further model evaluation.

### 1.4.1 Statistical model evaluation

For statistical model evaluation the following accuracy measures were calculated. For all concentration-time values available the mean relative deviation (MRD) as well as the median symmetric accuracy (MSA) according to Eq. (S1.3) and (S1.4) were calculated.

Equation : Mean relative deviation

$$x = \sqrt{\frac{\sum_{i=1}^N (\log_{10} c_{obs} - \log_{10} c_{pred})^2}{N}}$$

$$MRD = 10^x \quad (S1.3)$$

$C_{obs}$  = observed Concentration  
 $C_{pred}$  = predicted concentration  
 $N$  = number of observations  
 MRD = mean relative deviation

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Equation : Median symmetric accuracy

$$Q_i = \frac{C_{pred}}{C_{obs}}$$

$$\zeta = 100 * (\exp(\text{Mdn}(|\ln(Q_i)|)) - 1) \quad (\text{S1.4})$$

$C_{obs}$  = observed Concentration  
 $C_{pred}$  = predicted concentration  
 $Q_i$  = accuracy ratio  
 $\zeta$  = median symmetric accuracy

Hereby a MRD value  $\leq 2$  characterizes an adequate prediction whereas the MSA gives a easy interpretable impression of the relative deviation of the model predictions compared to the observed values. Additionally, NCA ratios (Eq. (S1.5)) were calculated and compared.

Equation : Non-compartmental analysis ratios

$$NCA_{ratio} = \frac{NCA_{pred}}{NCA_{obs}} \quad (\text{S1.5})$$

$NCA_{ratio}$  = predicted versus observed NCA estimate ratio  
 $NCA_{pred}$  = predicted NCA estimate  
 $NCA_{obs}$  = observed NCA estimate

Values  $\leq 2$  or  $\geq 0.5$  are considered as sufficient. Furthermore, for each DDI and DGI observed and predicted NCA effect ratios were estimated as shown in Eq. (S1.6)).

Equation : Drug-drug interaction and drug-gene interaction effect ratios

$$Effect_{ratio} = \frac{NCA_{pred,DDI/DGI}}{NCA_{pred,placebo}} / \frac{NCA_{obs,DDI/DGI}}{NCA_{obs,placebo}} \quad (\text{S1.6})$$

$Effect_{ratio}$  = predicted versus observed NCA estimate effect ratio  
 $NCA_{pred,DDI/DGI}$  = predicted NCA estimate under DDI and / or DGI conditions  
 $NCA_{pred,placebo}$  = predicted NCA estimate under placebo condition  
 $NCA_{obs,DDI/DGI}$  = observed NCA estimate under DDI and / or DGI conditions  
 $NCA_{obs,placebo}$  = observed NCA Estimate Under Placebo Condition

Finally, as a quantitative measure of the prediction accuracy for each DDI and DGI interaction as well as for all placebo concentration-time profiles the geometric mean fold error (GMFE) was calculated according to Eq. (S1.7):

## 1 General information

Equation : Geometric mean fold error

$$x = \frac{\sum |\log_{10}(\frac{NCA, value_{pred}}{NCA, value_{obs}})|}{N}$$

$$GMFE = 10^x \quad (S1.7)$$

N = number of observations  
 NCA<sub>pred</sub> = predicted NCA estimate  
 NCA<sub>obs</sub> = observed NCA estimate

**1.4.2 Graphical model evaluation**

For graphical model evaluation a set of different figures were generated. For all concentration-time profiles visual predictive checks (VPCs) were created using the virtual populations for model evaluation as described in Chapter 1.5 of the ESM and the subsequently calculated median and 90 % CI profiles. Furthermore, goodness of fit (GOF) plots like observed values versus predicted values were generated. Moreover, predicted versus observed NCA ratios as well as DDI and DGI effect ratios, as calculated in Chapter 1.4.1 of the ESM, were evaluated using the twofold limits, half-fold limits and / or the limits proposed by Guest et al [17].

**1.4.3 Local sensitivity analysis**

NCA parameter sensitivities of the final PBPK models were calculated as relative changes of the AUC of one dosing interval in steady-state conditions for simulations of the highest recommended doses. For this purpose, a mean individual as described in Chapter 1.3 of the ESM was used. Parameters were included into the analysis if they had to be optimized, if they might have a strong influence due to calculation methods used in the model (fraction unbound) or if they had significant impact in former models (solubility).

The sensitivity for the NCA parameter on the input parameter of interest was then calculated as the ratio of the relative change of the NCA parameter and the relative variation of the input parameter (see Eq. (S1.8)). For reasons of numerical stability, sensitivities were calculated as the average of several sensitivities based on different variations (see Eq. (S1.8)). The relative variations are defined by multiplication of the value in the simulation with variation factors (k). For each sensitivity analysis 9 ks were taken. The average of the sensitivities were following visualized as a tornado plot [18].

## 1 General information

Equation : Parameter sensitivity

$$S_{i,j} = \frac{\Delta PK_j}{\Delta p_i} * \frac{p_i}{PK_j} \quad (S1.8)$$

$$S_{i,j} = \frac{\sum_{k=1}^n \frac{\Delta_k PK_j}{\Delta_k p_i} * \frac{p_i}{PK_j}}{n} \quad (S1.8)$$

- PK<sub>j</sub> = pharmacokinetic parameter of a certain output  
 p<sub>i</sub> = input parameter  
 S<sub>i,j</sub> = sensitivity of a pharmacokinetic parameter of a certain output to an input parameter  
 k = variation factors

## 1.5 Virtual populations

For each profile with individual demographics available a virtual population containing 100 individuals with demographic properties (gender, age, weight, height, race) adapted to the mean of each respective study was created based on the International Commission on Radiological Protection (ICRP) database. If data were missing mean values as described in Chapter 1.3 of the ESM were used. Enzyme variability was removed except for protein ontogeny information if available. Subsequently, profiles were simulated and population mean and 90% CI were calculated.

## 2 PBPK modeling of simvastatin

### 2.1 Introduction

Simvastatin is an oral HMG-CoA reductase inhibitor and is among the ten most prescribed drugs in industrial nations [19]. Although statins and especially simvastatin have an excellent cost effectiveness and benefit risk ratio [20, 21] over-dosage can lead to rhabdomyolysis which is a feared and a potentially deadly adverse-drug event [22]. DDIs and DGIs are well known triggers leading to changed simvastatin PK and subsequently raise the risk of over-dosages [23, 24, 25, 26]. This is because simvastatin has a complex PK with high inter-individual variability, which involves many different drug transporters and metabolic enzymes. Hence, if DDIs or DGIs alter transporter or enzyme activity, simvastatin's PK can change dramatically. Simvastatin is given in its oral prodrug form simvastatin lactone (SL). SL is a highly lipophilic class II biopharmaceutical classification system drug with a high permeability but low solubility [27]. After disintegration, it is hydrolyzed partly chemical in a pH dependent manner [28] and mostly enzymatically by paraoxonase 3 (PON3) to its active form simvastatin acid (SA) [29]. Apart from organic anion transporting polypeptide 1B1 (OATP1B1) for all metabolic enzymes and transporters involved in simvastatin's PK polymorphisms with altered activity are reported [26]. Moreover, several inhibitors or inducer, so called perpetrator drugs, for either one or multiple of the above mentioned transporters and enzymes are known [30], changing the PK of simvastatin as a victim drug. Additionally, on top of this SL as well as SA itself show in vitro perpetrator drug effects for a broad range of enzymes and transporters [31, 32, 33, 34, 35, 36, 37, 38].

#### 2.1.1 Included processes

An overview of the processes included in the final whole-body PBPK model is given in Fig. S2.1.

In the final model metabolism of SL (Fig. S2.1 process [1]) and SA (Fig. S2.1 process [2]) by cytochrome P450 3A4 (CYP3A4) as well as SA metabolism by cytochrome P450 2C8 (CYP2C8) (Fig. S2.1 process [14]) and SL metabolism by cytochrome P450 3A5 (CYP3A5) (Fig. S2.1 process [13]) were included. In addition, the transformation of SL to SA was realized by inclusion of SL PON3 metabolism (Fig. S2.1 process [5]) as well as SL chemical hydrolysis (Fig. S2.1 process [3]) and plasma hydrolysis (Fig. S2.1 process [4]). Moreover, the back reaction (SA lactonisation) mediated by acyl glucuronide intermediates (enzymatically

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(Fig. S2.1 process [7]) or spontaneously (chemically (Fig. S2.1 process [6])) was included. Furthermore, SL transportation by breast cancer resistance protein (BCRP) (Fig. S2.1 process [12]) was incorporated. For this, the predefined reverse transcription polymerase chain reaction (RT-PCR) BCRP expression profile was adapted to express BCRP also in red blood cells which reduced the blood to plasma ratio SL from values  $>3$  without BCRP expression to values  $<1$  with BCRP expression. Literature values for  $BP_{SL}$  are around 0.57[39]. For description of SA distribution, the relevant transport processes of SA by OATP1B1 (Fig. S2.1 process [8]), organic anion transporting polypeptide 1B3 (OATP1B3) (Fig. S2.1 process [9]) and P-glycoprotein (P-gp) (Fig. S2.1 process [11]) were implemented. To cover the PK for different oral formulations SL dissolution was described using a Weibull function whereas dissolution time of 50% dissolution and dissolution shape were optimized. Genotypes which were included and covered by the model were *solute carrier organic anion transporter family member 1B1 (SLCO1B1)* (rs4149056) c.521T/T, c.521C/C, and c.521T/C *ATP-binding cassette sub-family B member 1 (ABCB1)* (rs1128503, rs2032582 and rs1045642) c.1236T-c.2677T-c.3435T and c.1236C-c.2677G-c.3435C, *ATP-binding cassette sub-family G member 2 (ABCG2)* (rs2231142) c.421C/C, c.421C/A and c.421A/A and *CYP3A5* (rs776746) *CYP3A5\*3/\*3*, *CYP3A5\*3/\*1* and *CYP3A5\*3/\*1*.

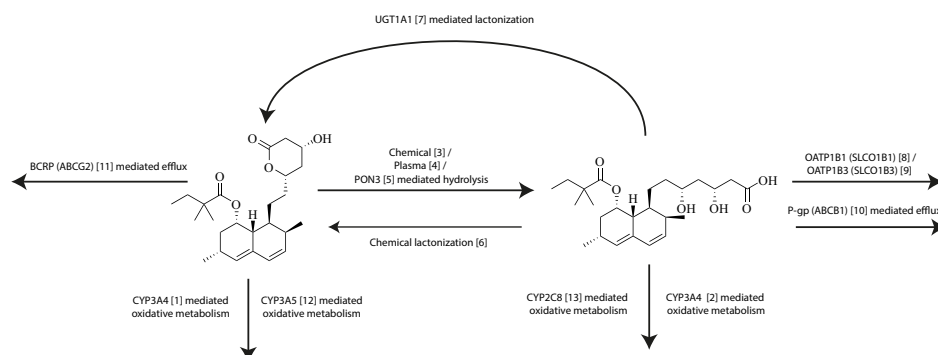


Figure S2.1: Included processes in the final whole-body PBPK model

### 2.1.2 Excluded processes

Few processes were excluded due to either lack of training data or ambiguous literature findings. Namely, no SL or SA multidrug resistance-associated protein 2 (MRP2) transportation was included. Although, there is some evidence that MRP2 and especially polymorphisms in the *ATP-binding cassette sub-family C member 2 (ABCC2)* gene are of relevance for simvastatin information available were too sparse to distinguish between other efflux processes [40, 36]. For the same reason and again because of ambiguous study information no P-gp efflux of SL was included [41, 42, 43]. The same applies to potential relevance of further influx transporters.

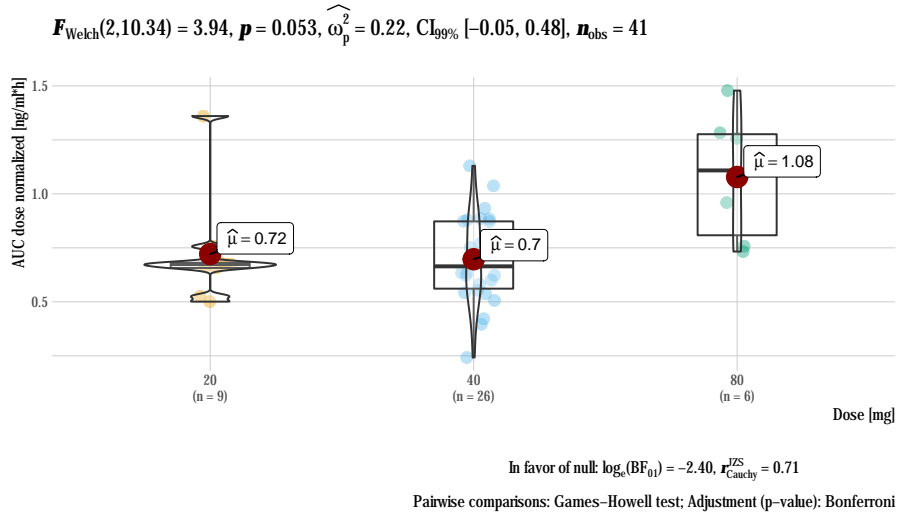


## 2.2 Simvastatin model development

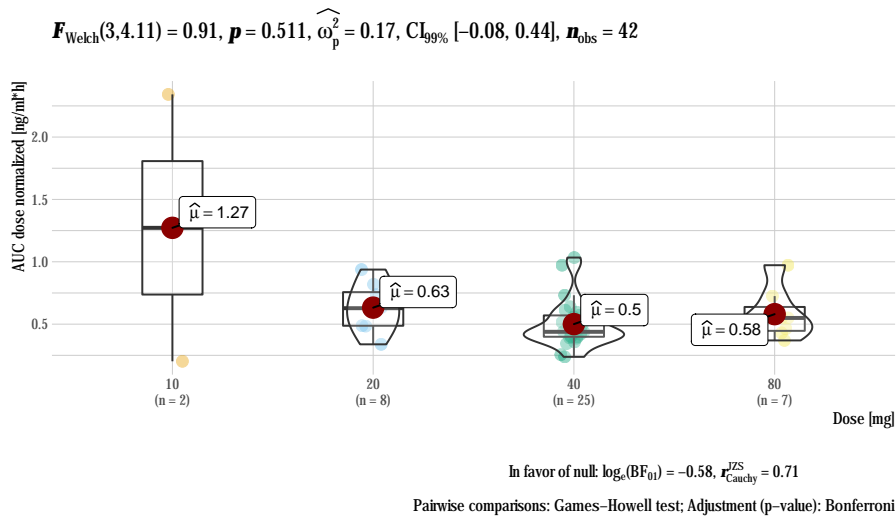
### 2.2.1 Clinical studies

For placebo and DGI model development and evaluation mean profiles from 57 studies were extracted including 59 SL and 57 SA pharmacokinetic profiles which represent information from in total 1271 study participants. An overview of all mean study demographics available can be found in Table S2.1. Doses available ranged from 10 mg to 80 mg after single and multiple doses. Dose linearity was found for SA and likely also for SL as shown in Fig. S2.2, S2.3 based on analysis of variance (ANOVA) analysis and pairwise comparison of dose normalized AUC and  $C_{\max}$  values from placebo single dose profiles.

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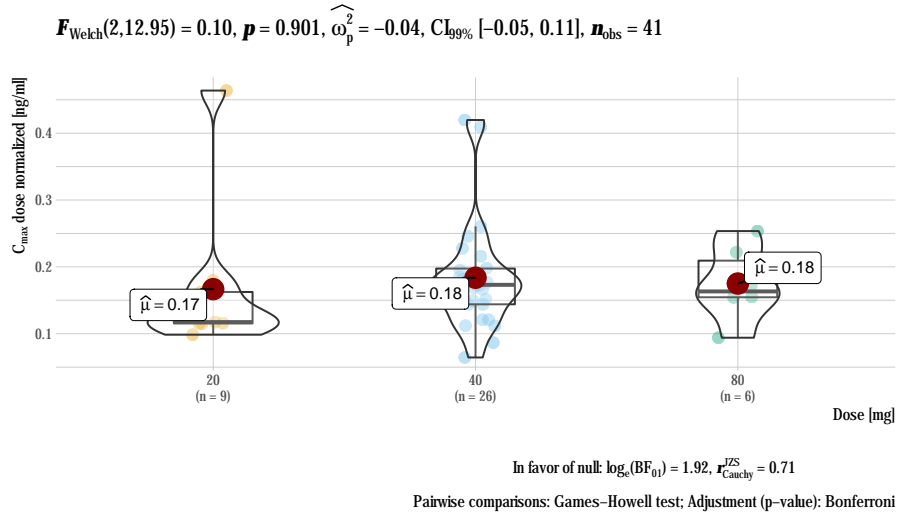
(1) Parameter: AUC Simvastatin Lactone



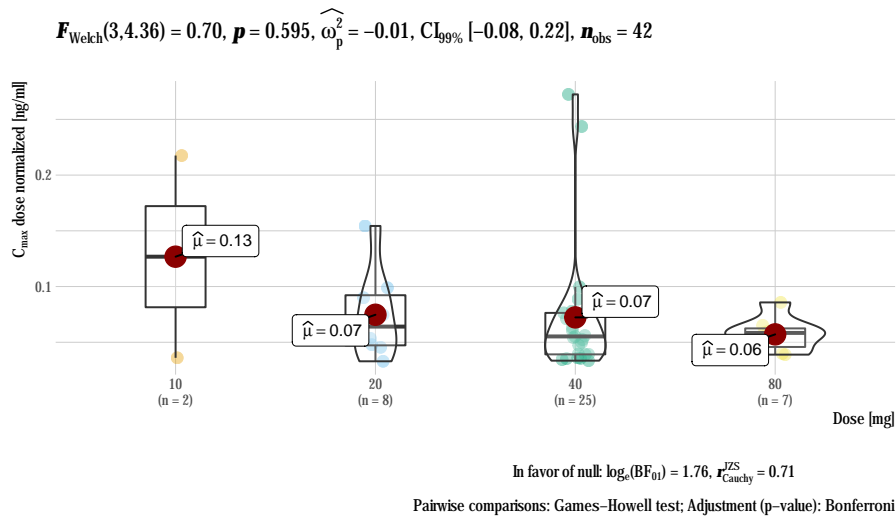
(2) Parameter: AUC Simvastatin Acid

Figure S2.2: Dose normalized AUC and  $C_{max}$  values: AUC Simvastatin Lactone, AUC Simvastatin Acid

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(1) Parameter:  $C_{max}$  Simvastatin Lactone



(2) Parameter:  $C_{max}$  Simvastatin Acid

Figure S2.3: Dose normalized AUC and  $C_{max}$  values:  $C_{max}$  Simvastatin Lactone,  $C_{max}$  Simvastatin Acid

Table S2.1: Mean study data used for simvastatin lactone and simvastatin acid placebo model development

Route	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
10 mg SL p.o. (Lipovas, fasted) s.d.	16	0	33 (26–44)	64 (56–75)	-	Test	77, 78	[44]
20 mg SL p.o. (Unknown, fasted) s.d.	12	25	31 (21–40)	-	-	Test	182, 183	[45]
20 mg SL p.o. (Unknown, fasted) s.d.	11	36	33	-	-	Test	196	[46]
20 mg SL p.o. (Unknown, fasted) s.d.	40	0	25	64	-	Training	318, 319	[47]
20 mg SL p.o. (Unknown, fasted) s.d.	40	0	32	66	-	Test	320, 321	[47]
20 mg SL p.o. (Unknown, fasted) s.d.	40	0	24	68	-	Test	322, 323	[47]
20 mg SL p.o. (Unknown, fasted) s.d.	40	0	26	78	-	Training	324, 325	[47]
20 mg SL p.o. (Zocor, fed) daily	31	19	38	72	-	Test	12	[48]
20 mg SL p.o. (Unknown, fasted) s.d.	10	50	-	-	-	Test	234	[49]
20 mg SL p.o. (Zocor, fasted) b.i.d.	11	0	(20–35)	-	-	Test	250, 251	[50]
20 mg SL p.o. (Zocor, fasted) s.d.	7	14	30 (26–42)	77 (70–84)	-	Training	91, 92	[51]
20 mg SL p.o. (Unknown, fasted) s.d.	15	-	-	-	-	Test	139, 144	[52]
40 mg SL p.o. (Unknown, fasted) s.d.	9	0	31 (22–49)	68	168	Test	1	[53]
40 mg SL p.o. (Unknown, fasted) s.d.	25	8	34 (22–45)	74	-	Test	180, 181	[54]
40 mg SL p.o. (Zocor, Unknown) daily	14	0	23	66	172	Test	192, 193	[55]
40 mg SL p.o. (Unknown, Unknown) daily	24	0	42	83	-	Test	190	[56]
40 mg SL p.o. (Zocor, fasted) s.d.	22	23	32	83	-	Training	197, 198	[57]
40 mg SL p.o. (Zocor, fasted) s.d.	5	60	(22–26)	-	-	Test	6, 9	[58]

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Table S2.1: Mean study data used for simvastatin lactone and simvastatin acid placebo model development (*continued*)

Route	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
40 mg SL p.o. (Zocor, fasted) s.d.	22	0	35 (21–60)	-	-	Test	223, 224	[59]
40 mg SL p.o. (Zocor, Unknown) s.d.	14	0	36 (23–43)	-	-	Test	209, 210	[60]
40 mg SL p.o. (Zocor, fasted) s.d.	14	0	37 (21–45)	-	-	Test	219, 220	[61]
40 mg SL p.o. (Zocor, fasted) s.d.	12	33	(19–)	-	-	Test	236, 237	[62]
40 mg SL p.o. (Zocor, fasted) s.d.	10	40	24	68	-	Test	30, 31	[63]
40 mg SL p.o. (Unknown, fasted) s.d.	23	4	32 (21–43)	-	-	Test	277, 278	[64]
40 mg SL p.o. (Zocor, fed) daily	12	-	-	-	-	Test	289, 290	[65]
40 mg SL p.o. (Unknown, fasted) s.d.	35	34	(18–55)	-	-	Test	101, 102	[66]
40 mg SL p.o. (Zocor, Unknown) s.d.	10	10	(20–24)	(58–79)	-	Test	53, 52	[67]
40 mg SL p.o. (Zocor, fasted) s.d.	10	0	(20–34)	(63–80)	-	Test	54, 55	[68]
40 mg SL p.o. (Unknown, Unknown) s.d.	21	0	33	81	-	Test	272	[69]
40 mg SL p.o. (Denan, fed) s.d.	20	50	50	(53–111)	(158–192)	Test	135, 137	[70]
40 mg SL p.o. (Unknown, fasted) daily	52	23	38 (19–55)	75 (55–100)	169 (149–190)	Test	316, 317	[71]
40 mg SL p.o. (Zocor, Unknown) daily	24	0	30 (19–44)	84 (59–114)	178 (164–190)	Test	270	[72]
40 mg SL p.o. (Zocor, fasted) s.d.	28	29	39 (21–63)	73 (55–97)	170 (146–184)	Training	76, 75	[73]
40 mg SL p.o. (Zocor, fasted) s.d.	12	67	56 (28–72)	88 (63–111)	-	Test	264, 265	[74]
40 mg SL p.o. (Zocor, fasted) daily	18	28	29 (21–43)	75 (52–93)	175 (152–193)	Training	260, 261	[75]
40 mg SL p.o. (Unknown, Unknown) s.d.	16	19	36	75	-	Test	205, 206	[76]

Table S2.1: Mean study data used for simvastatin lactone and simvastatin acid placebo model development (*continued*)

Route	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
40 mg SL p.o. (Unknown, fasted) daily	85	40	39 (21–55)	72 (51–102)	174 (154–196)	Training	174, 177	[77]
40 mg SL p.o. (Unknown, fasted) s.d.	85	40	39 (21–55)	72 (51–102)	174 (154–196)	Training	168, 171	[77]
40 mg SL p.o. (Unknown, fasted) s.d.	20	-	-	-	-	Test	100	[78]
40 mg SL p.o. (Unknown, fasted) s.d.	10	0	39 (18–63)	-	-	Test	246, 247	[79]
40 mg SL p.o. (Unknown, fasted) daily	18	39	29 (19–40)	67 (54–80)	-	Test	281	[80]
60 mg SL p.o. (Zocor, fasted) s.d.	10	50	(18–30)	(55–101)	-	Test	43, 42	[81]
80 mg SL p.o. (Unknown, fasted) daily	24	25	31 (20–45)	74 (49–88)	175 (153–190)	Training	127, 128	[82]
80 mg SL p.o. (Zocor, fed) daily	12	25	-	-	-	Test	186, 187	[83]
80 mg SL p.o. (Zocor, fasted) s.d.	24	67	31 (20–45)	-	170 (152–189)	Training	201, 202	[84]
80 mg SL p.o. (Unknown, fasted) s.d.	30	57	56 (26–74)	86 (56–120)	164 (151–187)	Test	227	[85]
80 mg SL p.o. (Unknown, fasted) daily	24	17	30 (19–47)	68 (49–84)	-	Test	166	[86]
80 mg SL p.o. (Zocor, fasted) s.d.	24	25	32 (18–45)	(50–)	-	Training	131, 132	[87]
80 mg SL p.o. (Zocor, fasted) s.d.	58	7	41 (20–60)	74 (51–92)	173 (156–194)	Training	230, 231	[88]
80 mg SL p.o. (Zocor, fasted) s.d.	12	0	(17–31)	76 (66–93)	-	Training	81, 82	[89]
80 mg SL p.o. (Unknown, fasted) s.d.	36	50	24	69	176	Test	85, 85, 86, 86	[90]
80 mg SL p.o. (Unknown, fed) s.d.	29	52	32 (20–59)	68 (48–101)	170 (153–185)	Test	274, 275	[91]

*Note:*

Values for age, weight and height are given as mean (range); Wild-type genotype was assumed; -, not given; b.i.d., twice daily; n, number of individuals studied; po, oral; s.d., single dose

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Table S2.2: Mean study data used for simvastatin lactone and simvastatin acid DGI model development

Route	Genotype	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
10 mg SL p.o. (Unknown, fasted) s.d.	c.521C/C	2	50	14	61	157	Test	338	[92]
10 mg SL p.o. (Unknown, fasted) s.d.	c.521T/C	15	53	14	78	160	Test	337	[92]
10 mg SL p.o. (Unknown, fasted) s.d.	c.521T/T	15	53	14	83	160	Test	336	[92]
20 mg SL p.o. (Zocor, fasted) s.d.	c.1236C- c.2677G- c.3435C	12	42	22	68	178	Training	21, 20	[41]
20 mg SL p.o. (Zocor, fasted) s.d.	c.1236T- c.2677T- c.3435T	12	42	24	66	174	Training	23, 22	[41]
20 mg SL p.o. (Zocor, fasted) s.d.	*3/*1	8	-	25	68	172	Test	98	[93]
20 mg SL p.o. (Zocor, fasted) s.d.	*3/*3	10	-	25	71	173	Test	99	[93]
20 mg SL p.o. (Zocor, fasted) s.d.	*1/*1	4	-	25	69	172	Training	97	[93]
40 mg SL p.o. (Zocor, fasted) s.d.	c.421C/A	4	25	27	73	176	Test	28, 25	[94]
40 mg SL p.o. (Zocor, fasted) s.d.	c.421C/C	23	52	22	68	174	Test	29, 26	[94]
40 mg SL p.o. (Zocor, fasted) s.d.	c.421A/A	5	80	22	56	164	Training	27, 24	[94]
40 mg SL p.o. (Zocor, fasted) s.d.	c.521T/C	12	42	24	69	174	Test	72, 71	[95]
40 mg SL p.o. (Zocor, fasted) s.d.	c.521T/T	16	50	23	68	174	Test	74, 73	[95]
40 mg SL p.o. (Zocor, fasted) s.d.	c.521C/C	4	25	23	84	180	Training	70, 69	[95]
60 mg SL p.o. (Zocor,unknown) s.d.	c.521C/C	1	-	24.9	70.1	174.6	Test	- <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	c.521T/C	42	-	24.9	70.1	174.6	Test	- <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	c.521T/T	88	-	24.9	70.1	174.6	Test	- <sup>a</sup>	[96]

Table S2.2: Mean study data used for simvastatin lactone and simvastatin acid DGI model development (*continued*)

Route	Genotype	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
60 mg SL p.o. (Zocor,unknown) s.d.	*3/*1	52	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	*3/*3	71	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	*1/*1	9	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	c.421C/A	56	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	c.421C/C	64	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]
60 mg SL p.o. (Zocor,unknown) s.d.	c.421A/A	12	-	24.9	70.1	174.6	Test	-. <sup>a</sup>	[96]

*Note:*

Values for age, weight and height are given as mean; -, not given; n, number of individuals studied; po, oral; s.d., single dose

<sup>a</sup> Only SL / SA AUC and C<sub>max</sub> values were available.



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### 2.2.2 Drug-dependent parameters

Table S2.3 and S2.5 compare the drug-dependent model parameter used in the final model with median literature values and shows the relative deviation from them. Moreover, they mark parameters that were optimized. Table S2.4 and S2.6 lists all parameters that were extracted from literature with the corresponding references.

Table S2.3: Drug-dependent parameters of the final simvastatin lactone model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
BP	-	0.5445	0.57	Calculated	Blood to plasma ratio after administration of 40 mg simvastatin lactone at steady-state
fu	%	1.34	2.37 (1.09–6)	Literature	Fraction unbound plasma
Lipophilicity	-	4.68	4.595 (2.06–5.19)	Literature	Lipophilicity
MW	-	418.6	418.6	Literature	Molecular weight
Solubility	mg l <sup>-1</sup>	16.4	16.4 (1.4–61.94)	Literature	Solubility in FaSSIF (pH=5)
<b>Enzymes</b>					
CYP3A4 $k_{cat}$	min <sup>-1</sup>	5194	-	Optimized	CYP3A4 catalytic rate constant
CYP3A4 $K_M$	μmol l <sup>-1</sup>	21	2.55 (0.46–30)	Literature	CYP3A4 Michaelis-Menten constant
CYP3A5 $k_{cat}$ *1/*1	min <sup>-1</sup>	162300	-	Optimized	CYP3A5 catalytic rate constant for *1/*1 genotype
CYP3A5 $k_{cat}$ *1/*3	min <sup>-1</sup>	81140	-	Calculated	CYP3A5 catalytic rate constant for *1/*3 genotype
CYP3A5 $k_{cat}$ *3/*3	min <sup>-1</sup>	0	-	Literature	CYP3A5 catalytic rate constant for *3/*3 genotype
CYP3A5 $K_M$	μmol l <sup>-1</sup>	39.08	88 (62–91)	Optimized	CYP3A5 Michaelis-Menten constant
PON3 $K_M$	μmol l <sup>-1</sup>	840	840	Literature	PON3 Michaelis-Menten constant
PON3 $k_{cat}$	min <sup>-1</sup>	4952	-	Optimized	PON3 catalytic rate constant
<b>Transporters</b>					
BCRP ( <i>ABCG2</i> ) $k_{cat}$ c.421AA	min <sup>-1</sup>	7.501	-	Optimized	BCRP catalytic rate constant for c.421AA genotype

Table S2.3: Drug-dependent parameters of the final simvastatin lactone model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
BCRP ( <i>ABCG2</i> ) $k_{\text{cat}}$ c.421CA	$\text{min}^{-1}$	20.06	-	Calculated	BCRP catalytic rate constant for c.421CA genotype
BCRP ( <i>ABCG2</i> ) $k_{\text{cat}}$ c.421CC	$\text{min}^{-1}$	32.61	-	Optimized	BCRP catalytic rate constant for c.421CC genotype
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	5	-	Assumed	BCRP Michaelis-Menten constant (assumed from other statins)
<b>Inhibition</b>					
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	1.1	5.7 (1.1–12.3)	Literature	Concentration for half-maximal CYP2C8 competitive inhibition
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	0.16	2.1 (0.16–35)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
$K_i$ MRP2 ( <i>ABCC2</i> )	$\mu\text{mol l}^{-1}$	5	32.1 (5–132)	Literature	Concentration for half-maximal MRP2 competitive inhibition
$K_i$ OATP1B1 ( <i>SLCO1B1</i> )	$\mu\text{mol l}^{-1}$	5	7.85 (5–12.5)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	4.6	37.7 (4.6–209)	Literature	Concentration for half-maximal P-gp competitive inhibition
<b>Formulation</b>					
Density	$\text{g cm}^{-3}$	1.2	1.2	Literature	Drug density
Dissolution shape	-	1.297	-	Optimized	Weibull function dissolution shape
Dissolution time (50% dissolved)	min	86.38	-	Optimized	Weibull function dissolution time (50% dissolved)
<b>System</b>					

Table S2.3: Drug-dependent parameters of the final simvastatin lactone model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
BCRP blood cells	%	30.46	-	Optimized	BCRP relative expression in blood cells (normalized)
Chemical hydrolysis rate	$l \mu\text{mol}^{-1} \text{min}^{-1}$	0.00098	0.0008217 (1.667e-06–0.0196)	Literature	Chemical hydrolysis rate
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Plasma hydrolysis rate	$l \mu\text{mol}^{-1} \text{min}^{-1}$	0.0603	0.0603	Literature	Plasma hydrolysis rate
Specific intest. perm.	$\text{cm min}^{-1}$	0.001082	0.258	Optimized	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	$\text{cm min}^{-1}$	0.2561	-	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim Standard; organ-plasma partition coefficient calculation method: Berezhkovskiy; formulation parameter values were used for solid oral dosage forms only

Table S2.4: Extracted drug-dependent parameter literature values for simvastatin lactone

Parameter	Unit	Literature value	Standard deviation	Note	Reference
BP	-	0.57	-	Blood to plasma ratio	[39]
fu	%	6	-	-	[97]
fu	%	4	-	-	[98]
fu	%	1.09	0.04	Human plasma - ligand concentration: 0.5 µg/ml	[99]
fu	%	1.25	0.07	Human plasma - ligand concentration: 1 µg/ml	[99]
fu	%	1.34	0.03	Human plasma - ligand concentration: 2 µg/ml	[99]
fu	%	1.59	0.15	Human plasma - ligand concentration: 4 µg/ml	[99]
fu	%	1.77	0.16	Human plasma - ligand concentration: 6 µg/ml	[99]
fu	%	2.3	0.28	Dog plasma - ligand concentration: 0.5 µg/ml	[99]
fu	%	2.49	0.34	Dog plasma - ligand concentration: 1 µg/ml	[99]
fu	%	2.44	0.12	Dog plasma - ligand concentration: 2 µg/ml	[99]
fu	%	3.13	0.33	Dog plasma - ligand concentration: 4 µg/ml	[99]
fu	%	3.38	0.51	Dog plasma - ligand concentration: 6 µg/ml	[99]
Lipophilicity	-	4.7	-	log D pH=7.0	[98]
Lipophilicity	-	4.69	-	clogP	[100]
Lipophilicity	-	4.48	-	clogP	[100]
Lipophilicity	-	4.42	-	clogP	[100]
Lipophilicity	-	4.91	-	clogP	[100]
Lipophilicity	-	4.51	-	clogP	[100]
Lipophilicity	-	4.46	-	clogP	[100]
Lipophilicity	-	4.74	-	clogP	[100]
Lipophilicity	-	4.68	-	clogP	[100]
Lipophilicity	-	4.79	-	clogP	[100]
Lipophilicity	-	4.38	-	clogP	[100]
Lipophilicity	-	5.19	-	clogP	[100]

Table S2.4: Extracted drug-dependent parameter literature values for simvastatin lactone (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
Lipophilicity	-	2.06	-	logP at pH 7	[100]
Lipophilicity	-	4.47	-	logP at pH 2	[100]
Lipophilicity	-	4.4	-	log D pH=7.0	[101]
Lipophilicity	-	4.68	-	logP	[39]
MW	-	418.6	-	Calculated	[102]
Solubility	mg l <sup>-1</sup>	8.43	-	Intrinsic solubility calculated	[102]
Solubility	mg l <sup>-1</sup>	61.94	-	FaHIF	[58]
Solubility	mg l <sup>-1</sup>	1.4	-	pH=5	[98]
Solubility	mg l <sup>-1</sup>	30	-	Water	[103]
Solubility	mg l <sup>-1</sup>	1.45	-	Distilled water	[27]
Solubility	mg l <sup>-1</sup>	14.5	-	pH=1.2	[27]
Solubility	mg l <sup>-1</sup>	24.4	-	pH=7	[27]
Solubility	mg l <sup>-1</sup>	29.9	-	FeSSIF	[27]
Solubility	mg l <sup>-1</sup>	16.4	-	FaSSIF	[27]
Solubility	mg l <sup>-1</sup>	16.4	-	Intestine	[39]
Solubility	mg l <sup>-1</sup>	14.5	-	Stomach	[39]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	5472	-	-	[104]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	3899	-	L1	[97]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	5141	-	L2	[97]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	5800	-	L3	[97]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	889.9	-	Total clearance males	[105]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	1330	-	Total clearance females	[105]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	2870	-	-	[106]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	1697	-	-	[107]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	1.101	-	human liver microsomes corrected for fumic (0.218)	[105]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	1.144	-	human liver microsomes corrected for fumic (0.218)	[105]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.46	-	human liver microsomes corrected for fumic (0.218)	[105]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	1.485	-	human liver microsomes corrected for fumic (0.218)	[105]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	2.3	-	human liver microsomes corrected for fumic (0.1)	[106]

Table S2.4: Extracted drug-dependent parameter literature values for simvastatin lactone (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	4.27	-	human liver microsomes - 3',5'-dihydrodiol SV corrected for fumic (0.122)	[107]
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	2.55	-	human liver microsomes - 3'-hydroxy SV corrected for fumic (0.122)	[107]
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	4.416	-	human liver microsomes - 6'-exomethylene SV corrected for fumic (0.122)	[107]
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	30	6.4	Recombinant enzyme - 3',5'-dihydrodiol SV	[107]
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	7	2.9	Recombinant enzyme - 3'-hydroxy SV	[107]
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	25	0.1	Recombinant enzyme - 6'-exomethylene SV	[107]
CYP3A5 $K_M$	$\mu\text{mol l}^{-1}$	91	-	Recombinant enzyme - 3',5'-dihydrodiol SV	[107]
CYP3A5 $K_M$	$\mu\text{mol l}^{-1}$	62	-	Recombinant enzyme - 3'-hydroxy SV	[107]
CYP3A5 $K_M$	$\mu\text{mol l}^{-1}$	88	-	Recombinant enzyme - 6'-exomethylene SV	[107]
PON3 $K_M$	$\mu\text{mol l}^{-1}$	840	-	-	[108]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	2.8	-	Pravastatin (acid)	[109]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	10.1	-	Rosuvastatin (acid)	[110]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	5.73	-	Pitavastatin (acid)	[38]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	10.8	-	Rosuvastatin (acid)	[111]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	2.02	-	Rosuvastatin (acid)	[112]
BCRP ( <i>ABCG2</i> ) $K_M$	$\mu\text{mol l}^{-1}$	1.2	-	Pitavastatin (acid)	[113]
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	1.1	-	$K_i$	[114]
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	7.5	-	$K_i$	[114]
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	5.7	-	$K_i$	[114]
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	12.3	-	$K_i$	[114]
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	3.3	-	$K_i$	[114]
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	6.23	-	$K_i$	[115]
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	0.31	-	$K_i$	[116]
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	0.54	-	$K_i$	[116]
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	16.5	-	$K_i$	[116]
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	0.38	-	$K_i$	[116]

Table S2.4: Extracted drug-dependent parameter literature values for simvastatin lactone (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.16	-	K <sub>i</sub>	[116]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.81	-	K <sub>i</sub>	[116]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.37	-	K <sub>i</sub>	[116]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	35	-	K <sub>i</sub>	[116]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	30	-	K <sub>i</sub>	[117]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	2.13	0.14	K <sub>i</sub>	[31]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	2.1	0.56	K <sub>i</sub>	[118]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	10	-	K <sub>i</sub>	[107]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	56	-	IC50	[119]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	8.2	-	IC50	[36]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	5	-	K <sub>i</sub>	[36]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	132	-	IC50	[120]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	9.7	-	IC50	[35]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	5	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	12.5	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	6	-	IC50	[121]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	16.2	-	IC50	[122]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	209	-	IC50	[123]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	59.6	-	IC50	[37]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	4.9	-	IC50	[124]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	59	-	IC50	[43]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	9	-	IC50	[43]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	56	-	IC50	[43]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	8.9	-	IC50	[125]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	26.1	-	IC50	[125]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	56.8	-	IC50	[125]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	4.6	-	IC50	[126]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	49.3	-	IC50	[126]
Density	g cm <sup>-3</sup>	1.2	-	-	[39]
Chemical hydrolysis rate	min <sup>-1</sup>	2.16e-05	-	pH=5; T=60°C phosphate buffer	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.000112	-	pH=6; T=60°C phosphate buffer	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.00178	-	pH=7; T=60°C phosphate buffer	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0196	-	pH=8; T=60°C phosphate buffer	[127]



Table S2.4: Extracted drug-dependent parameter literature values for simvastatin lactone (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
Chemical hydrolysis rate	min <sup>-1</sup>	1.667e-06	-	pH=3; T=40°C phosphate puffer estimated from graph	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	6.667e-06	-	pH=5; T=40°C phosphate puffer estimated from graph	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	2.667e-05	-	pH=6; T=40°C phosphate puffer estimated from graph	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0003433	-	pH=7; T=40°C phosphate puffer estimated from graph	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.00159	-	pH=8; T=40°C phosphate puffer estimated from graph	[127]
Chemical hydrolysis rate	min <sup>-1</sup>	0.000433	0.01191	pH=6.8; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0006828	0.01343	pH=7; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0008445	0.01627	pH=7.2; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0008983	0.03727	pH=7.4; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001206	0.006918	pH=7.6; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001129	0.00979	pH=7.8; T=37°C phosphate buffer saline	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0003164	0.002644	pH=6.8; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0005424	0.004621	pH=7; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.0007989	0.003254	pH=7.2; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001105	0.0304	pH=7.4; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001261	0.006527	pH=7.6; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001342	0.003418	pH=7.8; T=37°C Human plasma	[28]
Chemical hydrolysis rate	min <sup>-1</sup>	0.001067	-	pH=7.4	[39]
Plasma hydrolysis rate	l μmol <sup>-1</sup> min <sup>-1</sup>	0.0603	-	-	[128]
Specific intest. perm.	cm min <sup>-1</sup>	0.258	-	Effective permeability calculated from apparent permeability	[39]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

Table S2.5: Drug-dependent parameters of the final simvastatin acid model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
BP	-	0.56	0.5741 (0.56–0.5882)	Calculated	Blood to plasma ratio after administration of 40 mg simvastatin lactone at steady-state (range 0.54–0.58)
fu	%	5.68	6.255 (5.48–9.61)	Literature	Fraction unbound plasma
Lipophilicity	-	1.45	3.82 (1.45–4.7)	Literature	Lipophilicity
MW	-	436.6	436.6	Literature	Molecular weight
pKa	-	4.2	4.205 (4.18–5.5)	Literature	Acid dissociation constant (acidic)
Solubility	mg l <sup>-1</sup>	13.09	45.1 (0.1263–51.5)	Literature	Solubility at pH=6.84
<b>Enzymes</b>					
CYP2C8 k <sub>cat</sub>	1/min	52.3	-	Literature	CYP2C8 catalytic rate constant (calculated from V <sub>max</sub> )
CYP2C8 K <sub>M</sub>	μmol l <sup>-1</sup>	38.55	38.55 (16–88)	Literature	CYP2C8 Michaelis-Menten constant
CYP3A4 k <sub>cat</sub>	1/min	31	-	Literature	CYP3A4 catalytic rate constant (calculated from V <sub>max</sub> )
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	26	26 (21–29)	Literature	CYP3A4 Michaelis-Menten constant
UGT1A1 k <sub>cat</sub>	1/min	6.5	-	Literature	UGT1A1 catalytic rate constant (calculated from V <sub>max</sub> )
UGT1A1 K <sub>M</sub>	μmol l <sup>-1</sup>	349	349	Literature	UGT1A1 Michaelis-Menten constant
UGT1A3 k <sub>cat</sub>	1/min	6.5	-	Literature	UGT1A3 catalytic rate constant (calculated from V <sub>max</sub> )

Table S2.5: Drug-dependent parameters of the final simvastatin acid model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
UGT1A3 $K_M$	$\mu\text{mol l}^{-1}$	349	349	Literature	UGT1A3 Michaelis-Menten constant
<b>Transporters</b>					
OATP1B1 ( <i>SLCO1B1</i> ) $k_{\text{cat}}$ c.521CC	1/min	1.025	-	Optimized	OATP1B1 catalytic rate constant for c.521CC genotype
OATP1B1 ( <i>SLCO1B1</i> ) $k_{\text{cat}}$ c.521TC	1/min	5.637	-	Calculated	OATP1B1 catalytic rate constant for c.521TC genotype
OATP1B1 ( <i>SLCO1B1</i> ) $k_{\text{cat}}$ c.521TT	1/min	10.25	-	Optimized	OATP1B1 catalytic rate constant for c.521TT genotype
OATP1B1 ( <i>SLCO1B1</i> ) $K_M$	$\mu\text{mol l}^{-1}$	2	1.99 (1.17–2.53)	Literature	OATP1B1 Michaelis-Menten constant
OATP1B3 ( <i>SLCO1B3</i> ) $k_{\text{cat}}$	1/min	2.145	-	Optimized	OATP1B3 catalytic rate constant for c.521TT genotype
OATP1B3 ( <i>SLCO1B3</i> ) $K_M$	$\mu\text{mol l}^{-1}$	2	-	Assumed	OATP1B3 Michaelis-Menten constant
P-gp ( <i>ABCB1</i> ) $k_{\text{cat}}$	1/min	50	-	Optimized	P-gp catalytic rate constant for unknown genotype
P-gp ( <i>ABCB1</i> ) $k_{\text{cat}}$ c.1236Cc.- 2677G-c.3435C	1/min	4.64	-	Optimized	P-gp catalytic rate constant for c.1236Cc.-2677G-c.3435C genotype
P-gp ( <i>ABCB1</i> ) $k_{\text{cat}}$ c.1236Tc.- 2677T-c.3435T	1/min	50	-	Optimized	P-gp catalytic rate constant for c.1236Tc.-2677T-c.3435T genotype
P-gp ( <i>ABCB1</i> ) $K_M$	$\mu\text{mol l}^{-1}$	10	-	Assumed	P-gp Michaelis-Menten constant

**Inhibition**

Table S2.5: Drug-dependent parameters of the final simvastatin acid model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
$K_i$ BCRP ( <i>ABCG2</i> )	$\mu\text{mol l}^{-1}$	18	18	Literature	Concentration for half-maximal BCRP competitive inhibition
$K_i$ CYP2C8	$\mu\text{mol l}^{-1}$	41.1	41.1	Literature	Concentration for half-maximal CYP2C8 competitive inhibition
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	69.6	56.1 (42.6–69.6)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
$K_i$ OATP1B1 ( <i>SLCO1B1</i> )	$\mu\text{mol l}^{-1}$	3.6	3.6	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Liver lactonization rate	$1 \mu\text{mol}^{-1} \text{min}^{-1}$	0.002433	-	Literature	Liver lactonization rate (calculated from liver S9 <a href="#">\cite{Tsamandouras2015}</a> )
Specific intest. perm.	$\text{cm min}^{-1}$	5.925e-07	-	Calculated	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	$\text{cm min}^{-1}$	0.0001171	-	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permeabilities calculation method: Schmitt; organ-plasma partition coefficient calculation method: Charge-dependent Schmitt normalized to PK-Sim

Table S2.6: Extracted drug-dependent parameter literature values for simvastatin acid

Parameter	Unit	Literature value	Standard deviation	Note	Reference
BP	-	0.5882	-	Blood to plasma ratio	[98]
BP	-	0.56	-	Blood to plasma ratio	[39]
fu	%	5.66	0.56	Human plasma - ligand concentration: 0.5 µg/ml	[99]
fu	%	5.92	0.22	Human plasma - ligand concentration: 1 µg/ml	[99]
fu	%	5.48	0.12	Human plasma - ligand concentration: 2 µg/ml	[99]
fu	%	5.68	0.17	Human plasma - ligand concentration: 4 µg/ml	[99]
fu	%	5.79	0.03	Human plasma - ligand concentration: 6 µg/ml	[99]
fu	%	9.61	0.7	Dog plasma - ligand concentration: 0.5 µg/ml	[99]
fu	%	8.35	0.22	Dog plasma - ligand concentration: 1 µg/ml	[99]
fu	%	6.96	0.2	Dog plasma - ligand concentration: 2 µg/ml	[99]
fu	%	6.59	0.1	Dog plasma - ligand concentration: 4 µg/ml	[99]
fu	%	7.32	0.007	Dog plasma - ligand concentration: 6 µg/ml	[99]
Lipophilicity	-	3.85	-	Calculated	[102]
Lipophilicity	-	4.22	-	Calculated	[129]
Lipophilicity	-	3.79	-	Calculated	[129]
Lipophilicity	-	1.8	-	logD pH=7	[130]
Lipophilicity	-	4.7	-	LogP	[98]
Lipophilicity	-	2.1	-	logD pH=7.0	[98]
Lipophilicity	-	1.45	-	logD pH=7.4	[39]
Lipophilicity	-	4.54	-	LogP	[39]
MW	-	436.6	-	Calculated	[129]
pKa	-	4.21	-	-	[129]
pKa	-	4.2	-	-	[130]
pKa	-	5.5	-	-	[98]
pKa	-	4.18	-	-	[131]

Table S2.6: Extracted drug-dependent parameter literature values for simvastatin acid (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
Solubility	mg l <sup>-1</sup>	51.5	-	Intrinsic solubility	[102]
Solubility	mg l <sup>-1</sup>	50	-	pH=1.7	[102]
Solubility	mg l <sup>-1</sup>	40.2	-	Calculated	[129]
Solubility	mg l <sup>-1</sup>	0.1263	0.000842	pH=6.84	[58]
CL <sub>int</sub> CYP2C8	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	9.4	-	Human liver microsomes	[132]
CL <sub>int</sub> CYP3A4	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	57	-	Human liver microsomes	[132]
CL <sub>int</sub>	μl min <sup>-1</sup> mg <sup>-1</sup> mic.protein	55	-	Human liver microsomes	[132]
CYP2C8 K <sub>M</sub>	μmol l <sup>-1</sup>	88	-	CYP2C8 - 3',5'-dihydrodiol SV	[132]
CYP2C8 K <sub>M</sub>	μmol l <sup>-1</sup>	36	-	CYP2C8 - 3'-hydroxy SV	[132]
CYP2C8 K <sub>M</sub>	μmol l <sup>-1</sup>	16	-	CYP2C8 - 6'-exomethylene SV	[132]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	21	-	CYP3A4 - 6'-exomethylene SA	[133]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	26	-	CYP3A4 - 3',5'-dihydrodiol SA	[132]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	29	-	CYP3A4 - 3'-hydroxy SA	[132]
K <sub>i</sub> CYP2C8	pmol mg <sup>-1</sup> mic.protein	41.1	-	K <sub>i</sub>	[32]
K <sub>M</sub>	μmol l <sup>-1</sup>	76	35	human liver microsomes - 3',5'-dihydrodiol SA	[132]
K <sub>M</sub>	μmol l <sup>-1</sup>	47	12	human liver microsomes - 3' - hydroxy SA	[132]
K <sub>M</sub>	μmol l <sup>-1</sup>	47	21	human liver microsomes - 6'-exomethylene SA	[132]
UGT1A1 K <sub>M</sub>	μmol l <sup>-1</sup>	349	-	Human liver microsomes corrected for fumic (0.8341)	[133]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	33.2	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	18.3	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	124	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	21.7	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	20.2	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	33.6	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	31.7	-	-	[134]
UGT1A1 protein expression	pmol mg <sup>-1</sup> mic.protein	34.3	-	-	[134]
UGT1A3 K <sub>M</sub>	μmol l <sup>-1</sup>	349	-	Human liver microsomes corrected for fumic (0.8341)	[133]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	17.3	-	-	[134]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	9.9	-	-	[134]

Table S2.6: Extracted drug-dependent parameter literature values for simvastatin acid (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	20.6	-	-	[134]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	0.4	-	-	[134]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	123.1	-	-	[134]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	8.2	-	-	[134]
UGT1A3 protein expression	pmol mg <sup>-1</sup> mic.protein	6.3	-	-	[134]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	2.09	1	1a	[135]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	1.99	1.02	2b	[135]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	1.69	2.58	5	[135]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	1.17	1.67	15	[135]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	2.53	1.38	18	[135]
OATP3A1 ( <i>SLCO3A1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	0.017	0.002	-	[136]
CYP2C8 K <sub>M</sub>	μmol l <sup>-1</sup>	41.1	-	-	[32]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	18	-	K <sub>i</sub>	[38]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	69.6	5.2	K <sub>i</sub>	[31]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	42.6	4.3	IC50	[137]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	3.6	-	IC50	[35]

*Note:*

If IC50 values could not be used for K<sub>i</sub> value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities) K<sub>i</sub> = IC50 was assumed

## 2.3 Simvastatin model evaluation

For simvastatin model evaluation various graphical and statistical evaluation techniques were used. Figures S2.4–S2.26 display the VPCs for the training and test dataset used for model development and evaluation. Figure S2.27 and S2.28 show the predicted versus observed plasma-concentration time values for the training and test dataset, respectively. Figures S2.29–S2.32 compare the calculated predicted versus observed NCA values the training and test data. In addition, Tables S2.7 and S2.8 summarize statistical quality measures like MRD, MSA and GMFE. Finally, Fig. S2.33 shows the results of the performed sensitivity analysis.

### 2.3.1 Profiles



2 PBPK modeling of simvastatin

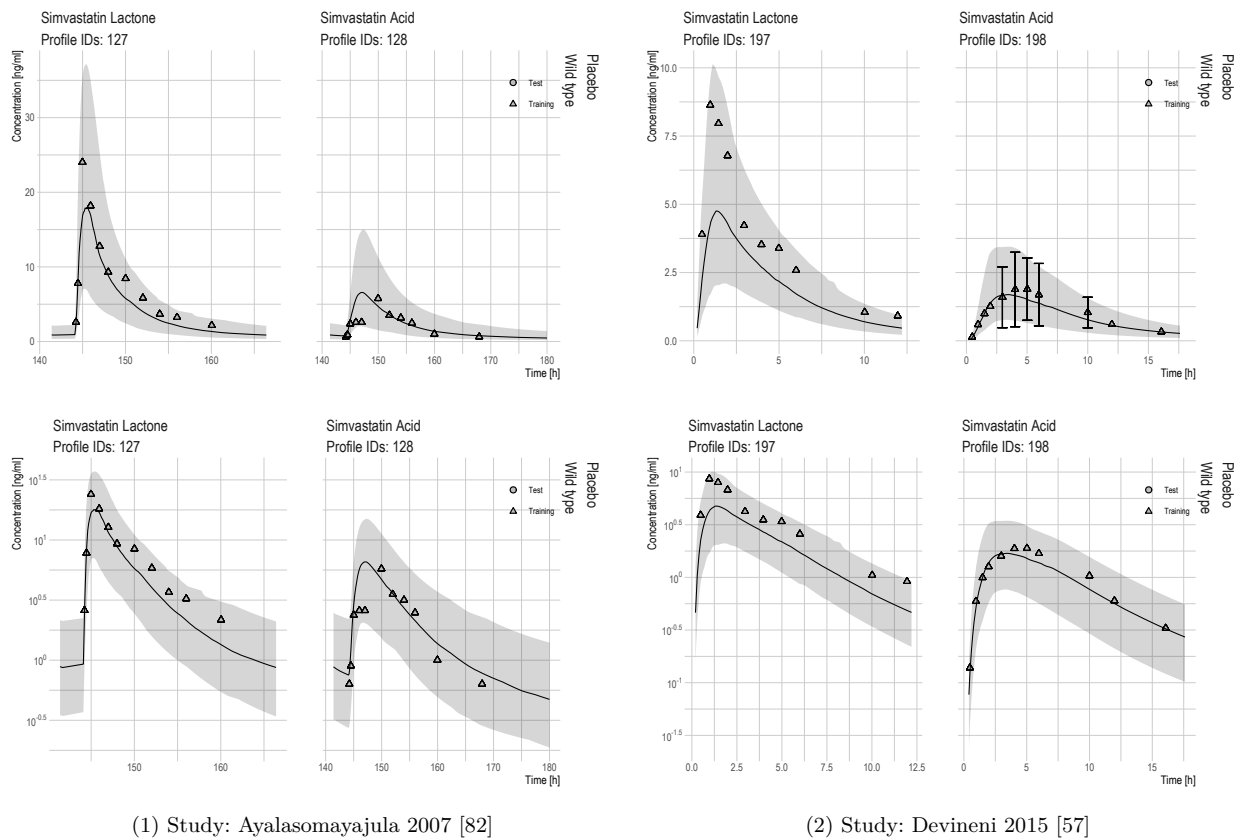


Figure S2.4: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90 % CI: Ayalasomayajula 2007 [82], Devineni 2015 [57]

2 PBPK modeling of simvastatin

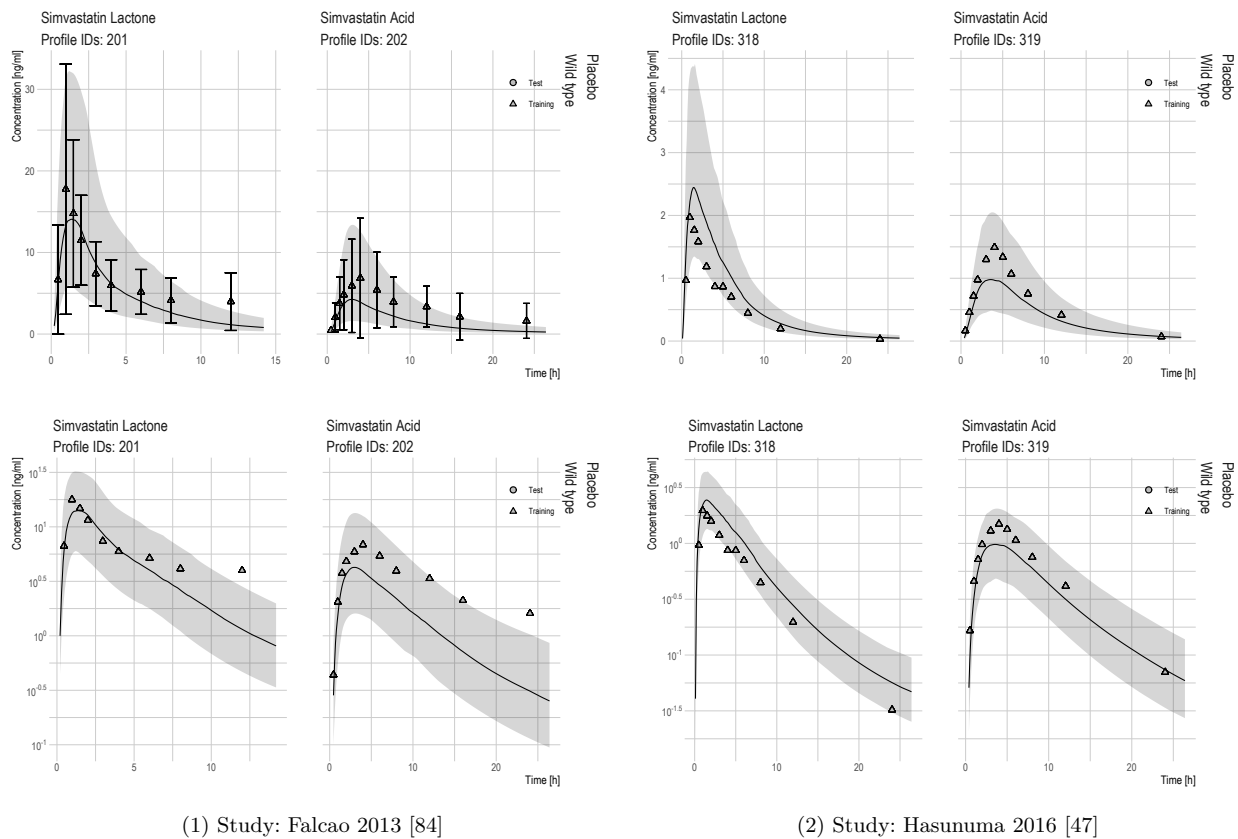


Figure S2.5: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90 % CI: Falcao 2013 [84], Hasunuma 2016 [47]

2 PBPK modeling of simvastatin

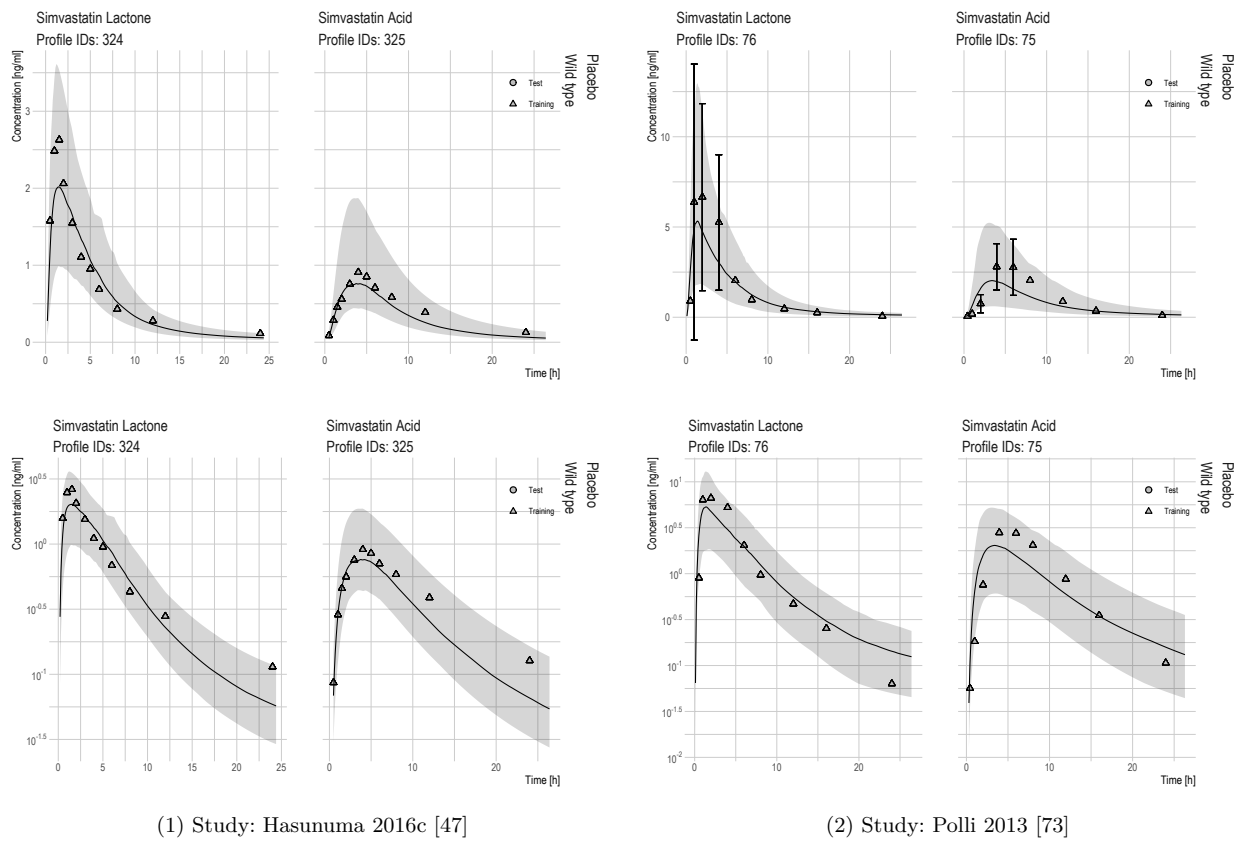


Figure S2.6: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90 % CI: Hasunuma 2016c [47], Polli 2013 [73]

2 PBPK modeling of simvastatin

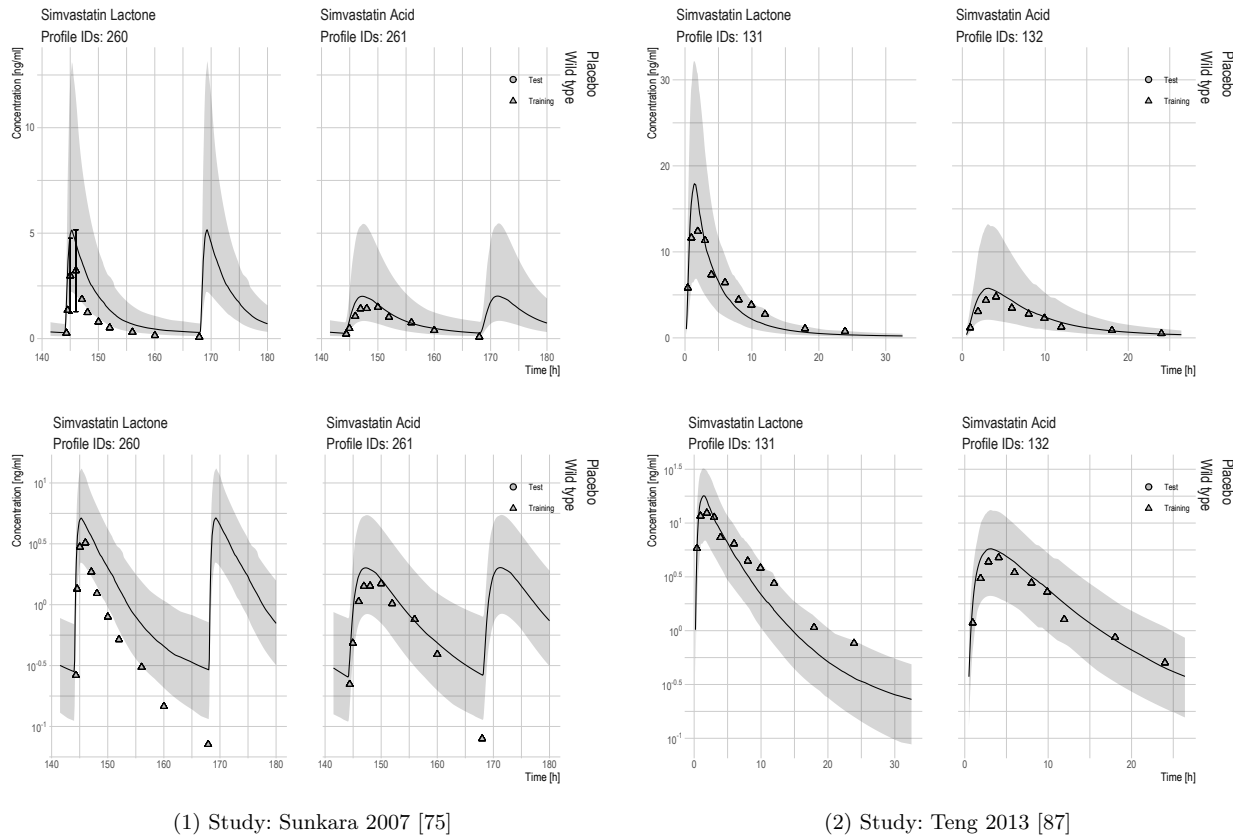


Figure S2.7: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90 % CI: Sunkara 2007 [75], Teng 2013 [87]

2 PBPK modeling of simvastatin

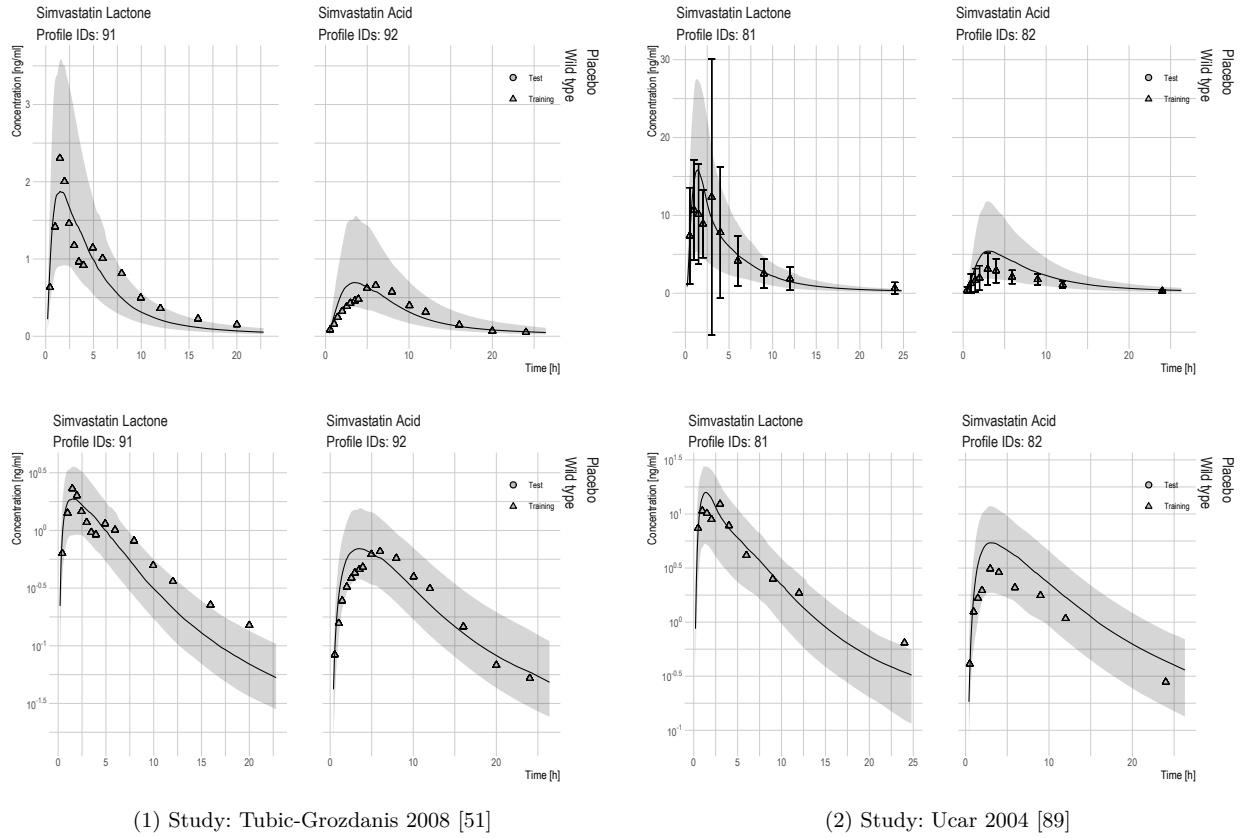


Figure S2.8: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90 % CI: Tubic-Grozdanic 2008 [51], Ucar 2004 [89]

## 2 PBPK modeling of simvastatin

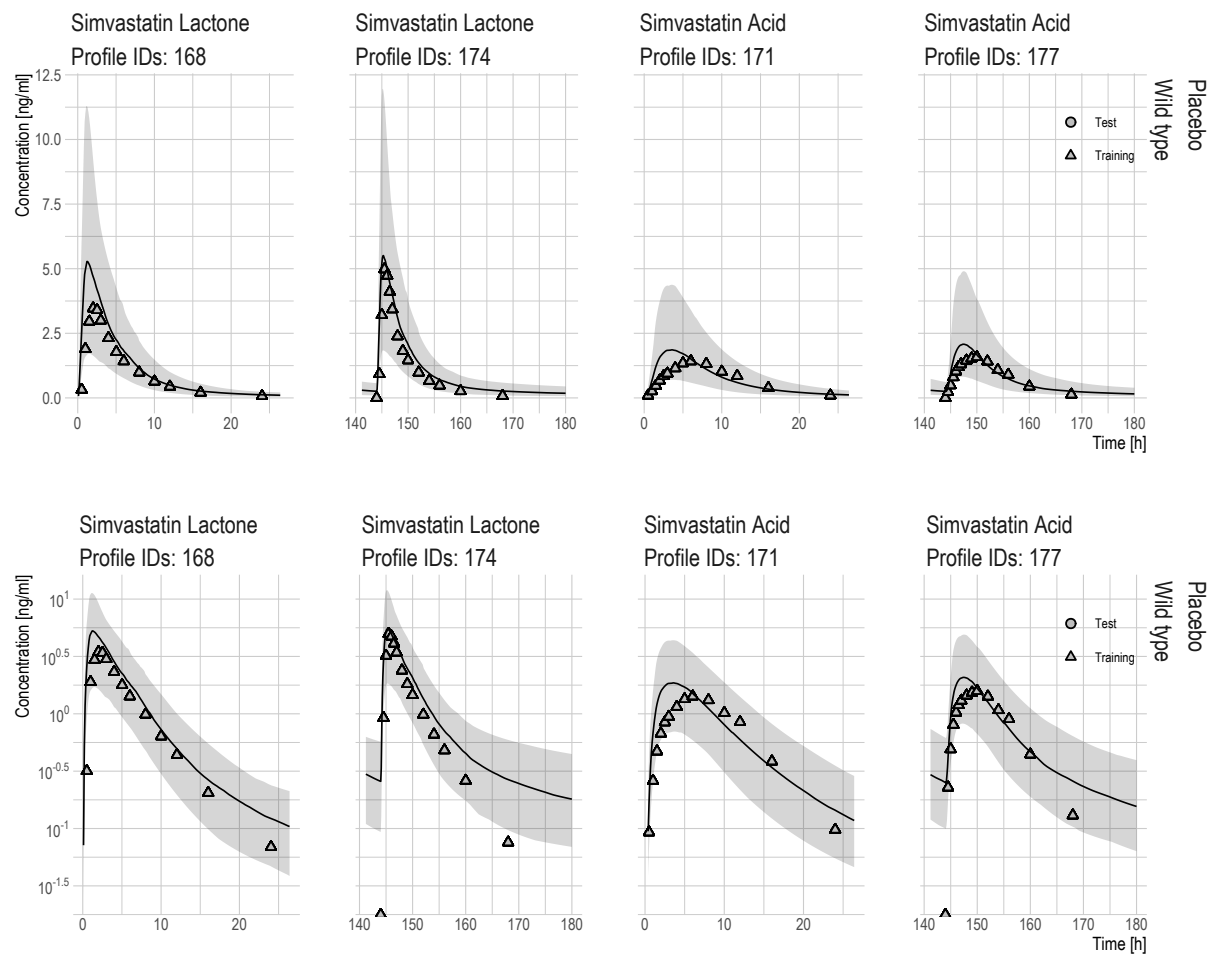


Figure S2.9: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the training dataset. Solid line and shaded area are predicted median and 90% CI: Winsemius 2014 [77]

## 2 PBPK modeling of simvastatin

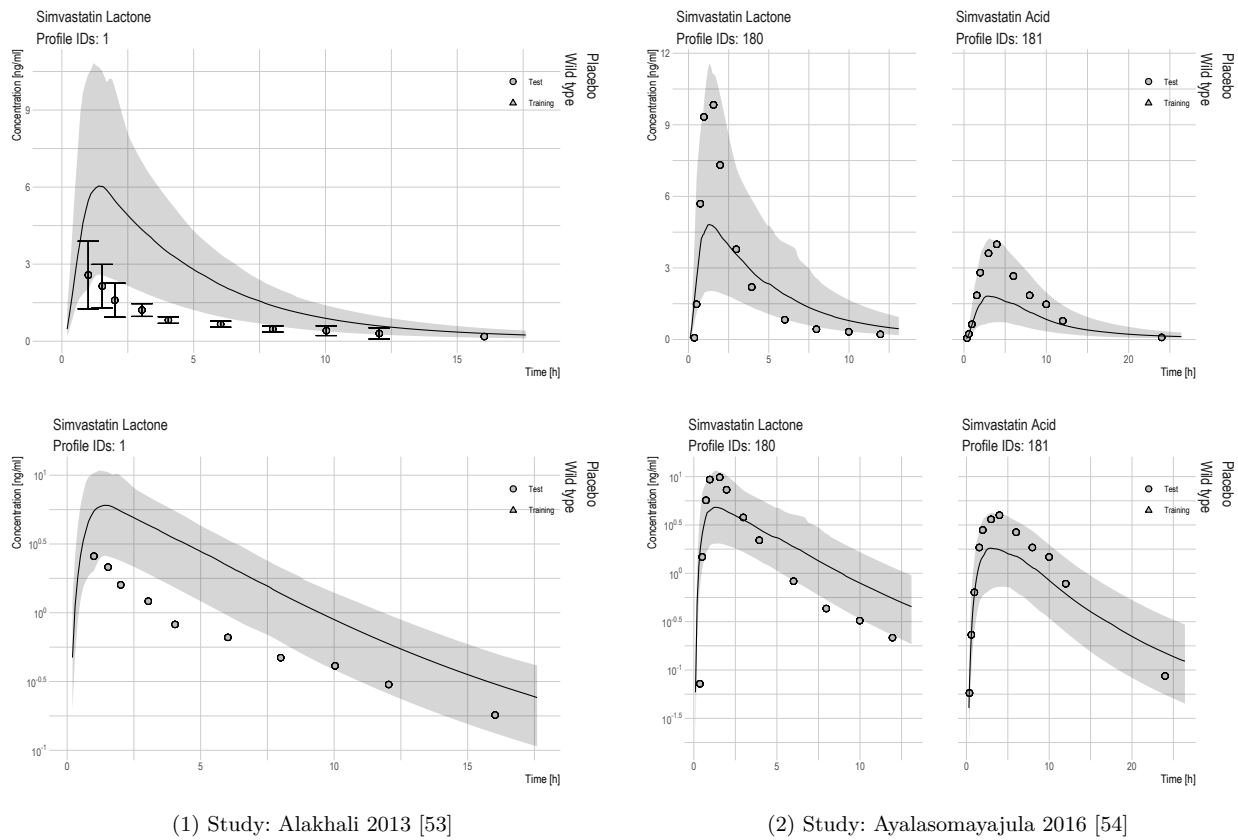


Figure S2.10: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Alakhali 2013 [53], Ayalasomayajula 2016 [54]

2 PBPK modeling of simvastatin

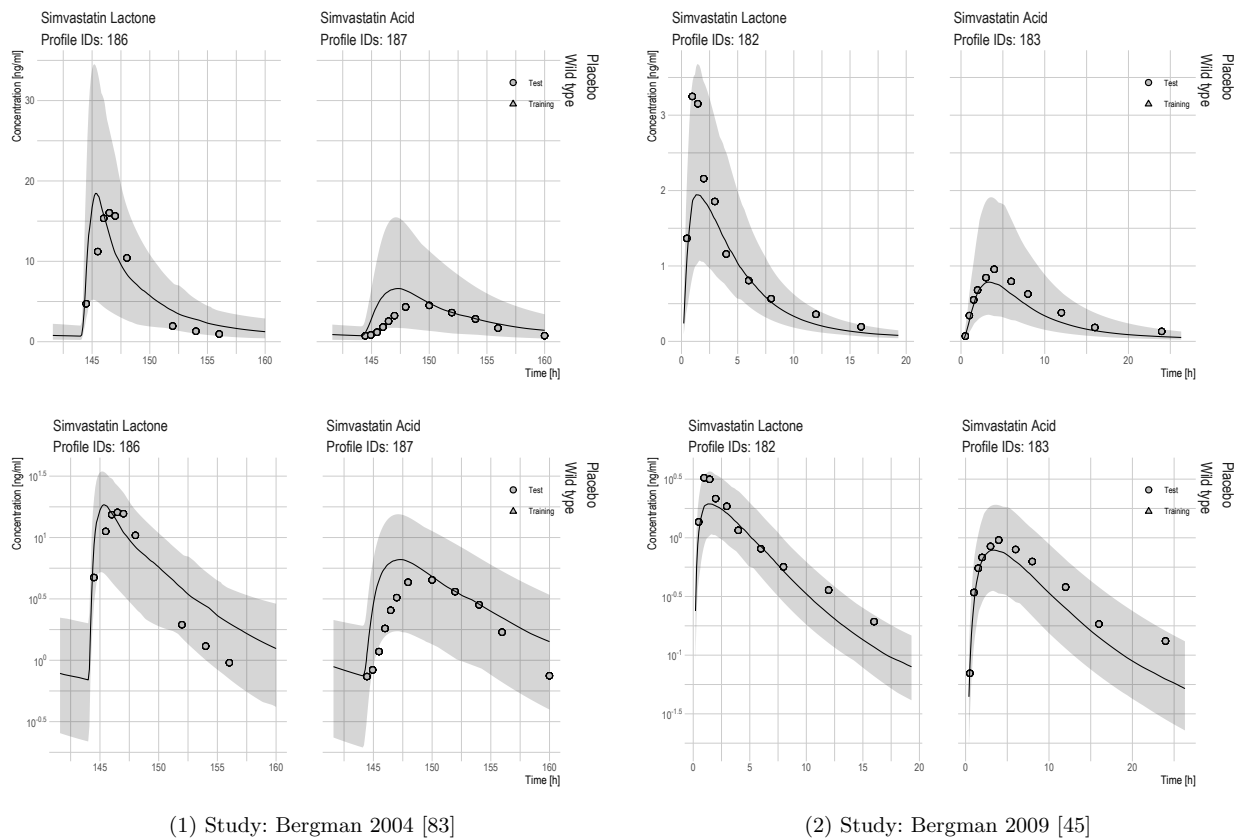


Figure S2.11: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Bergman 2004 [83], Bergman 2009 [45]



## 2 PBPK modeling of simvastatin

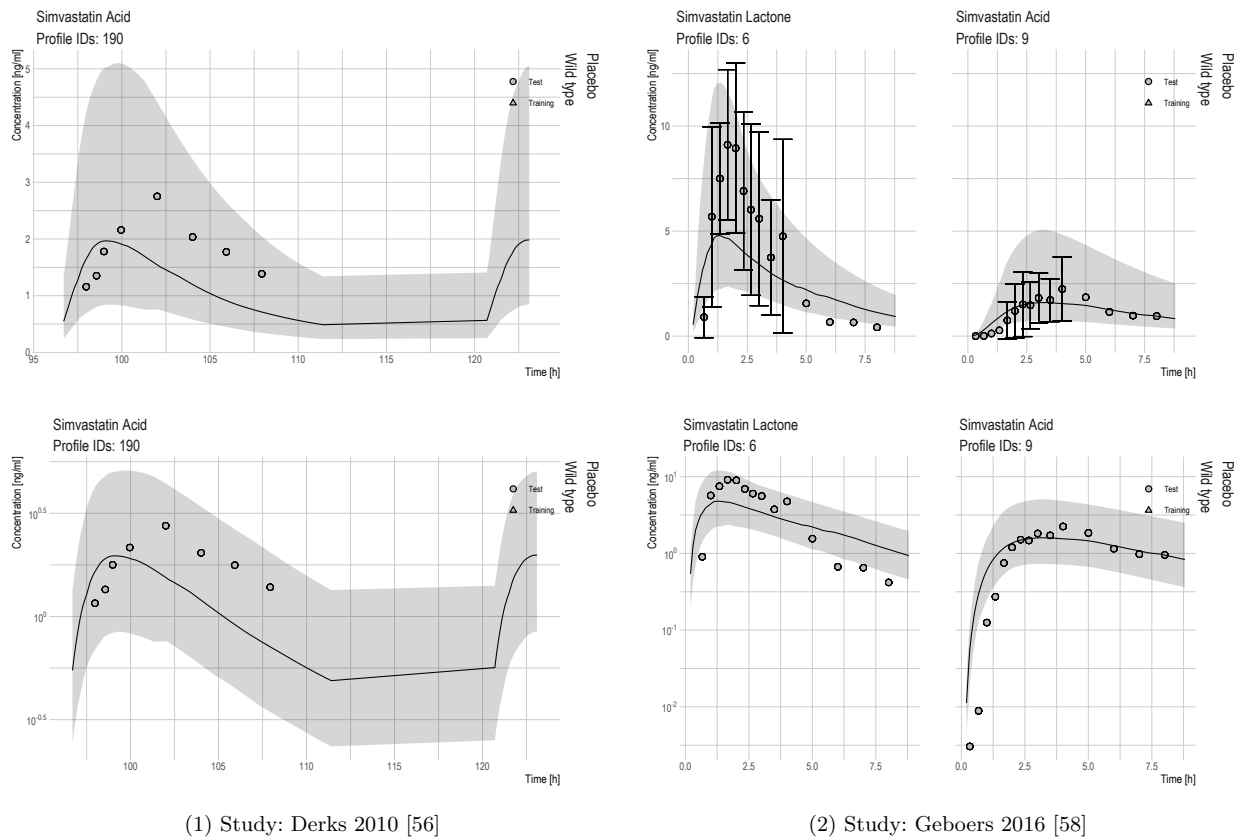


Figure S2.12: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Derks 2010 [56], Geboers 2016 [58]

2 PBPK modeling of simvastatin

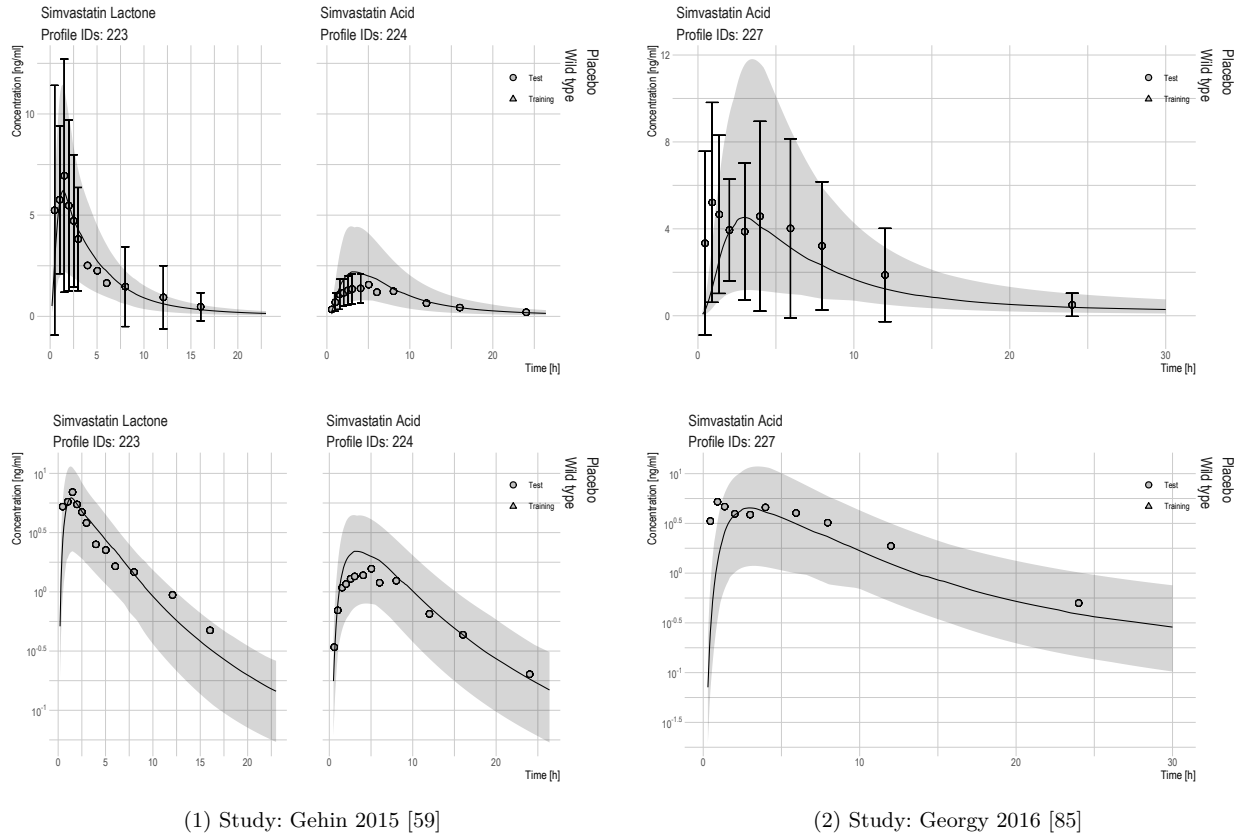


Figure S2.13: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Gehin 2015 [59], Georgy 2016 [85]

2 PBPK modeling of simvastatin

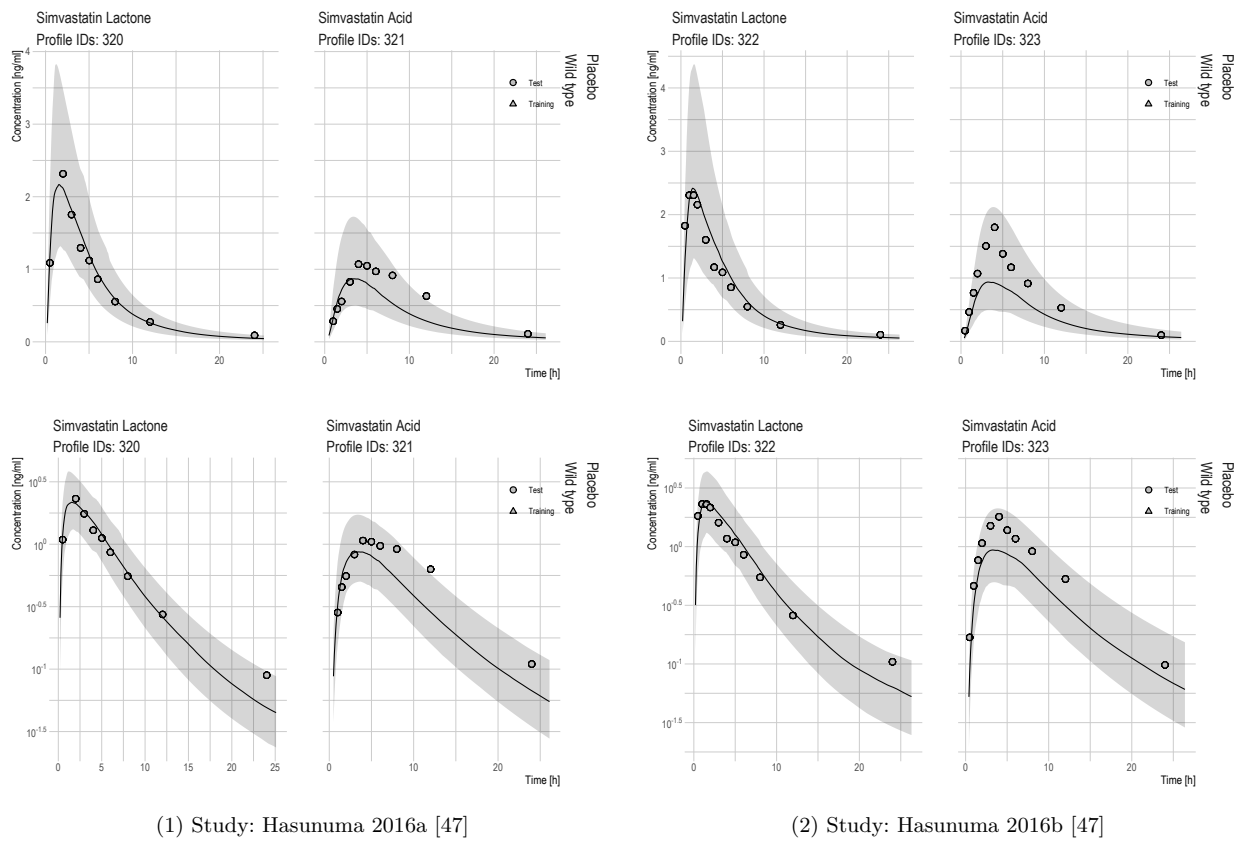


Figure S2.14: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Hasunuma 2016a [47], Hasunuma 2016b [47]

2 PBPK modeling of simvastatin

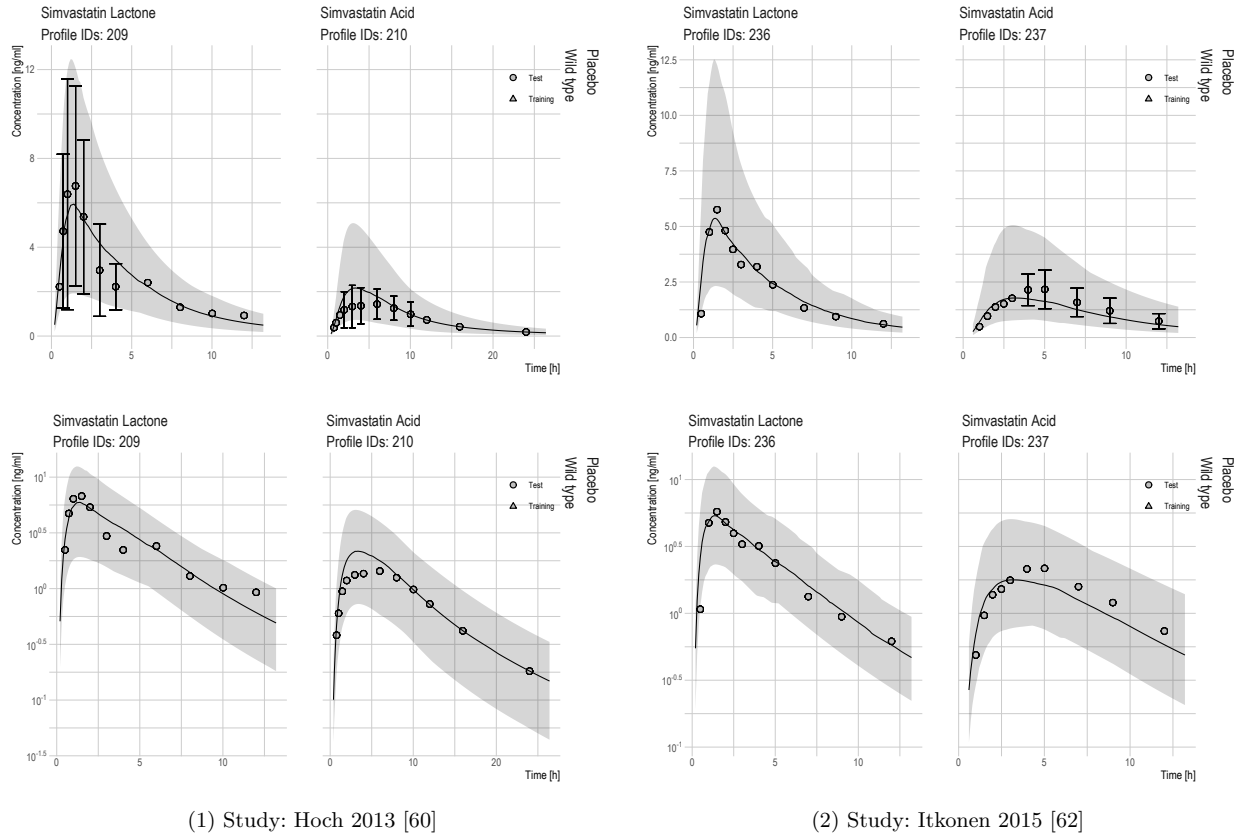


Figure S2.15: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Hoch 2013 [60], Itkonen 2015 [62]

2 PBPK modeling of simvastatin

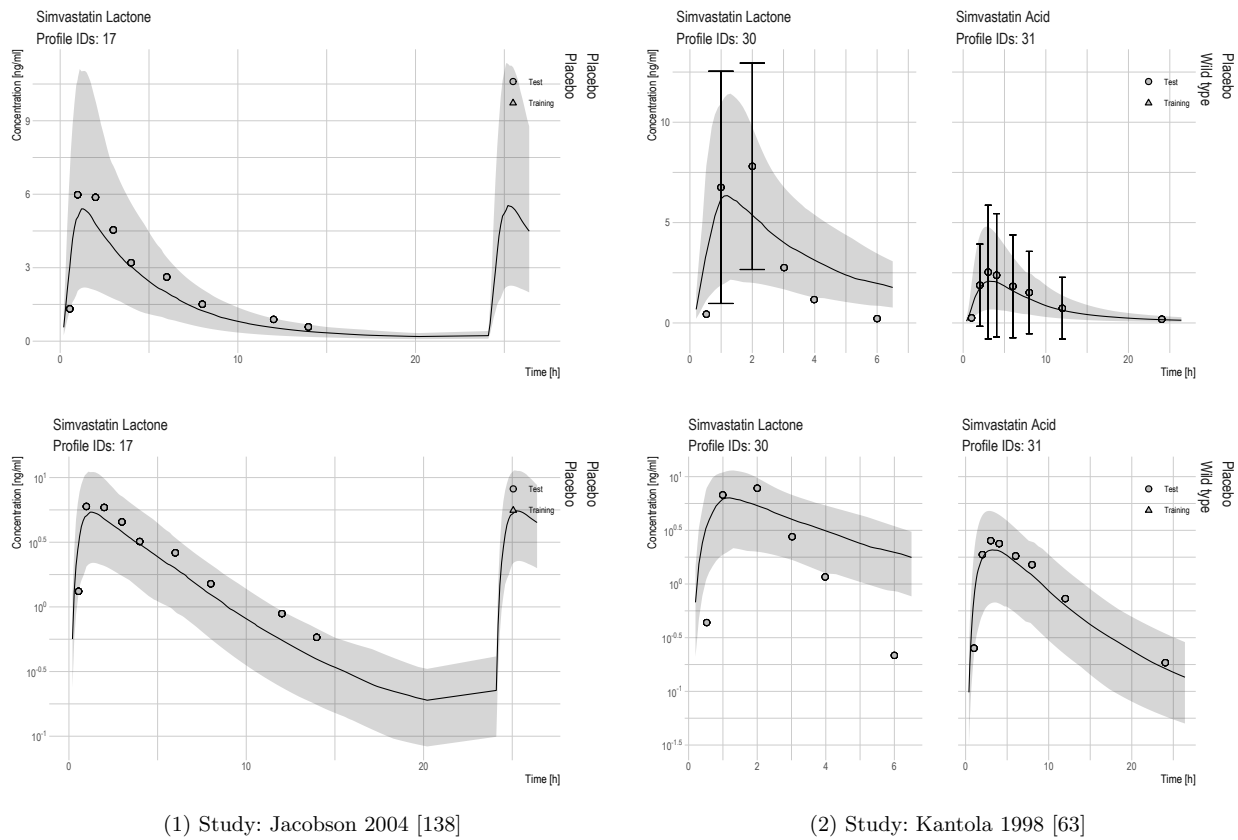


Figure S2.16: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Jacobson 2004 [138], Kantola 1998 [63]

2 PBPK modeling of simvastatin

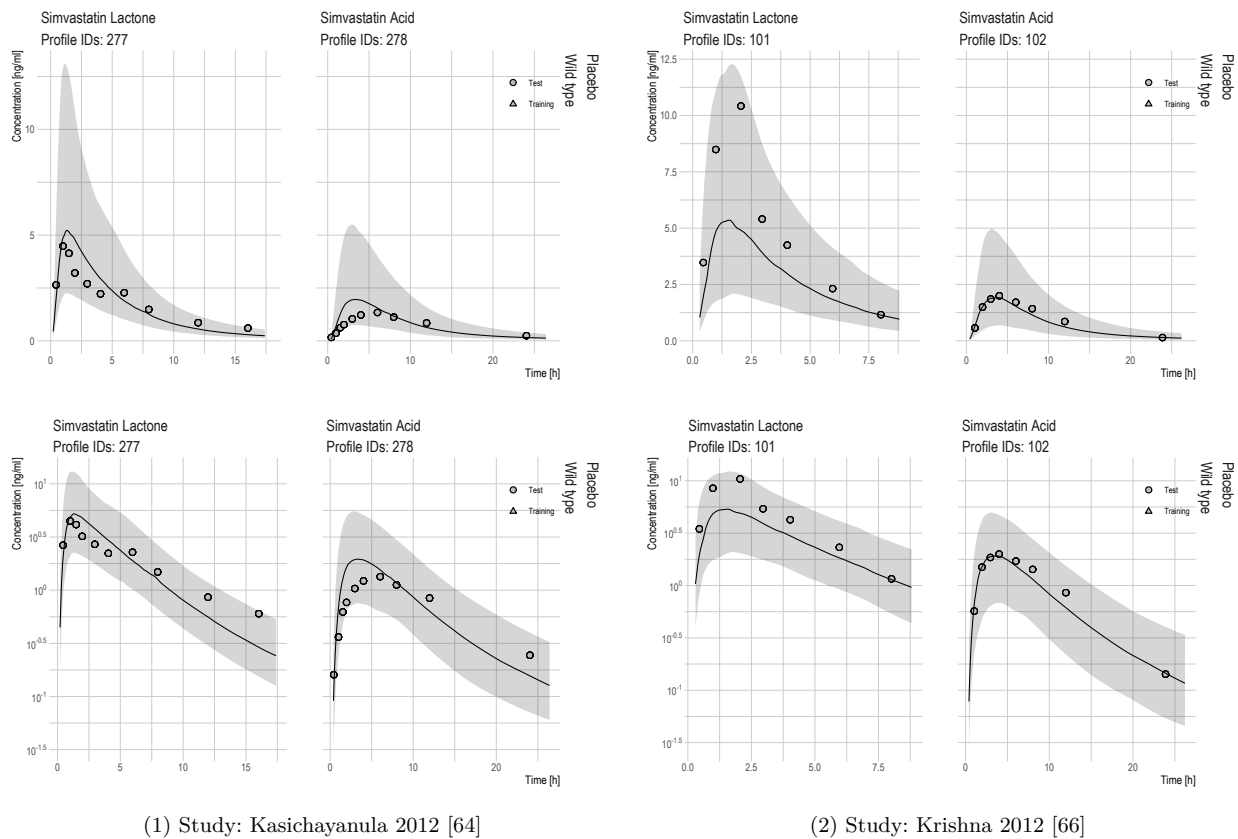


Figure S2.17: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Kasichayanula 2012 [64], Krishna 2012 [66]

## 2 PBPK modeling of simvastatin

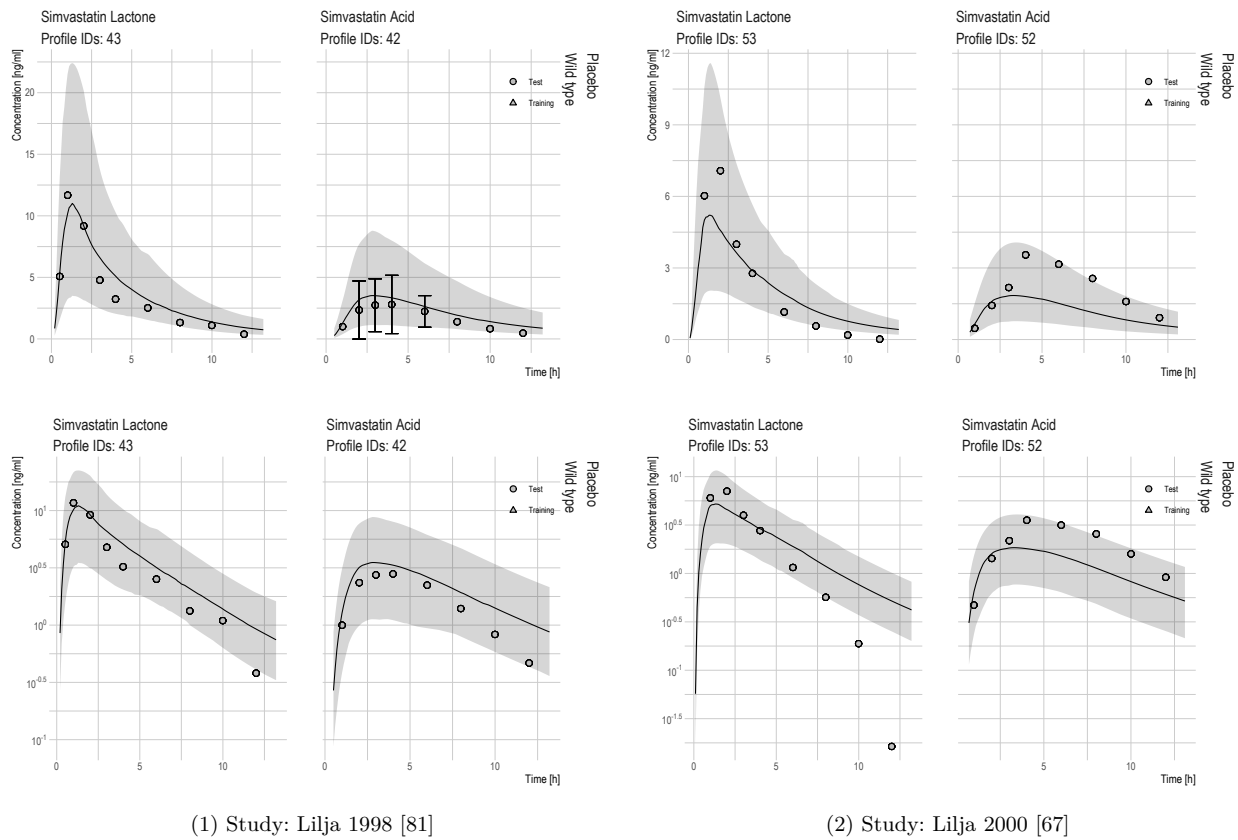


Figure S2.18: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Lilja 1998 [81], Lilja 2000 [67]

2 PBPK modeling of simvastatin

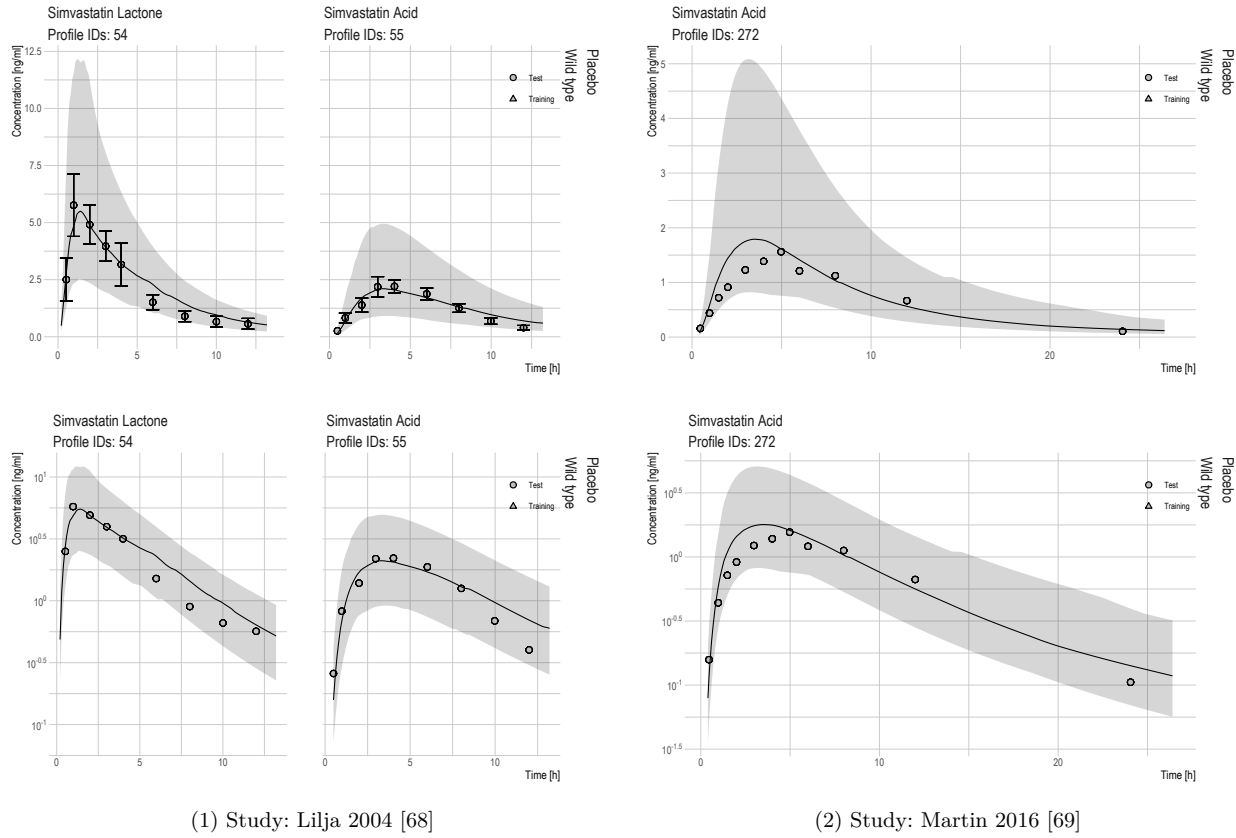


Figure S2.19: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Lilja 2004 [68], Martin 2016 [69]



2 PBPK modeling of simvastatin

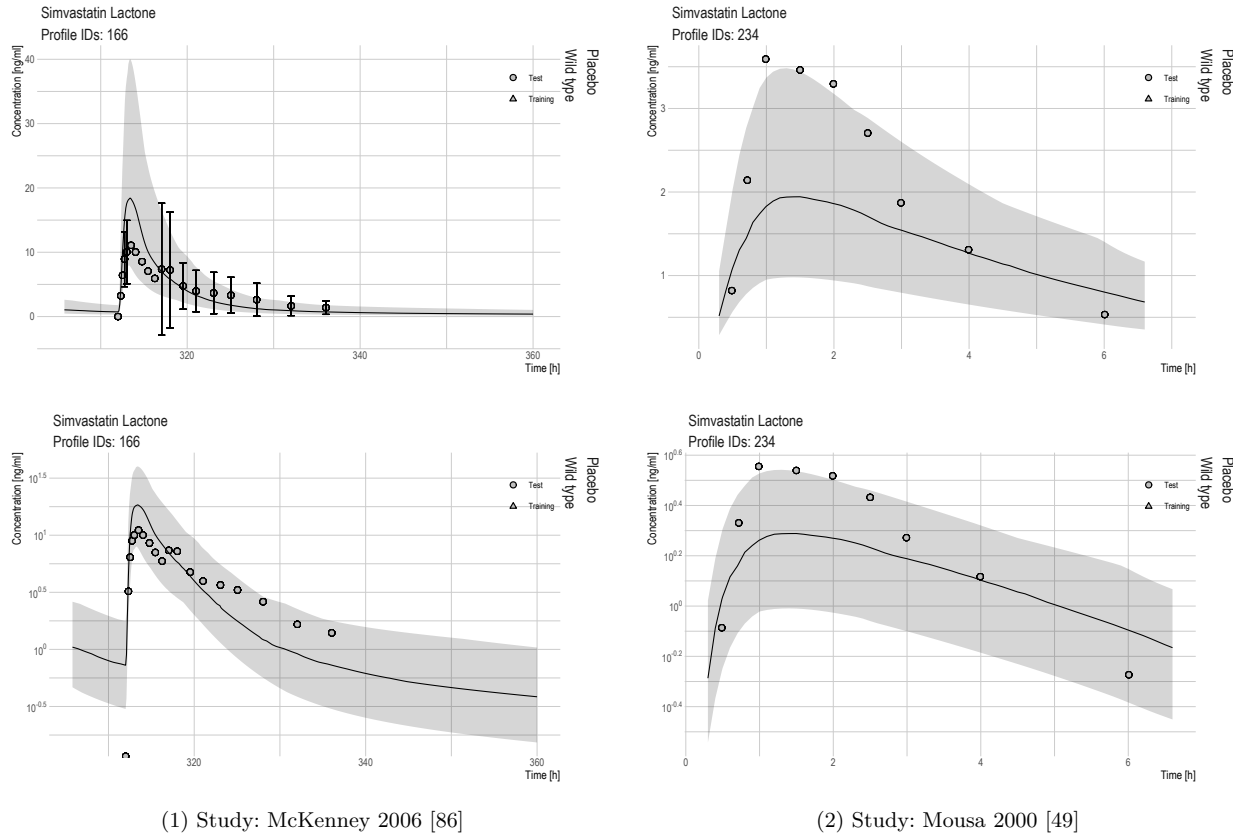


Figure S2.20: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: McKenney 2006 [86], Mousa 2000 [49]

2 PBPK modeling of simvastatin

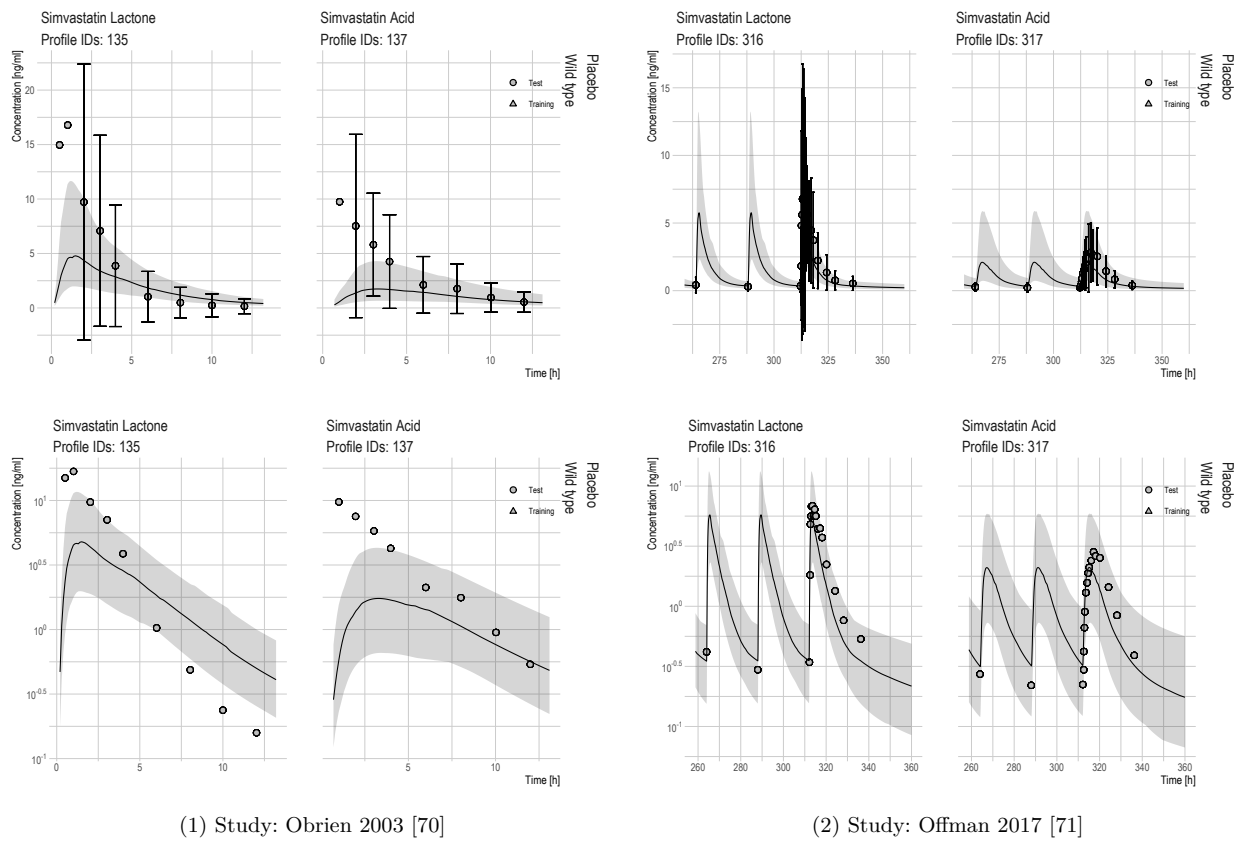


Figure S2.21: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: O'Brien 2003 [70], Offman 2017 [71]

2 PBPK modeling of simvastatin

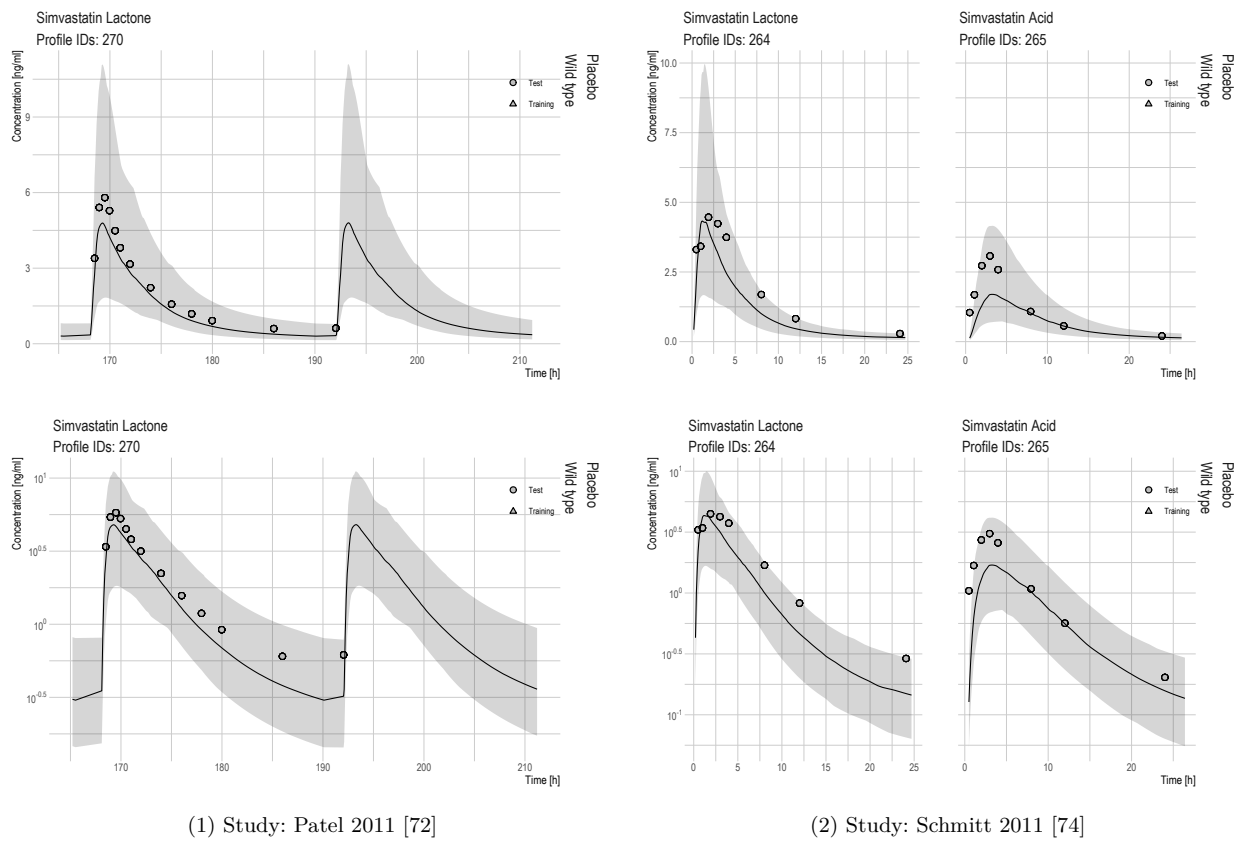
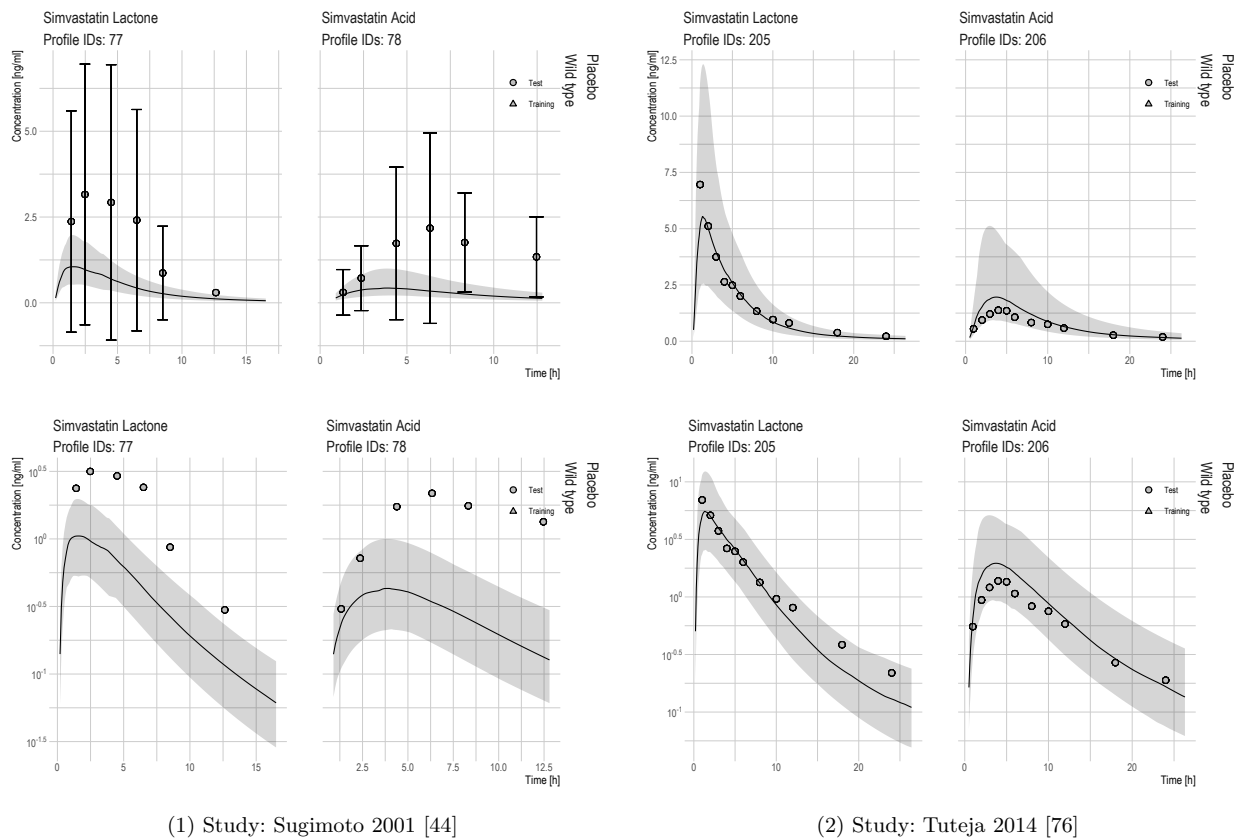


Figure S2.22: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Patel 2011 [72], Schmitt 2011 [74]

2 PBPK modeling of simvastatin



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Figure S2.23: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Sugimoto 2001 [44], Tuteja 2014 [76]

2 PBPK modeling of simvastatin

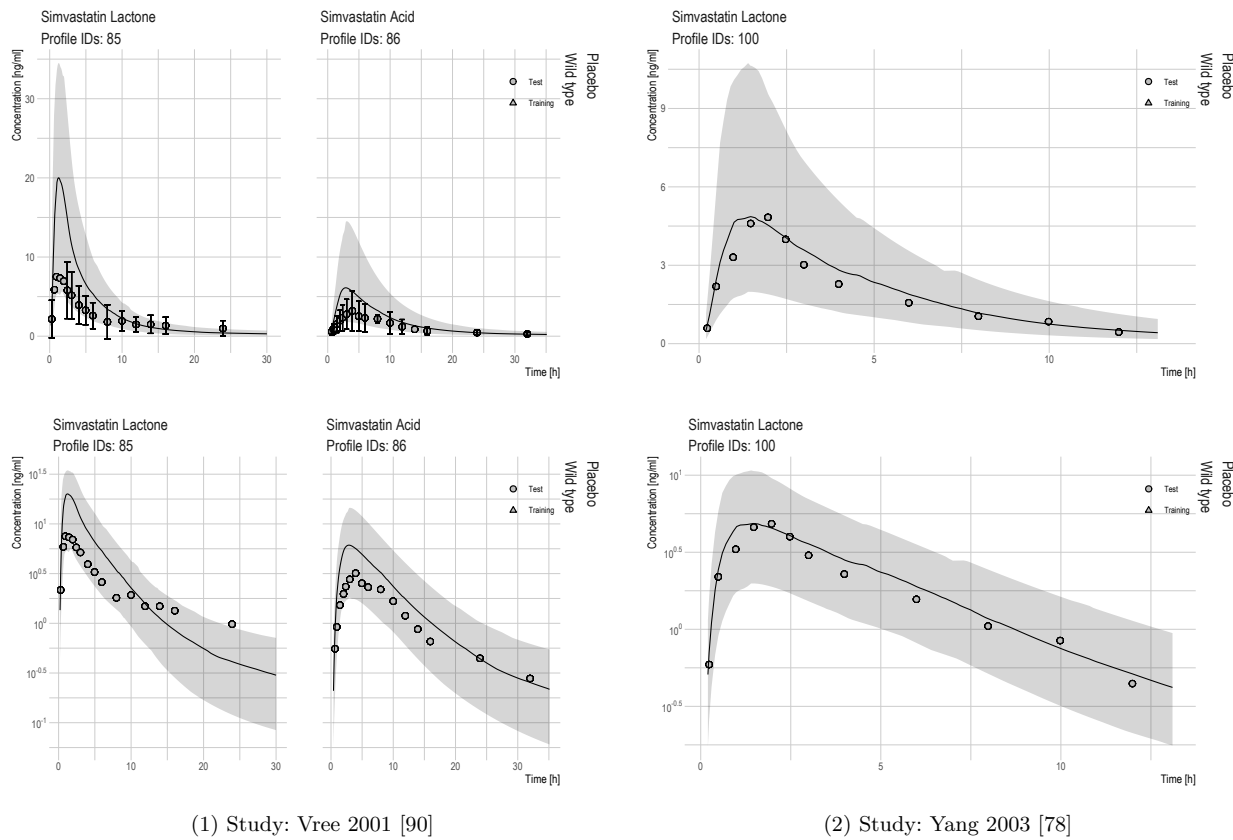
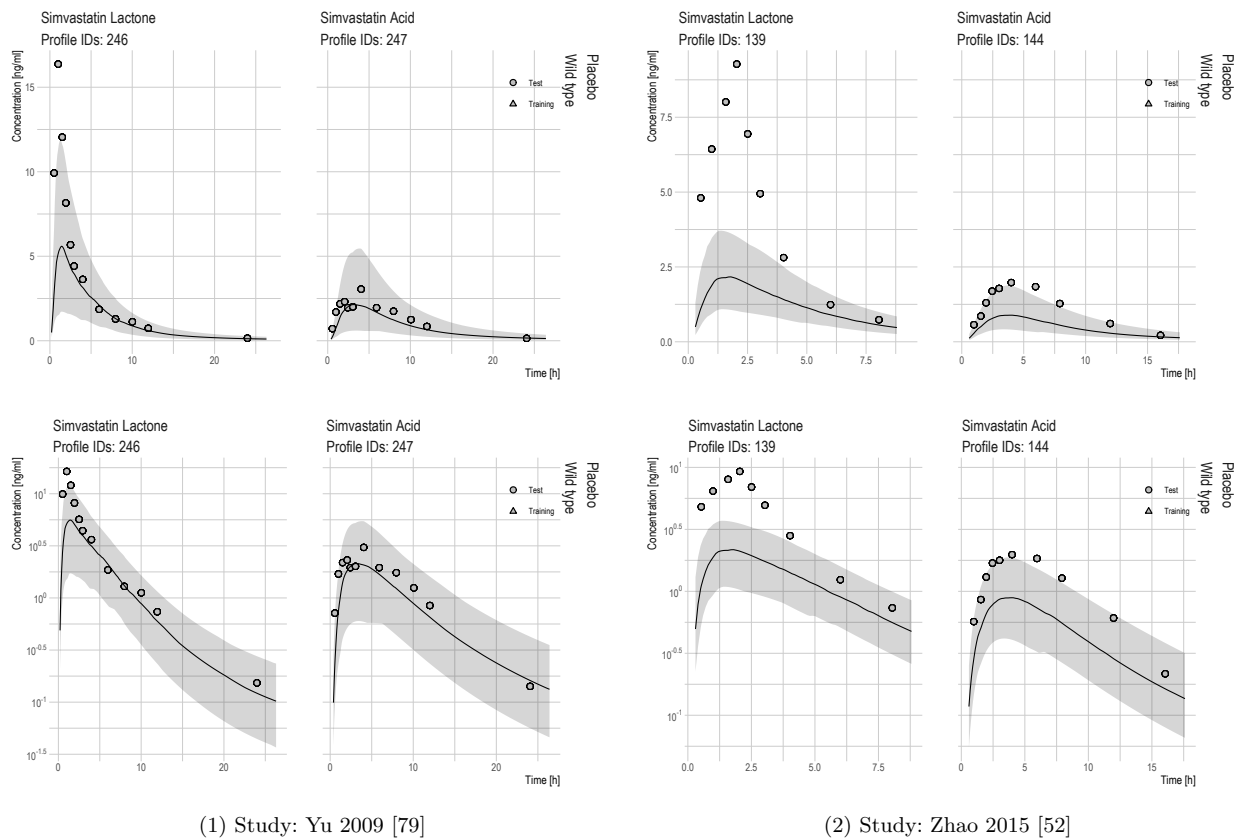


Figure S2.24: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Vree 2001 [90], Yang 2003 [78]

2 PBPK modeling of simvastatin



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Figure S2.25: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Yu 2009 [79], Zhao 2015 [52]

2 PBPK modeling of simvastatin

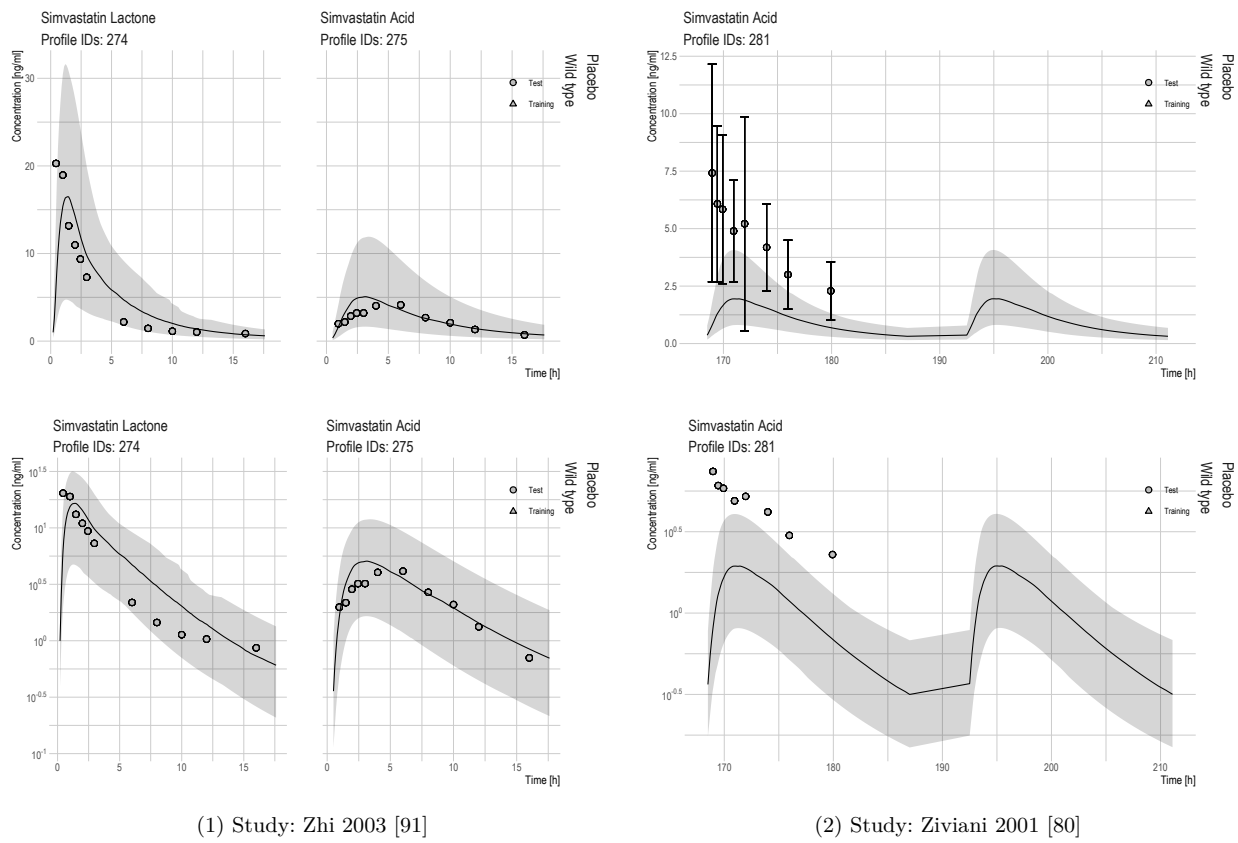


Figure S2.26: Linear and semi-logarithmic VPCs of the plasma concentration-time values in the test dataset. Solid line and shaded area are predicted median and 90 % CI: Zhi 2003 [91], Ziviani 2001 [80]

## 2 PBPK modeling of simvastatin

## 2.3.2 Predicted concentrations versus observed concentrations GOF plots

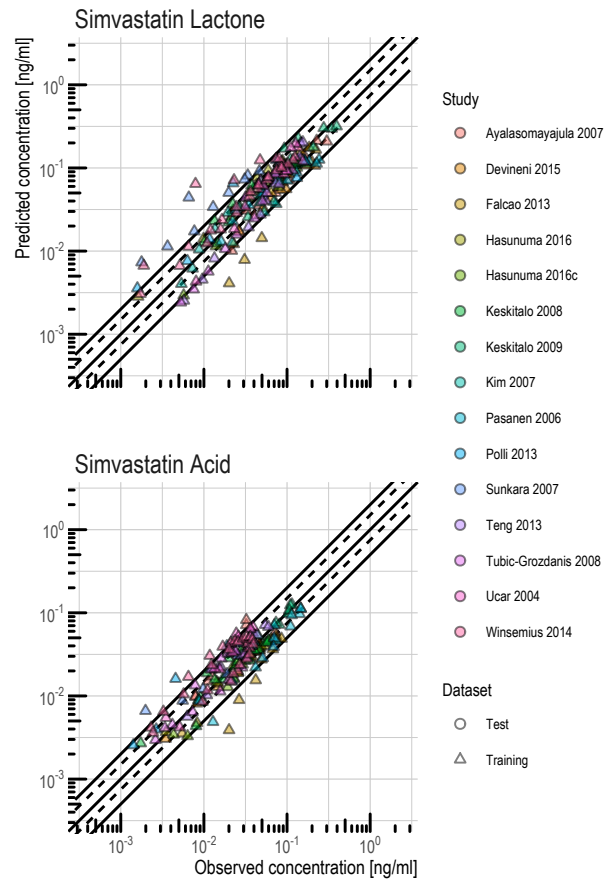


Figure S2.27: Goodness of fit plots - observed versus predicted plasma concentration-time values in the training dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations



## 2 PBPK modeling of simvastatin

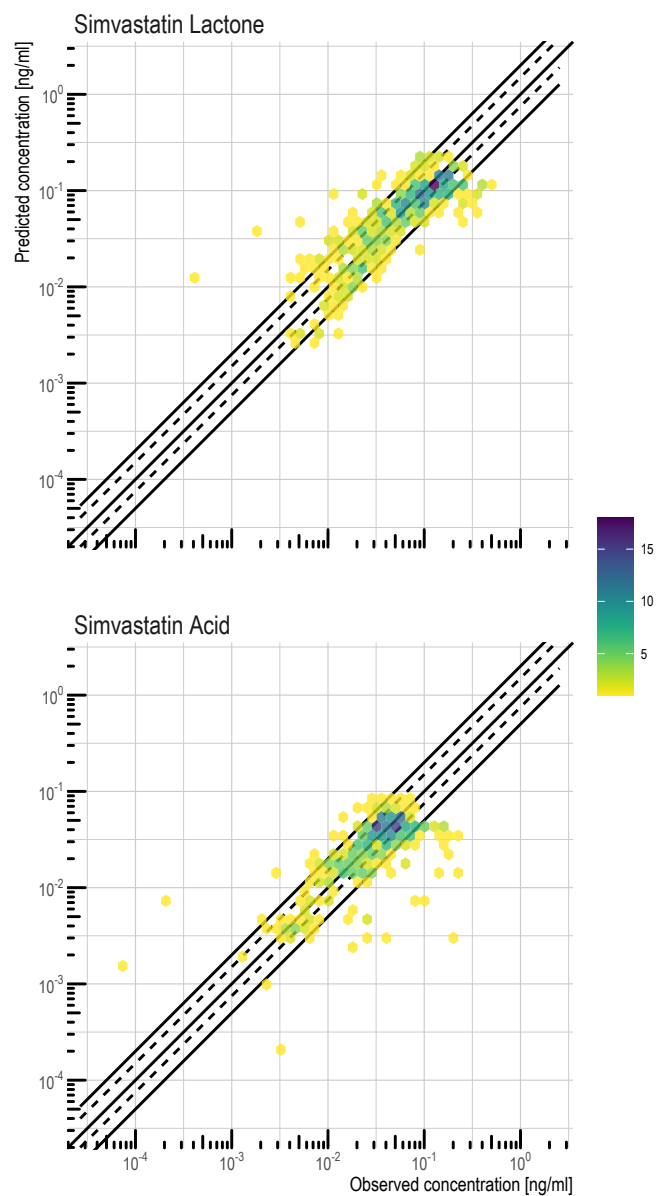


Figure S2.28: Goodness of fit plots - binned observed versus predicted plasma concentration-time values in the test dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations. The color of a hexagon reflects the binned number of observations in the respective neighborhood

## 2 PBPK modeling of simvastatin

## 2.3.3 NCA GOF plots

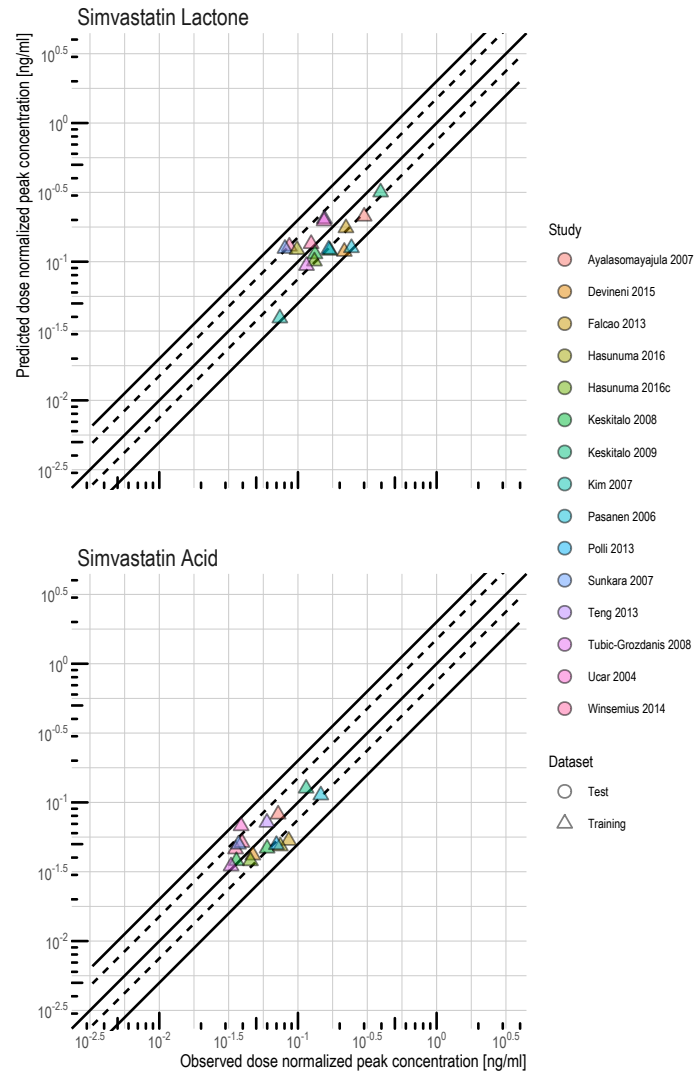


Figure S2.29: NCA ratios of the training dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations.: Training  $C_{max}$

## 2 PBPK modeling of simvastatin

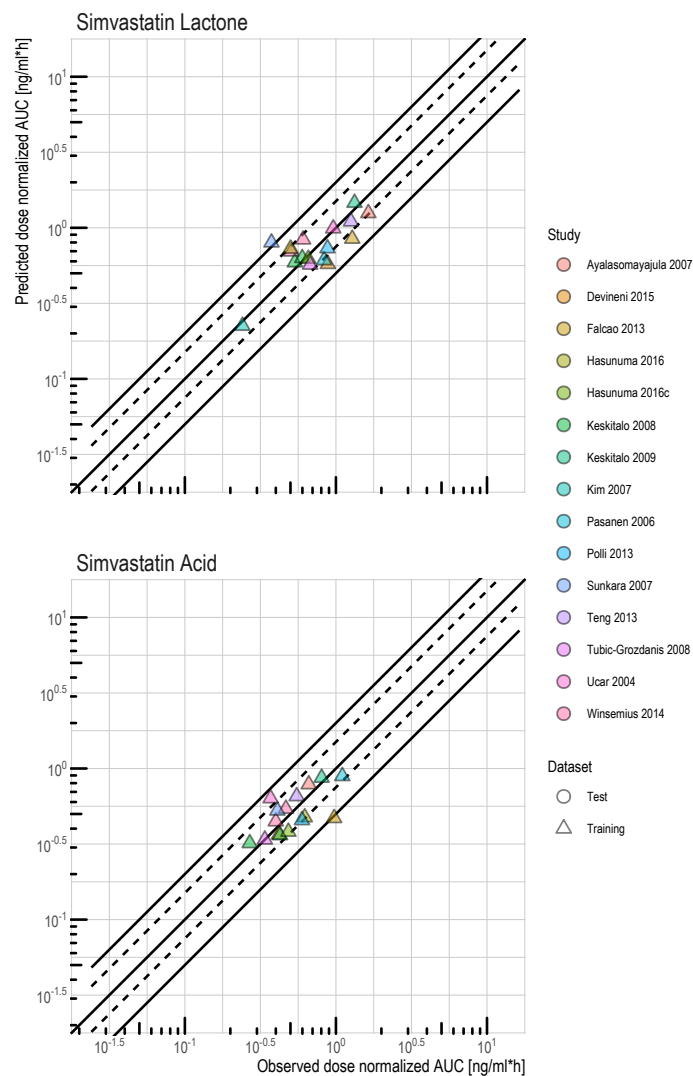


Figure S2.30: NCA ratios of the training dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations.: Training AUC

## 2 PBPK modeling of simvastatin

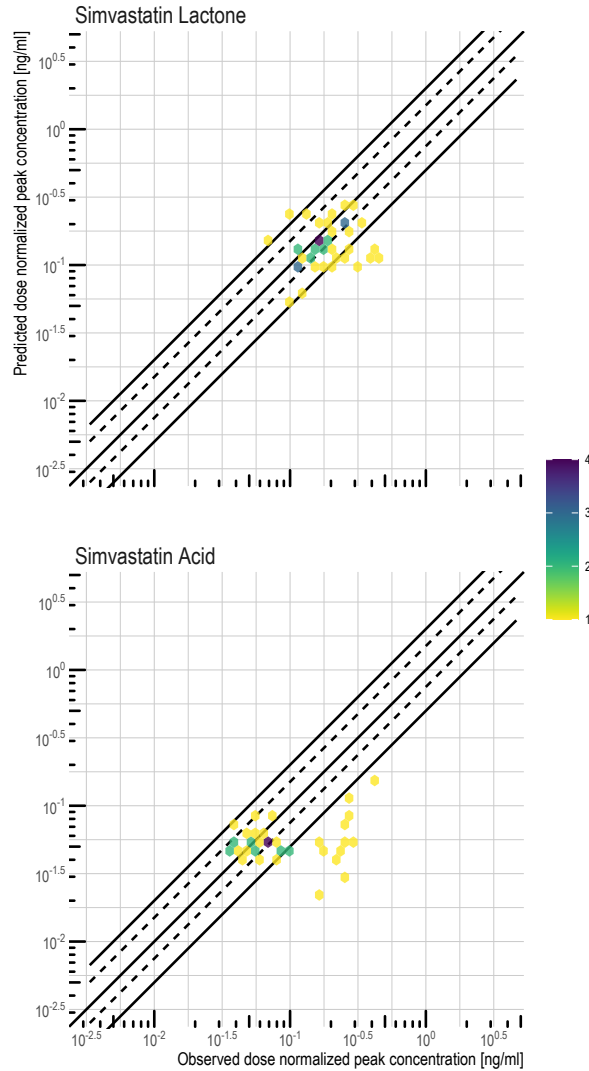


Figure S2.31: NCA ratios of the test dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations. The color of a hexagon reflects the binned number of observations in the respective neighborhood.: Test  $C_{max}$

## 2 PBPK modeling of simvastatin

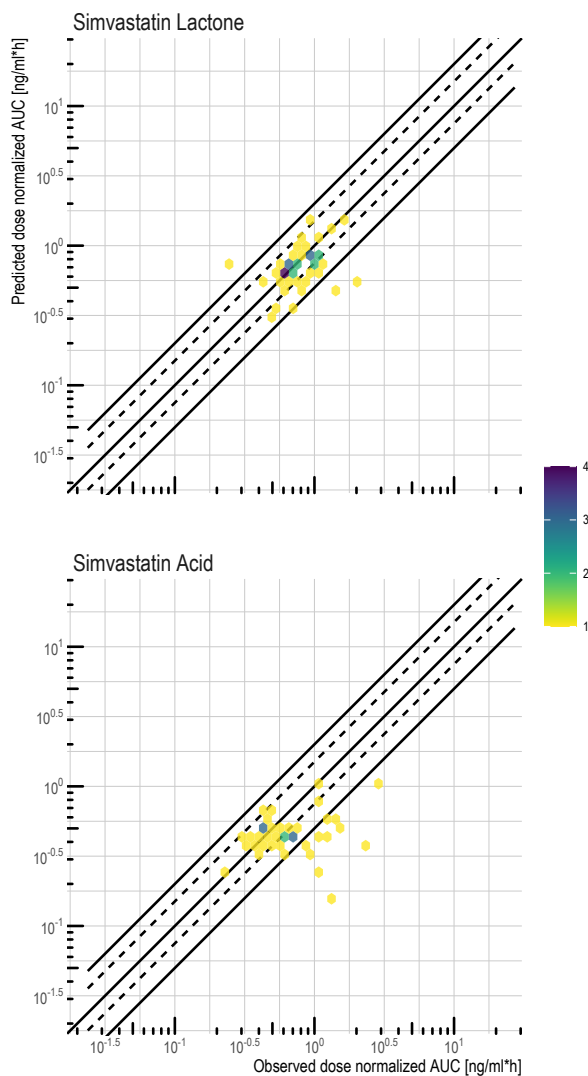


Figure S2.32: NCA ratios of the test dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations. The color of a hexagon reflects the binned number of observations in the respective neighborhood.: Test AUC

## 2 PBPK modeling of simvastatin

## 2.3.4 MRD and MSA of plasma concentration predictions

Table S2.7: Summary of the statistical placebo model evaluation (MSA and MRD)

Molecule	MRD mean (sd)	MSA mean (sd)
Simvastatin Lactone	1.87 (0.709) N = 42 (N MRD > 2 = 11)	57 (56.3) N = 42 (N MSA > 100 = 7)
Simvastatin Acid	1.98 (1.17) N = 40 (N MRD > 2 = 9)	60.6 (78.5) N = 40 (N MSA > 100 = 4)

## 2.3.5 NCA ratios and GMFE of NCA values

Table S2.8: Summary of the statistical placebo model evaluation (NCA ratio and GMFE)

Parameter	NCA ratio mean (sd)	GMFE
<b>Simvastatin Lactone</b>		
AUC	0.998 (0.401) N = 63 (N ratio > 2   ratio < 0.5 = 5)	1.3
$C_{max}$	0.869 (0.419) N = 63 (N ratio > 2   ratio < 0.5 = 10)	1.47
<b>Simvastatin Acid</b>		
AUC	0.851 (0.362) N = 58 (N ratio > 2   ratio < 0.5 = 12)	1.53
$C_{max}$	0.832 (0.43) N = 58 (N ratio > 2   ratio < 0.5 = 14)	1.71

## 2.3.6 Local sensitivity analysis

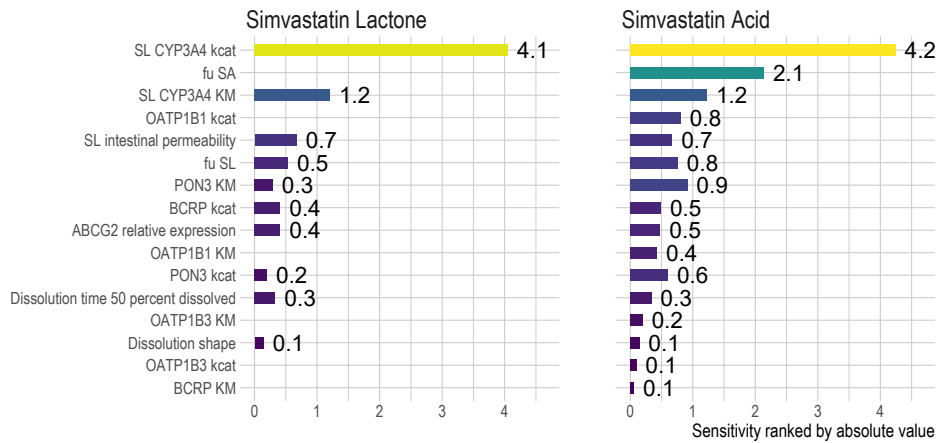


Figure S2.33: Simvastatin PBPK model sensitivity analysis. Sensitivity to single parameter perturbation, measured as change of the simulated AUC in steady-state of a 80 mg oral dose

## 2.4 Simvastatin DGIs

### 2.4.1 OATP1B1 (SLCO1B1)

The *SLCO1B1* polymorphism (rs4149056) is one of the best described and most important SL pharmacogene (PGx) [139]. Available data included one mean study profile from Pasanen et al. [95] for the c.521T/T, c.521T/C and c.521C/C genotypes, respectively. Detailed descriptions are presented in Table S2.2. It has to be noted, that for the c.521C/C genotype from Pasanen et al. [95] an unexpected elevation in the SL plasma concentration-time profile was observed. Interestingly, the elevation was comparable with the study from Keskitalo et al. [94] investigating the influence of rs2231142 in the *ABCG2* gene. In the study by Keskitalo et al. [94], participants hetero- or homozygous for the *ABCG2* c.421A/A genotype (non-functional) were extremely likely also heterozygous for rs4149056 polymorphism (88 % for c.421C/A and c.421A/A compared to 30 % in the c.421C/C group). Unfortunately, in the study from Pasanen et al. [95], participants were not screened for polymorphisms in the *ABCG2* gene. Moreover, a large difference in mean body weight in the c.521C/C and c.421A/A (84 kg versus 56 kg) was observed. As shown by Tsamandouras et al. [26] body weight is a significant covariate on the volume of distribution of simvastatin and thus, this could further obscure the covariate-adjusted difference in both groups.

A comparison of the model predicted an observed plasma concentration-time profile for each profile is shown in Figs. S2.34 and S2.35.

2 PBPK modeling of simvastatin

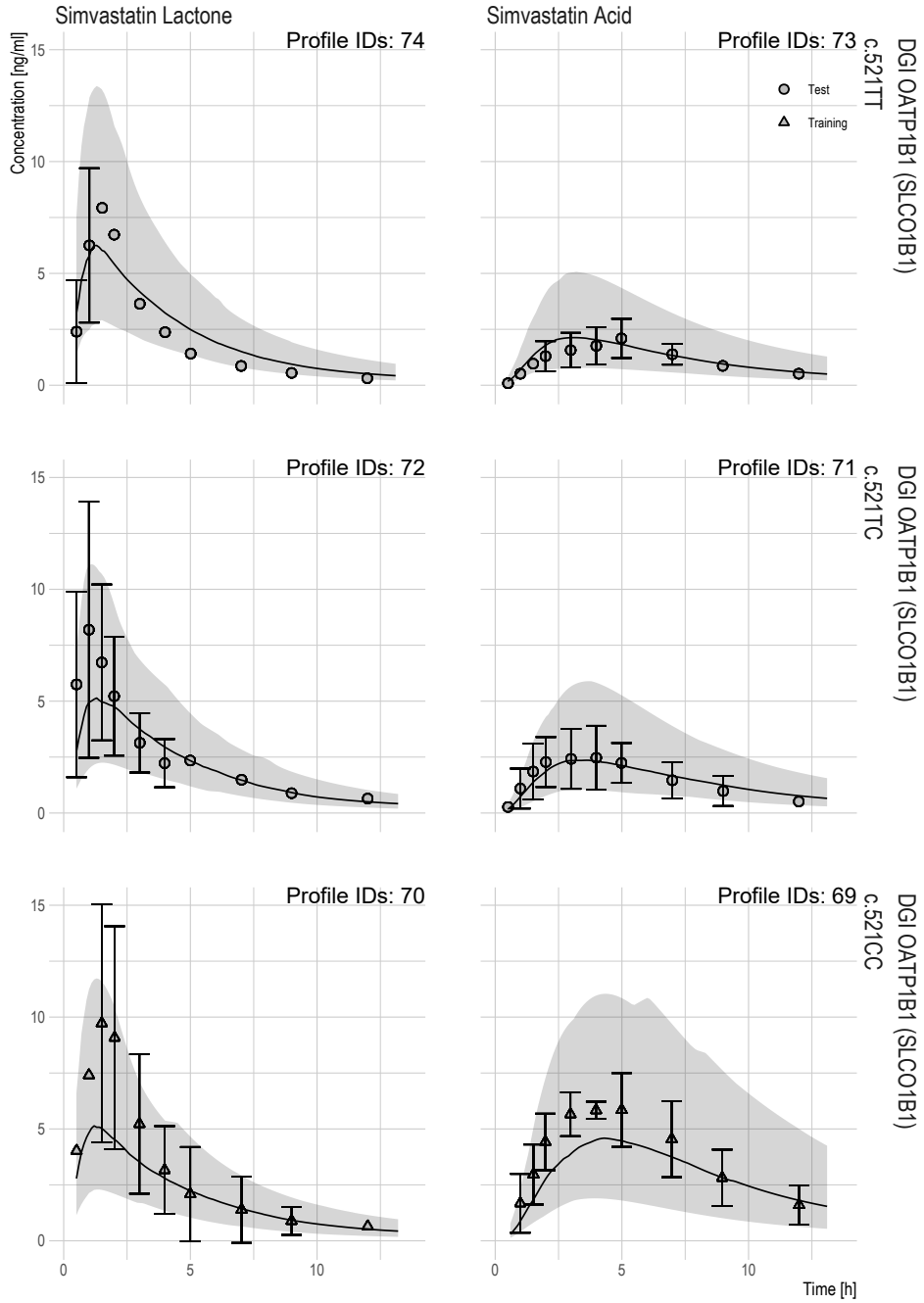


Figure S2.34: Linear VPCs of the plasma concentration-time profiles for investigated DGIs: OATP1B1 *SLCO1B1*. Solid line and shaded area are predicted median and 90% CI



2 PBPK modeling of simvastatin

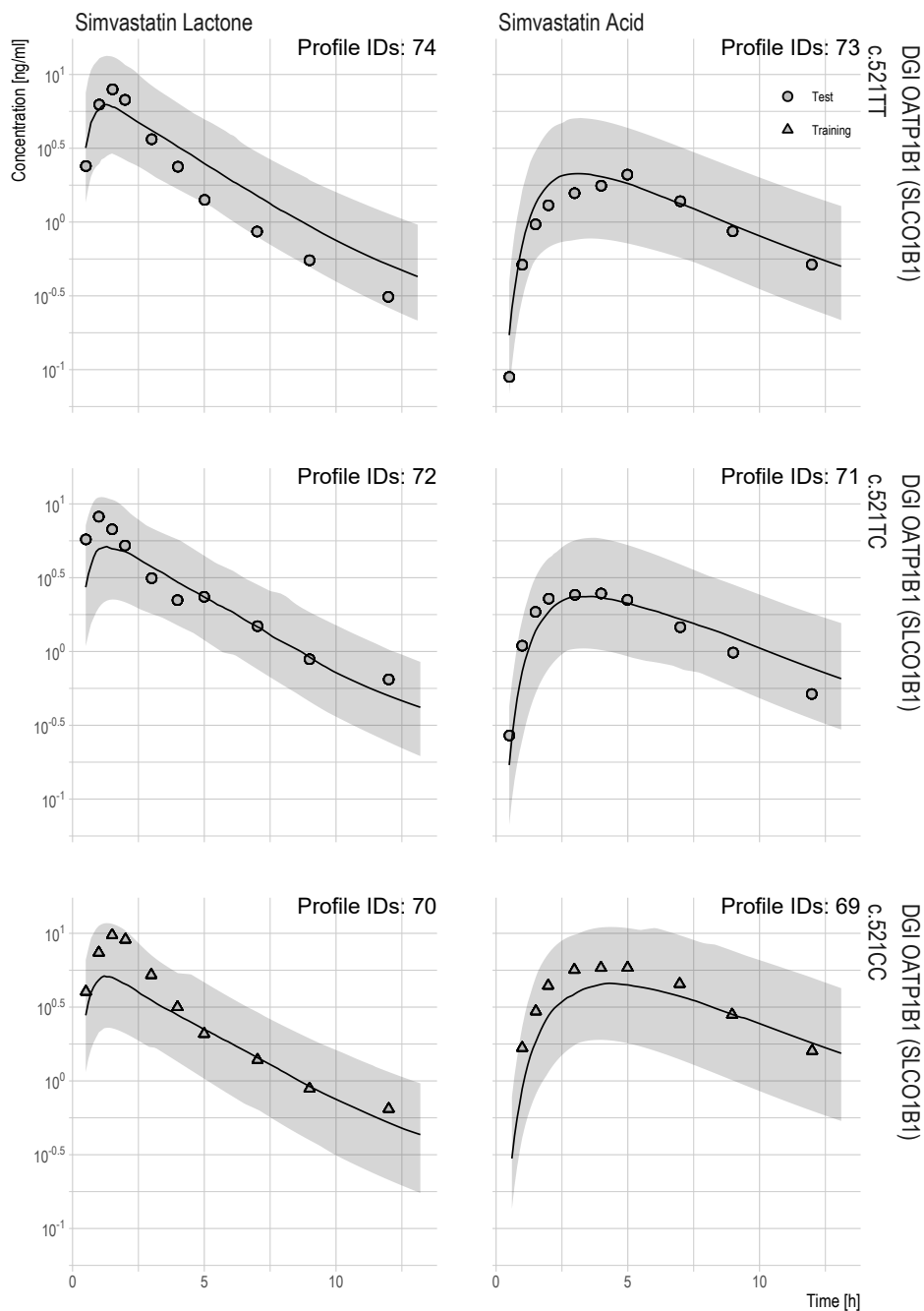


Figure S2.35: Semilogarithmic VPCs of the plasma concentration-time profiles for investigated DGIs: OATP1B1 *SLCO1B1*. Solid line and shaded area are predicted median and 90% CI

## 2 PBPK modeling of simvastatin

## 2.4.2 BCRP (ABCG2)

Although identified as significant covariate in two studies [94, 26] the rs2231142 polymorphism in *ABCG2* ranks only as level 3 clinical annotation on pharmgkb [140]. Nevertheless, it was included in the PBPK model assuming solely an impact on SL PK. Furthermore, for each study arm an individual OATP1B1 activity was calculated based on the observed *SLCO1B1* rs4149056 genotypes assuming an additive relationship. Data available included one SL and SA study mean profile from Keskitalo et al. [94] for the c.421C/C, c.421C/A and c.421A/A genotypes, respectively. Detailed descriptions are presented in Table S2.2. Despite the observed and well predicted effects, further studies should be performed to confirm the relevance and impact of BCRP (*ABCG2*) on SL and SA PK. The model capability to describe and predict the effect is shown in Figs. S2.36 and S2.37.

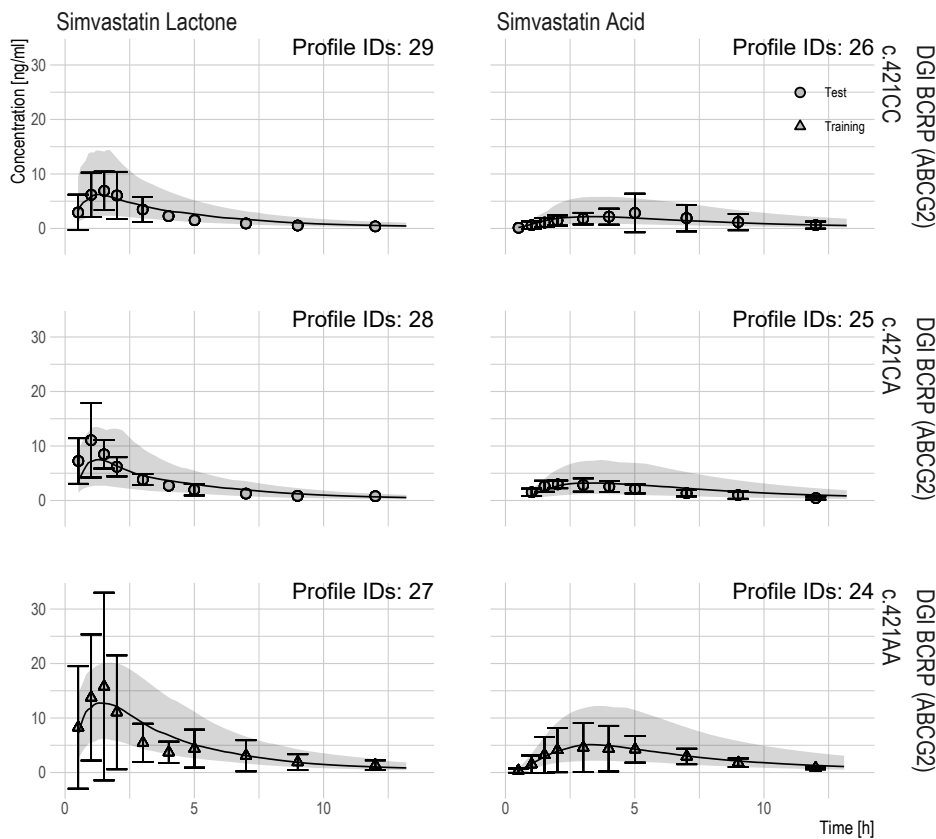


Figure S2.36: Linear VPCs of the plasma concentration-time profiles for investigated DGIs: BCRP *ABCG2*. Solid line and shaded area are predicted median and 90% CI

2 PBPK modeling of simvastatin

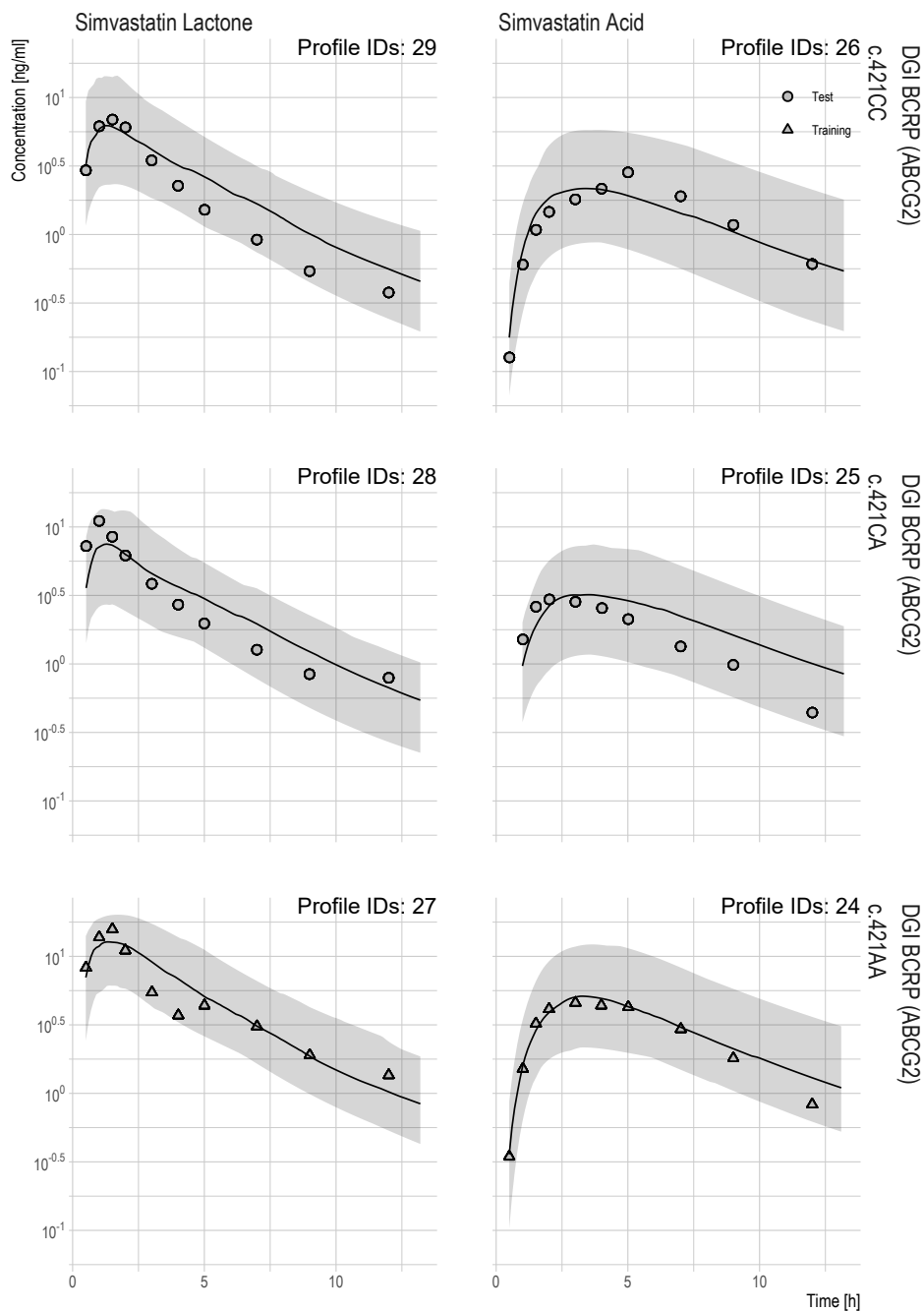


Figure S2.37: Semilogarithmic VPCs of the plasma concentration-time profiles for investigated DGIs: BCRP *ABCG2*. Solid line and shaded area are predicted median and 90 % CI

## 2 PBPK modeling of simvastatin

## 2.4.3 P-gp (ABCB1)

The different P-gp *ABCB1* genotypes were only descriptively included, since no study arm for evaluation was on hand. Hereby, one SL and SA study mean profile from Keskitalo et al. [41] for the *ABCB1* c.1236T-c.2677T-c.3435T and one for the c.1236C-c.2677G-c.3435C were available. Details are shown in Table S2.2. Nevertheless, by solely adapting the P-gp  $k_{cat}$  the different PK profiles of SA could be described accurately as shown in Figs. S2.38 and S2.39.

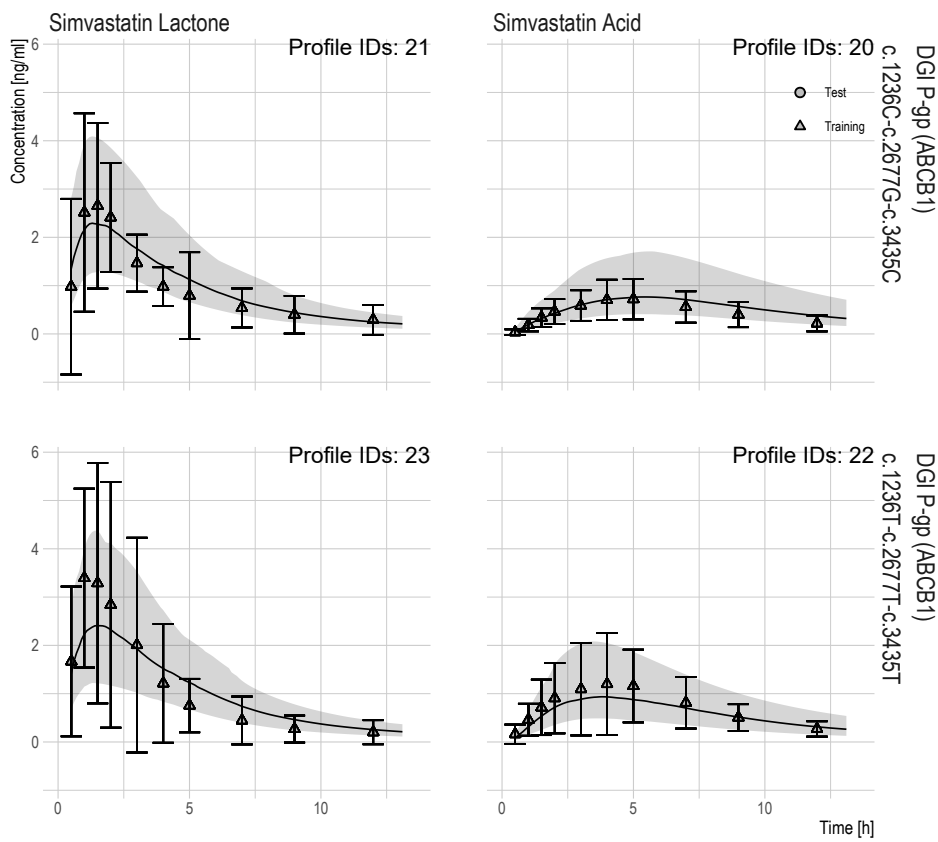


Figure S2.38: Linear VPCs of the plasma concentration-time profiles for investigated DGIs: P-gp *ABCB1*. Solid line and shaded area are predicted median and 90% CI

2 PBPK modeling of simvastatin

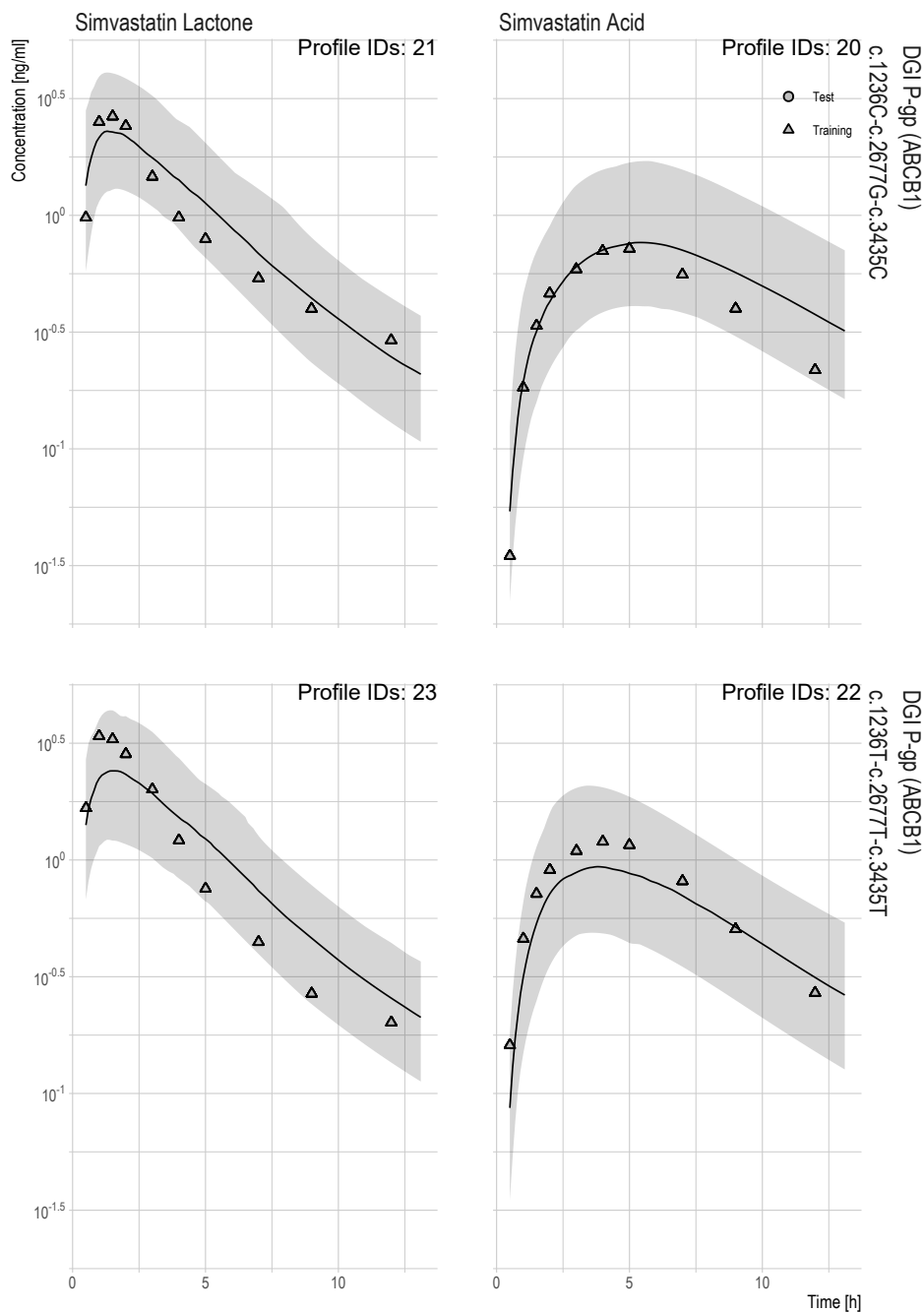


Figure S2.39: Semilogarithmic VPCs of the plasma concentration-time profiles for investigated DGIs: P-gp *ABCB1*. Solid line and shaded area are predicted median and 90% CI

## 2 PBPK modeling of simvastatin

## 2.4.4 CYP3A5 (CYP3A5 gene)

Unfortunately, in the only available study investigating the effects of the *CYP3A5* polymorphism on simvastatin, no SA PK were measured [93]. Following, SA metabolism by CYP3A5 could not be estimated and hence, was not included in the model [132]. Nevertheless, for SL the model showed good accuracy in describing the homozygous *CYP3A5*\*1/\*1 and predicting the heterozygous *CYP3A5*\*1/\*3 genotype as presented in Fig. S2.40.

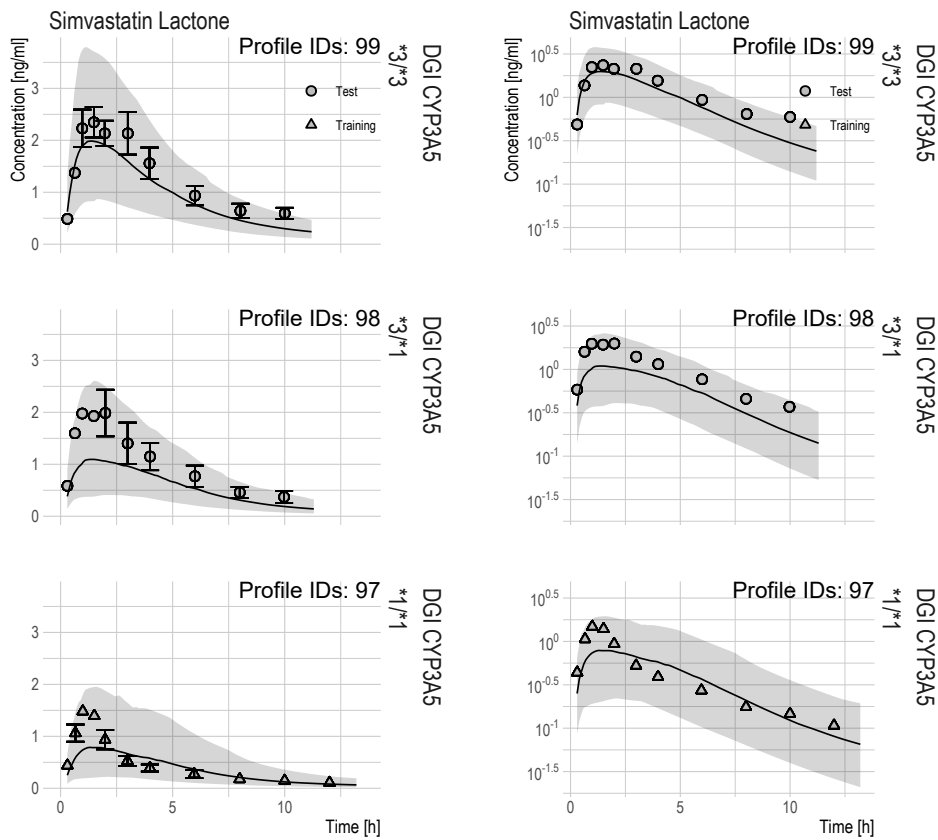


Figure S2.40: Linear (left) and semilogarithmic (right) VPCs of the plasma concentration-time profiles for investigated DGIs: *CYP3A5*. Solid line and shaded area are predicted median and 90% CI

## 2 PBPK modeling of simvastatin

## 2.4.5 MRD and MSA of plasma concentration predictions

Table S2.9 summarizes the statistical quality measures MRD and MSA exclusively for the DGIs data.

Table S2.9: Summary of the statistical DGI model evaluation (MSA and MRD)

Molecule	MRD mean (sd)	MSA mean (sd)
Simvastatin Lactone	1.45 (0.121) N = 11 (N MRD > 2 = 0)	33.3 (16.1) N = 11 (N MSA > 100 = 0)
Simvastatin Acid	1.36 (0.134) N = 8 (N MRD > 2 = 0)	24.3 (11.6) N = 8 (N MSA > 100 = 0)

## 2.4.6 NCA ratios and GMFE of NCA values

Table S2.10 summarizes the NCA ratios and GMFE values exclusively for the DGIs data.

Table S2.10: Summary of the statistical DGI model evaluation (NCA ratio and GMFE)

Parameter	NCA ratio mean (sd)	GMFE
<b>Simvastatin Lactone</b>		
AUC	0.958 (0.265) N = 20 (N ratio > 2   ratio < 0.5 = 1)	1.25
$C_{max}$	0.754 (0.214) N = 20 (N ratio > 2   ratio < 0.5 = 2)	1.43
<b>Simvastatin Acid</b>		
AUC	0.703 (0.363) N = 17 (N ratio > 2   ratio < 0.5 = 7)	1.81
$C_{max}$	0.583 (0.365) N = 17 (N ratio > 2   ratio < 0.5 = 9)	2.23

## 3 Simvastatin DDIs

### 3.1 Clinical studies

For DDI network evaluation mean profiles from 6 studies were extracted including 9 SL and 7 SA pharmacokinetic profiles which represent information from 75 study participants in total. An overview of all mean study demographics available can be found in Table S3.1.



Table S3.1: Mean study simvastatin pharmacokinetic profiles used for drug-drug interaction model evaluation

Route	Route co-medication	N	Females [%]	Age [years]	Weight [kg]	Height [cm]	Dataset	Profile Ids	References
2 mg SL p.o. (Solution, NA) s.d.	Placebo	12	-	-	-	-	Test	306, 307	[141]
40 mg SL p.o. (Zocor, fasted) s.d.	600 mg gemfibrozil p.o. b.i.d.	10	50	22 (20–31)	62 (41–81)	-	Test	4, 5	[142]
40 mg SL p.o. (Zocor, fasted) s.d.	Placebo	10	50	22 (20–31)	62 (41–81)	-	Test	2, 3	[142]
40 mg SL p.o. (Zocor, fasted) s.d.	600 mg rifampicin p.o. daily	18	56	39	73	-	Test	295	[143]
40 mg SL p.o. (Zocor, fasted) s.d.	Placebo	18	56	39	73	-	Test	293	[143]
40 mg SL p.o. (Unknown, Unknown) daily	500 mg clarithromycin p.o. daily	15	300	(18–60)	-	-	Test	15	[138]
40 mg SL p.o. (Unknown, Unknown) daily	Placebo	15	300	(18–60)	-	-	Test	14	[138]
40 mg SL p.o. (Unknown, Unknown) s.d.	Placebo	15	300	(18–60)	-	-	Test	18	[138]
40 mg SL p.o. (Zocor, fasted) s.d.	600 mg rifampicin p.o. daily	10	50	(21–29)	(59–81)	-	Test	38, 39	[144]
40 mg SL p.o. (Zocor, fasted) s.d.	Placebo	10	50	(21–29)	(59–81)	-	Test	36, 37	[144]
40 mg SL p.o. (Zocor, fasted) s.d.	Placebo	10	30	22 (19–29)	69 (52–86)	-	Test	59, 60, 58	[145]

*Note:*

Values for age, weight and height are given as mean (range); -, not given; b.i.d., twice daily; n, number of individuals studied; po, oral; s.d., single dose

## 3.2 Clarithromycin

The antibiotic clarithromycin is a strong inhibitor of CYP3A4 and P-gp as well as an inhibitor of OATP1B1 and OATP1B3 [146]. A previously developed clarithromycin PBPK model was extended by  $K_i$  values to model the competitive inhibition of OATP1B1 and OATP1B3 [16]. The parameters of the extended clarithromycin model are given in the Tables S3.2 and S3.3. For DDI evaluation one SL plasma concentration-time profile and one SA AUC under clarithromycin co-treatment were available [138]. Linear and semilogarithmic VPCs are shown in Figs. S3.1 and S3.2.

### 3.2.1 Drug-dependent parameters

Table S3.2: Drug-dependent parameters of the final clarithromycin model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	29.9	30 (28–40)	Literature	Fraction unbound plasma
Lipophilicity	-	2.3	2.3	Literature	Lipophilicity
MW	-	748	748	Literature	Molecular weight
pKa	-	8.99	6.845 (4.7–8.99)	Literature	Acid dissociation constant (basic)
Solubility	mg l <sup>-1</sup>	12170	12170	Literature	Solubility at pH=2.4
<b>Enzymes</b>					
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	76.5	76.5	Literature	CYP3A4 catalytic rate constant
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	48.7	48.7	Literature	CYP3A4 Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	6.04	5.765 (2.25–39.2)	Literature	Concentration for half-maximal inactivation (MBI)
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	10	12.58 (3.44–96)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	9.8	9.8	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	4.1	31 (3.8–434)	Literature	Concentration for half-maximal P-gp competitive inhibition
K <sub>inact</sub> CYP3A4	min <sup>-1</sup>	0.04	0.06 (0.04–0.23)	Literature	Maximum inactivation rate constant (MBI)
<b>Formulation</b>					
Dissolution shape	-	2.9	2.9	Literature	Weibull function dissolution shape

Table S3.2: Drug-dependent parameters of the final clarithromycin model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
Dissolution time (50 % dissolved)	min	5	5	Literature	Weibull function dissolution time (50% dissolved)
<b>System</b>					
CL <sub>Ren</sub>	ml/min	100	100	Literature	Renal plasma clearance
EHC	-	1	1	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	1	Assumed	Fraction of filtered drug reaching the urine
Perm. Into blood cells	cm min <sup>-1</sup>	0.000362	3.62e-06	Literature	Plasma to blood cells permeability
Perm. Out of blood cells	cm min <sup>-1</sup>	1.04e-05	1.04e-07	Literature	Blood cells to plasma permeability
Specific intest. perm.	cm min <sup>-1</sup>	1.23e-05	1.23e-07	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	3.28e-05	3.28e-07	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permeabilities calculation method: PK-Sim Standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland; formulation parameter values were used for solid oral dosage forms only

Table S3.3: Extracted drug-dependent parameter literature values for clarithromycin

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	28	-	-	[2]
fu	%	30	-	-	[2]
fu	%	40	-	-	[2]
Lipophilicity	-	2.3	-	-	[2]
MW	-	748	-	-	[2]
Perm. Into blood cells	cm min <sup>-1</sup>	3.62e-06	-	-	[2]
Perm. Out of blood cells	cm min <sup>-1</sup>	1.04e-07	-	-	[2]
pKa	-	4.7	-	-	[2]
pKa	-	8.99	-	-	[2]
Solubility	mg l <sup>-1</sup>	12170	-	-	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	76.5	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	48.7	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	2.25	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	4.12	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	5.49	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	6.04	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	29.5	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	39.2	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	8.26	-	K <sub>i</sub>	[147]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	3.44	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	16.9	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	5.1	-	IC50	[149]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	26.2	-	IC50	[150]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	96	-	IC50	[151]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	5.3	-	IC50	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	75	-	IC50	[152]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	9.8	-	IC50	[151]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	34	-	IC50	[153]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	66	-	IC50	[153]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	7	-	IC50	[154]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	28	-	IC50	[154]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	39.7	-	IC50	[123]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	34	-	IC50	[124]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	434	-	K <sub>i</sub>	[155]

Table S3.3: Extracted drug-dependent parameter literature values for clarithromycin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	4.1	-	IC50	[2]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	8.9	-	IC50	[146]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	3.8	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	7.2	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	15.1	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	23.8	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	50.2	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	56.1	-	IC50	[43]
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	86.7	-	IC50	[43]
$K_{\text{inact}}$ CYP3A4	$\text{min}^{-1}$	0.04	-	-	[2]
$K_{\text{inact}}$ CYP3A4	$\text{min}^{-1}$	0.05	-	-	[2]
$K_{\text{inact}}$ CYP3A4	$\text{min}^{-1}$	0.07	-	-	[2]
$K_{\text{inact}}$ CYP3A4	$\text{min}^{-1}$	0.23	-	-	[2]
Dissolution shape	-	2.9	-	-	[2]
Dissolution time (50 % dissolved)	min	5	-	-	[2]
$CL_{\text{Ren}}$	m/ min	100	-	-	[2]
EHC	-	1	-	-	[2]
GFR	-	1	-	-	[2]
Specific intest. perm.	$\text{cm min}^{-1}$	1.23e-07	-	-	[2]
Specific organ perm.	$\text{cm min}^{-1}$	3.28e-07	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

## 3 Simvastatin DDIs

## 3.2.2 Profiles

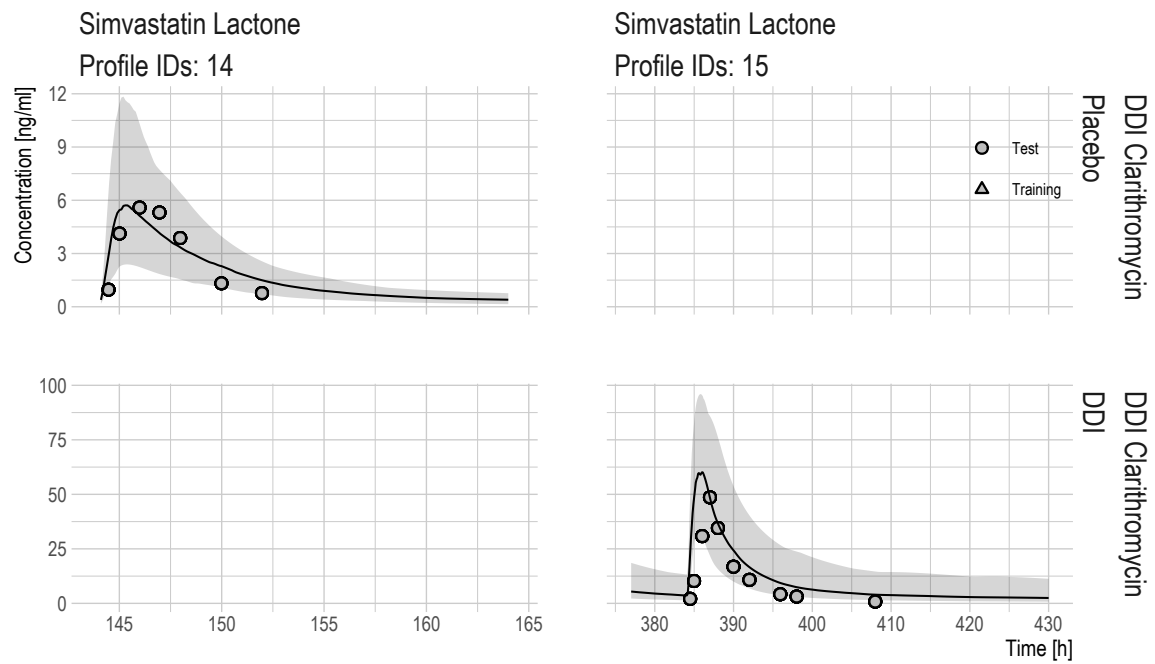


Figure S3.1: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Clarithromycin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90 % CI

3 Simvastatin DDIs

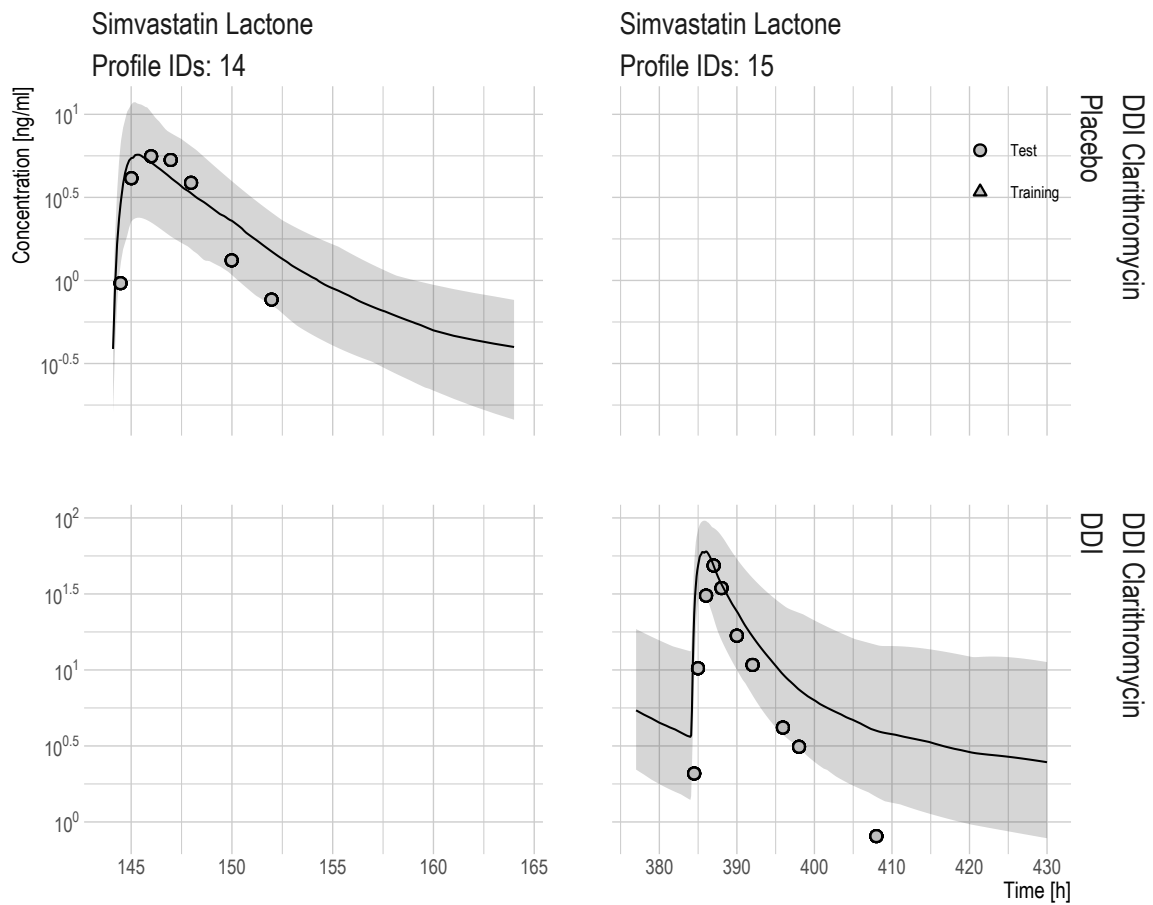


Figure S3.2: Semi-logarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Clarithromycin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90% CI



### 3.3 Rifampicin

The antibiotic rifampicin is a strong inducer and inhibitor of different metabolic enzymes and transporters [156]. A previously developed rifampicin PBPK model was extended by interaction constants [16]. Furthermore, since Hanke et al. [16] found an induction effect on the esterase arylacetamide deacetylase (AADAC) also for the SL relevant esterase PON3 an induction with the same parameter values was assumed. The parameters of the extended rifampicin model are given in Tables S3.4 and S3.5. Results of the simvastatin-rifampicin DDI interaction are shown in Figs. S3.3 - S3.8.

#### 3.3.1 Drug-dependent parameters

Table S3.4: Drug-dependent parameters of the final rifampicin model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
BP	-	0.89	0.895 (0.89–0.9)	Literature	Blood to plasma ratio
fu	%	17	17 (11–17.5)	Literature	Fraction unbound plasma
Lipophilicity	-	2.5	2.5 (1.3–2.7)	Literature	Lipophilicity
MW	g mol <sup>-1</sup>	822.9	822.9	Literature	Molecular weight
pKa	-	1.7	4.8 (1.7–7.9)	Literature	Acid dissociation constant (acidic)
	-	7.9	4.8 (1.7–7.9)	Literature	Acid dissociation constant (basic)
Solubility	mg l <sup>-1</sup>	2800	2670 (1100–3350)	Literature	Solubility at pH=7.5
<b>Enzymes</b>					
AADAC k <sub>cat</sub>	min <sup>-1</sup>	9.87	9.87	Literature	AADAC catalytic rate constant
AADAC K <sub>M</sub>	μmol l <sup>-1</sup>	195.1	195.1	Literature	AADAC Michaelis-Menten constant
<b>Transporters</b>					
OATP1B1 ( <i>SLCO1B1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	7.8	7.8	Literature	OATP1B1 catalytic rate constant
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	1.5	1.5	Literature	OATP1B1 Michaelis-Menten constant
P-gp ( <i>ABCB1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	0.61	0.61	Literature	P-gp catalytic rate constant
P-gp ( <i>ABCB1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	55	55	Literature	P-gp Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	36	36 (14–461)	Literature	Concentration for half-maximal BCRP competitive inhibition

Table S3.4: Drug-dependent parameters of the final rifampicin model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
K <sub>i</sub> CYP2C8	μmol <sup>-1</sup>	30.2	30.2	Literature	Concentration for half-maximal CYP2C8 competitive inhibition
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	18.5	18.5 (-19.7)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	7.9	33.8 (7.9-144)	Literature	Concentration for half-maximal MRP2 competitive inhibition
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.22	1.235 (0.22-3948)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol <sup>-1</sup>	0.39	0.39	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	169	169 (4.3-279)	Literature	Concentration for half-maximal P-gp competitive inhibition
K <sub>i</sub> UGT1A1	μmol <sup>-1</sup>	33	33	Literature	Concentration for half-maximal UGT1A1 competitive inhibition
K <sub>i</sub> UGT1A3	μmol <sup>-1</sup>	600	600	Literature	Concentration for half-maximal UGT1A3 competitive inhibition
<b>Induction</b>					
E <sub>max</sub> AADAC	-	0.99	0.99	Literature	Maximum in vivo induction effect
E <sub>max</sub> CYP2C8	-	5	3.2 (1.3-5)	Literature	Maximum in vivo induction effect
E <sub>max</sub> CYP3A4	-	9	5 (2-30)	Literature	Maximum in vivo induction effect

Table S3.4: Drug-dependent parameters of the final rifampicin model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
E <sub>max</sub> CYP3A5	-	2	2	Literature	Maximum in vivo induction effect
E <sub>max</sub> OATP1B1 ( <i>SLCO1B1</i> )	-	0.38	0.38	Literature	Maximum in vivo induction effect
E <sub>max</sub> OATP1B3 ( <i>SLCO1B3</i> )	-	0.38	-	Assumed	Maximum in vivo induction effect
E <sub>max</sub> P-gp ( <i>ABCB1</i> )	-	2.5	2.5	Literature	Maximum in vivo induction effect
E <sub>max</sub> PON3	-	0.99	0.99	Assumed	Maximum in vivo induction effect
E <sub>max</sub> UGT1A1	-	1.3	1.3	Literature	Maximum in vivo induction effect
E <sub>max</sub> UGT1A3	-	1.4	1.4	Literature	Maximum in vivo induction effect
Induction EC <sub>50</sub>	μmol <sup>-1</sup>	0.34	-	Literature	Concentration for half-maximal induction
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	1.24e-05	1.24e-05	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	2.93e-05	2.93e-05	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim Standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland

Table S3.5: Extracted drug-dependent parameter literature values for rifampicin

Parameter	Unit	Literature value	Standard deviation	Note	Reference
BP	-	0.9	-	-	[2]
BP	-	0.89	-	-	[2]
fu	%	17	-	-	[2]
fu	%	11	-	-	[2]
fu	%	16	-	-	[2]
fu	%	17	-	-	[2]
fu	%	17.5	-	-	[2]
Lipophilicity	-	2.5	-	-	[2]
Lipophilicity	-	1.3	-	-	[2]
Lipophilicity	-	2.7	-	-	[2]
MW	g mol <sup>-1</sup>	822.9	-	-	[2]
pKa	-	1.7	-	acidic	[2]
pKa	-	7.9	-	basic	[2]
Solubility	mg l <sup>-1</sup>	2800	-	pH=7.5	[2]
Solubility	mg l <sup>-1</sup>	1100	-	pH=6.5	[2]
Solubility	mg l <sup>-1</sup>	1400	-	pH=6.8	[2]
Solubility	mg l <sup>-1</sup>	2540	-	pH=6.8	[2]
Solubility	mg l <sup>-1</sup>	2800	-	pH=7.5	[2]
Solubility	mg l <sup>-1</sup>	3350	-	pH=7.4	[2]
AADAC k <sub>cat</sub>	min <sup>-1</sup>	9.87	-	-	[2]
AADAC K <sub>M</sub>	μmol l <sup>-1</sup>	195.1	-	-	[2]
OATP1B1 ( <i>SLCO1B1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	7.8	-	-	[2]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	1.5	-	-	[2]
P-gp ( <i>ABCB1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	0.61	-	-	[2]
P-gp ( <i>ABCB1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	55	-	-	[2]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	56	-	IC50	[157]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	461	-	IC50	[158]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	18.8	-	K <sub>i</sub>	[159]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	36	-	K <sub>i</sub>	[159]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	14	-	IC50	[160]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	14	-	-	[161]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	36	-	-	[161]
K <sub>i</sub> CYP2C8	-	30.2	-	K <sub>i</sub>	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	19.7	-	IC50	[162]

Table S3.5: Extracted drug-dependent parameter literature values for rifampicin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	18.5	-	K <sub>i</sub>	[163]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	18.5	-	-	[2]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	27	-	IC50	[157]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	144	-	IC50	[158]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	83	-	IC50	[120]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	7.9	-	K <sub>i</sub>	[159]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	40.6	-	K <sub>i</sub>	[159]
K <sub>i</sub> MRP2 ( <i>ABCC2</i> )	μmol <sup>-1</sup>	14.7	-	IC50	[160]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.6	-	IC50	[164]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.99	-	IC50	[165]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.25	-	IC50	[166]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	4.61	-	IC50	[166]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.29	-	IC50	[167]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.77	-	IC50	[168]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	466.8	-	IC50	[169]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3948	-	IC50	[169]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.59	-	IC50	[170]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	5.16	-	IC50	[171]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	4.42	-	K <sub>i</sub>	[171]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3	-	IC50	[172]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.5	-	IC50	[172]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.41	-	K <sub>i</sub>	[173]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.3	-	K <sub>i</sub>	[174]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.22	-	K <sub>i</sub>	[158]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.5	-	IC50	[175]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.3	-	IC50	[176]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.477	-	K <sub>i</sub>	[147]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.75	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.585	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	6.96	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.922	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.694	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.423	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.05	-	K <sub>i</sub>	[178]

Table S3.5: Extracted drug-dependent parameter literature values for rifampicin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.442	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.358	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.07	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.653	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.598	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.952	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.23	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.377	-	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.355	-	IC50	[179]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.79	-	IC50	[180]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.2	-	IC50	[181]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	10.46	-	IC50	[182]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.4	-	IC50	[183]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	4.9	-	IC50	[183]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.56	-	IC50	[183]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	4.1	-	IC50	[183]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	12.2	-	K <sub>i</sub>	[184]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.278	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.391	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.08	-	IC50	[185]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	11.9	-	IC50	[186]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	120	-	IC50	[187]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	32.9	-	IC50	[188]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	22.9	-	IC50	[188]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	-	-	K <sub>i</sub>	[188]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.91	-	IC50	[189]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.3	-	IC50	[149]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.74	-	IC50	[190]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.24	-	IC50	[190]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.9	-	IC50	[191]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	-	-	K <sub>i</sub>	[191]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.6	-	IC50	[160]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.1	-	IC50	[160]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.24	-	IC50	[192]

Table S3.5: Extracted drug-dependent parameter literature values for rifampicin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.29	-	IC50	[192]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.55	-	IC50	[193]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.1	-	IC50	[193]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.66	-	IC50	[194]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.79	-	K <sub>i</sub>	[194]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.6	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.65	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.2	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.62	-	K <sub>i</sub>	[195]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.39	-	IC50	[196]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	5.65	-	IC50	[197]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.88	-	IC50	[197]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.818	-	IC50	[198]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	1.2	-	IC50	[198]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.94	-	IC50	[199]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.2	-	IC50	[200]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	50	-	IC50	[200]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	0.477	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.49	-	IC50	[201]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	3.2	-	IC50	[202]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	17	-	K <sub>i</sub>	[203]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.2	-	IC50	[204]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	6.75	-	K <sub>i</sub>	[161]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	2.4	-	IC50	[205]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	8.8	-	IC50	[152]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	55.29	-	IC50	[206]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol <sup>-1</sup>	0.39	-	-	[195]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	29	-	IC50	[157]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	279	-	IC50	[158]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	4.3	-	K <sub>i</sub>	[159]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	23	-	K <sub>i</sub>	[159]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	175	-	IC50	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	169	-	IC50	[207]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	169	-	-	[2]



Table S3.5: Extracted drug-dependent parameter literature values for rifampicin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> UGT1A1	μmol <sup>-1</sup>	33	-	-	[170]
K <sub>i</sub> UGT1A3	μmol <sup>-1</sup>	600	-	-	[208]
E <sub>max</sub> AADAC	-	0.99	-	-	[2]
E <sub>max</sub> CYP2C8	-	4	-	-	[209]
E <sub>max</sub> CYP2C8	-	1.3	-	-	[210]
E <sub>max</sub> CYP2C8	-	3	-	-	[211]
E <sub>max</sub> CYP2C8	-	5	-	-	[128]
E <sub>max</sub> CYP2C8	-	3.2	-	-	[2]
E <sub>max</sub> CYP3A4	-	3.13	-	-	[212]
E <sub>max</sub> CYP3A4	-	10.5	-	-	[213]
E <sub>max</sub> CYP3A4	-	13	-	-	[209]
E <sub>max</sub> CYP3A4	-	6.2	-	-	[214]
E <sub>max</sub> CYP3A4	-	2.8	-	-	[215]
E <sub>max</sub> CYP3A4	-	2	-	-	[216]
E <sub>max</sub> CYP3A4	-	2	-	-	[217]
E <sub>max</sub> CYP3A4	-	18.5	-	-	[218]
E <sub>max</sub> CYP3A4	-	7.5	2.1	-	[219]
E <sub>max</sub> CYP3A4	-	4.3	-	-	[162]
E <sub>max</sub> CYP3A4	-	7.9	2.9	-	[220]
E <sub>max</sub> CYP3A4	-	4.1	-	-	[221]
E <sub>max</sub> CYP3A4	-	9.6	-	-	[222]
E <sub>max</sub> CYP3A4	-	12	4	-	[65]
E <sub>max</sub> CYP3A4	-	5	-	-	[211]
E <sub>max</sub> CYP3A4	-	2.8	0.5	-	[223]
E <sub>max</sub> CYP3A4	-	2.2	0.3	-	[223]
E <sub>max</sub> CYP3A4	-	3.2	0.2	-	[223]
E <sub>max</sub> CYP3A4	-	5.2	3.3	-	[223]
E <sub>max</sub> CYP3A4	-	10	-	-	[224]
E <sub>max</sub> CYP3A4	-	3.2	2.3	-	[225]
E <sub>max</sub> CYP3A4	-	30	-	-	[226]
E <sub>max</sub> CYP3A4	-	4	-	-	[227]
E <sub>max</sub> CYP3A4	-	14.5	-	-	[228]
E <sub>max</sub> CYP3A4	-	14.6	2	-	[229]
E <sub>max</sub> CYP3A4	-	2	-	-	[230]

Table S3.5: Extracted drug-dependent parameter literature values for rifampicin (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
$E_{\max}$ CYP3A4	-	2	-	-	[230]
$E_{\max}$ CYP3A4	-	10	-	-	[128]
$E_{\max}$ CYP3A4	-	4.12	0.09	-	[231]
$E_{\max}$ CYP3A4	-	14	-	-	[232]
$E_{\max}$ CYP3A4	-	3.8	-	-	[233]
$E_{\max}$ CYP3A4	-	9	-	-	[2]
$E_{\max}$ CYP3A4	-	4	-	-	[234]
$E_{\max}$ CYP3A4	-	5.9	-	-	[235]
$E_{\max}$ CYP3A4	-	8.9	-	-	[235]
$E_{\max}$ CYP3A4	-	3.2	-	-	[235]
$E_{\max}$ CYP3A4	-	2.1	0.3	-	[236]
$E_{\max}$ CYP3A5	-	2	-	-	[230]
$E_{\max}$ OATP1B1 ( <i>SLCO1B1</i> )	-	0.38	-	-	[2]
$E_{\max}$ P-gp ( <i>ABCB1</i> )	-	2.5	-	-	[2]
$E_{\max}$ PON3	-	0.99	-	-	[2]
$E_{\max}$ UGT1A1	-	1.3	-	-	[233]
$E_{\max}$ UGT1A3	-	1.4	-	-	[237]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.37	0.1	-	[238]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.12	0.02	-	[220]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.6	-	-	[239]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.14	0.02	-	[231]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	3.2	-	-	[240]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.34	-	-	[2]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.18	-	-	[241]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.51	-	-	[241]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.18	-	-	[235]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	1.1	-	-	[235]
Induction $EC_{50}$	$\mu\text{mol l}^{-1}$	0.65	-	-	[235]
Specific intest. perm.	$\text{cm min}^{-1}$	1.24e-05	-	-	[2]
Specific organ perm.	$\text{cm min}^{-1}$	2.93e-05	-	-	[2]

*Note:*

If  $IC_{50}$  values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC_{50}$  was assumed

3 Simvastatin DDIs

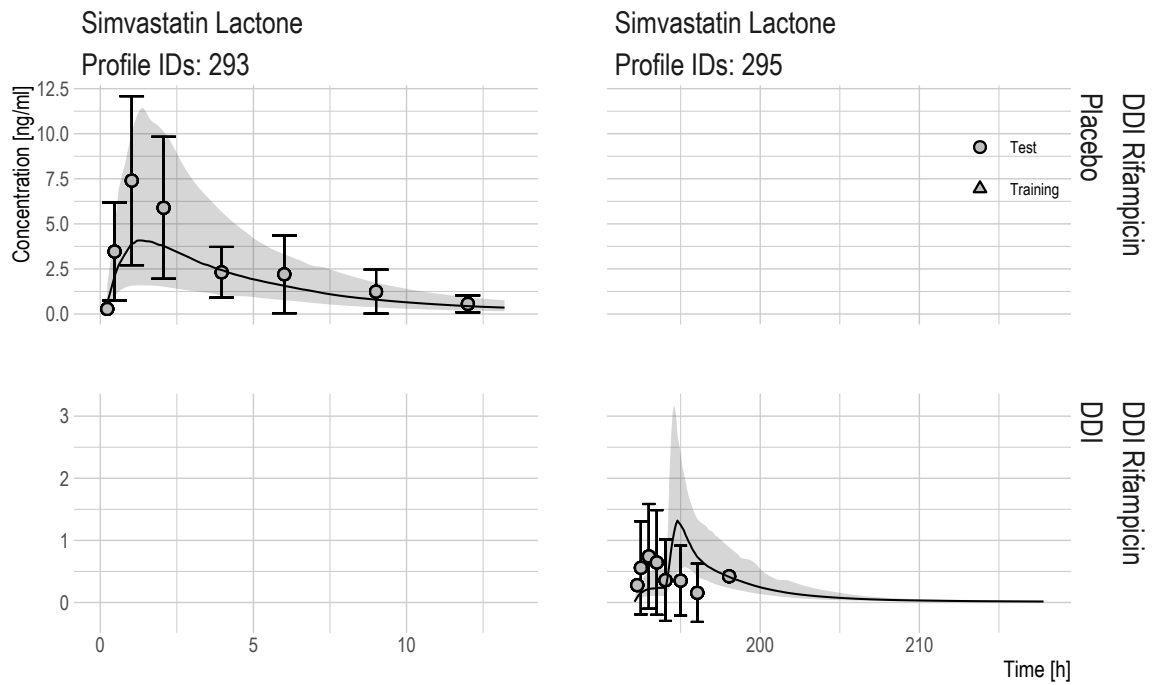


Figure S3.3: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90% CI

3 Simvastatin DDIs

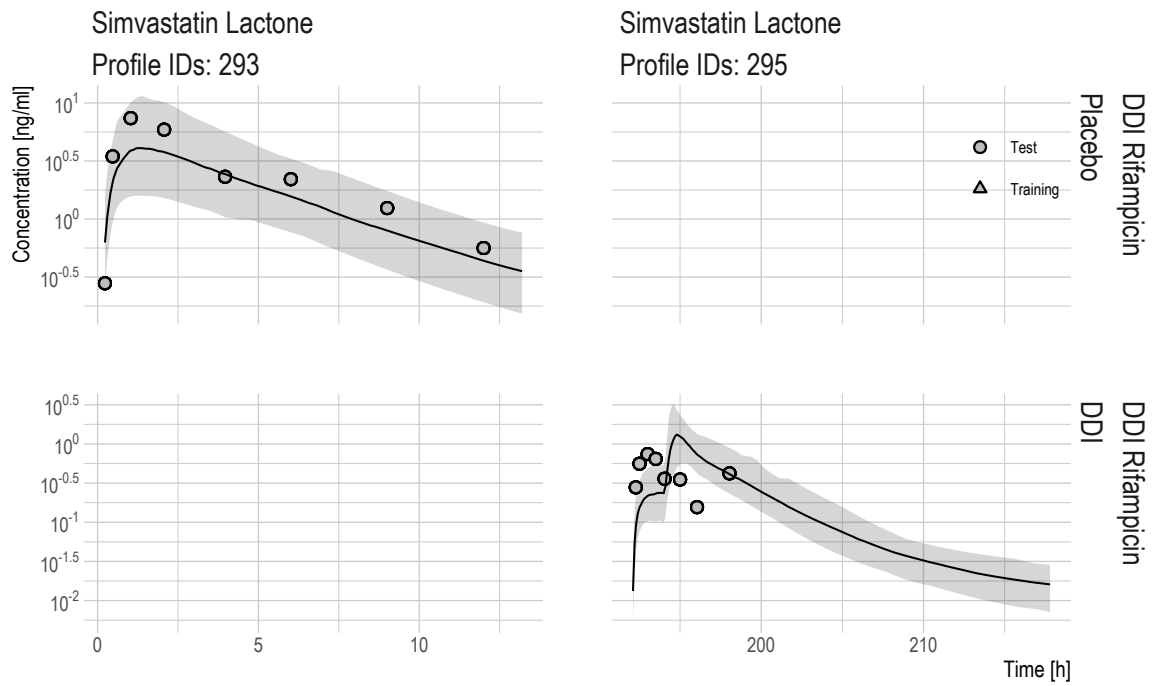


Figure S3.4: Semilogarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90 % CI

## 3 Simvastatin DDIs

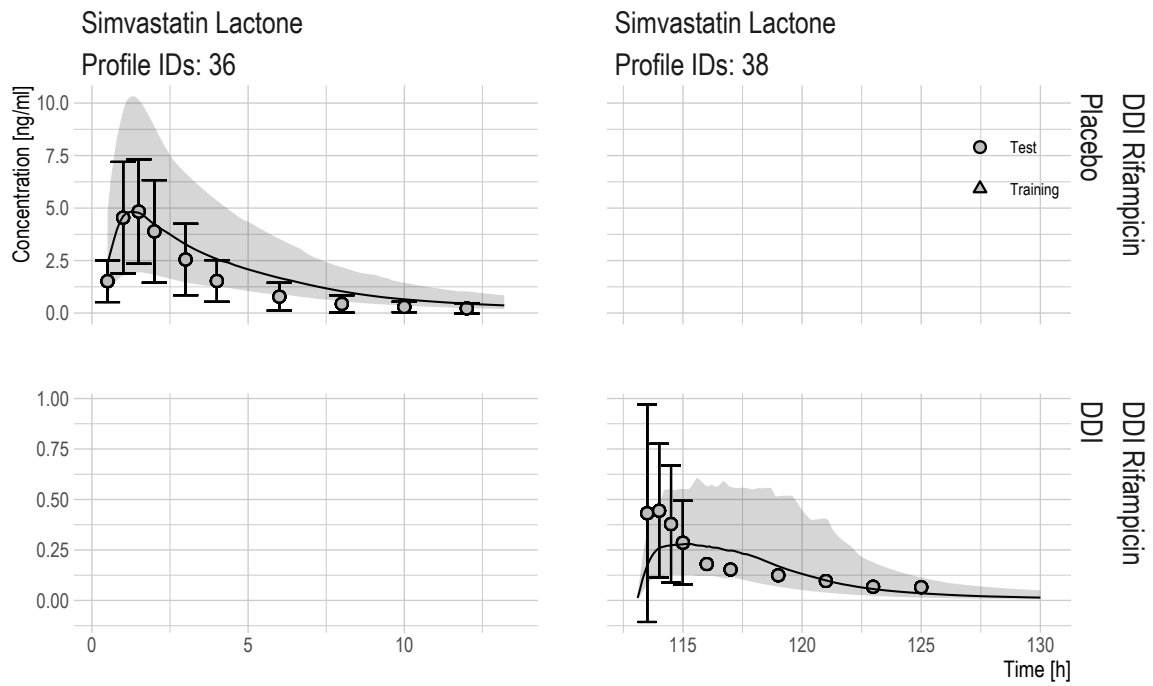


Figure S3.5: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90% CI

3 Simvastatin DDIs

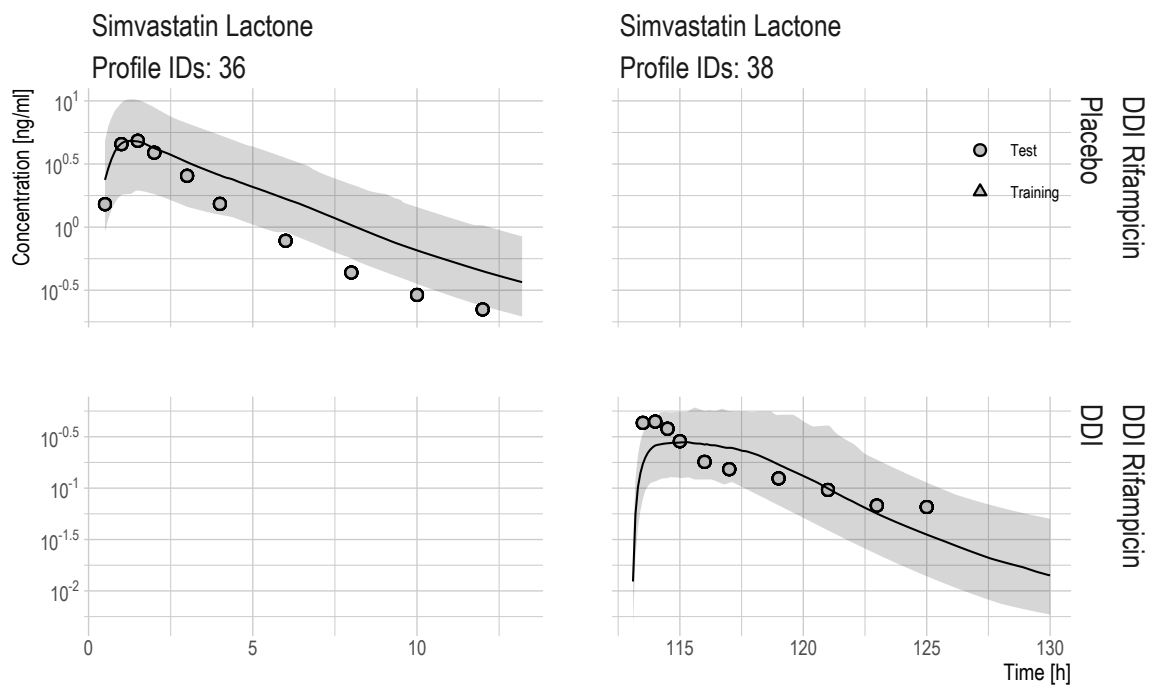


Figure S3.6: Semilogarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin - Simvastatin Lactone. Solid line and shaded area are predicted median and 90 % CI

3 Simvastatin DDIs

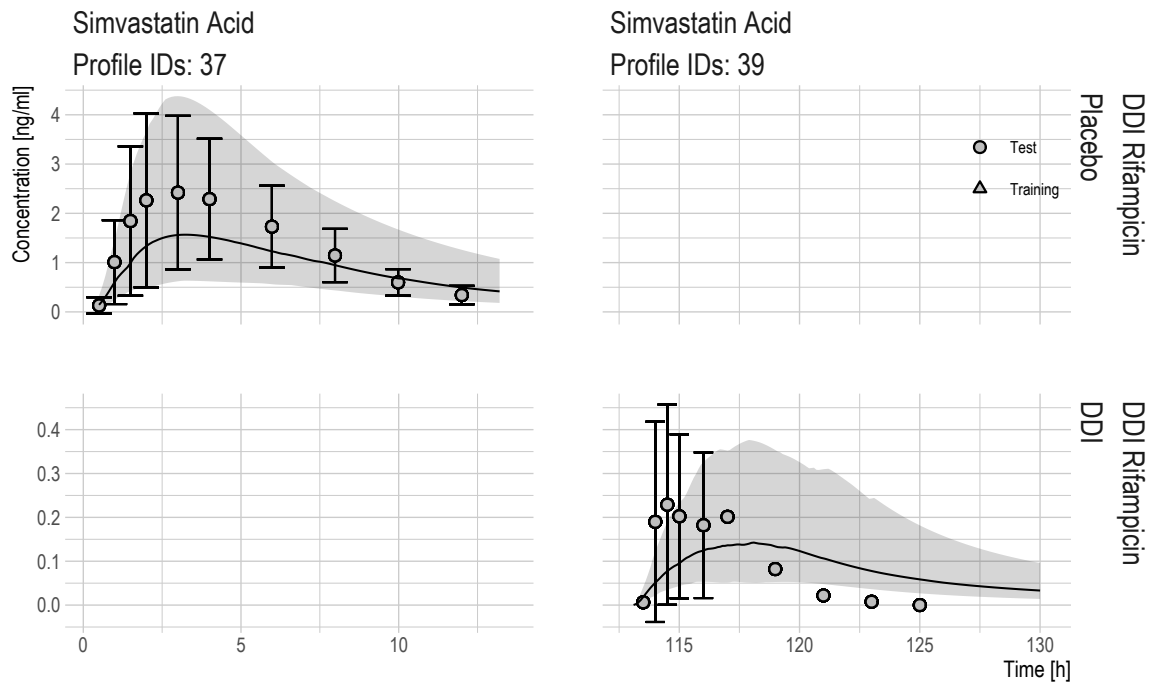


Figure S3.7: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin Simvastatin Acid. Solid line and shaded area are predicted median and 90% CI

3 Simvastatin DDIs

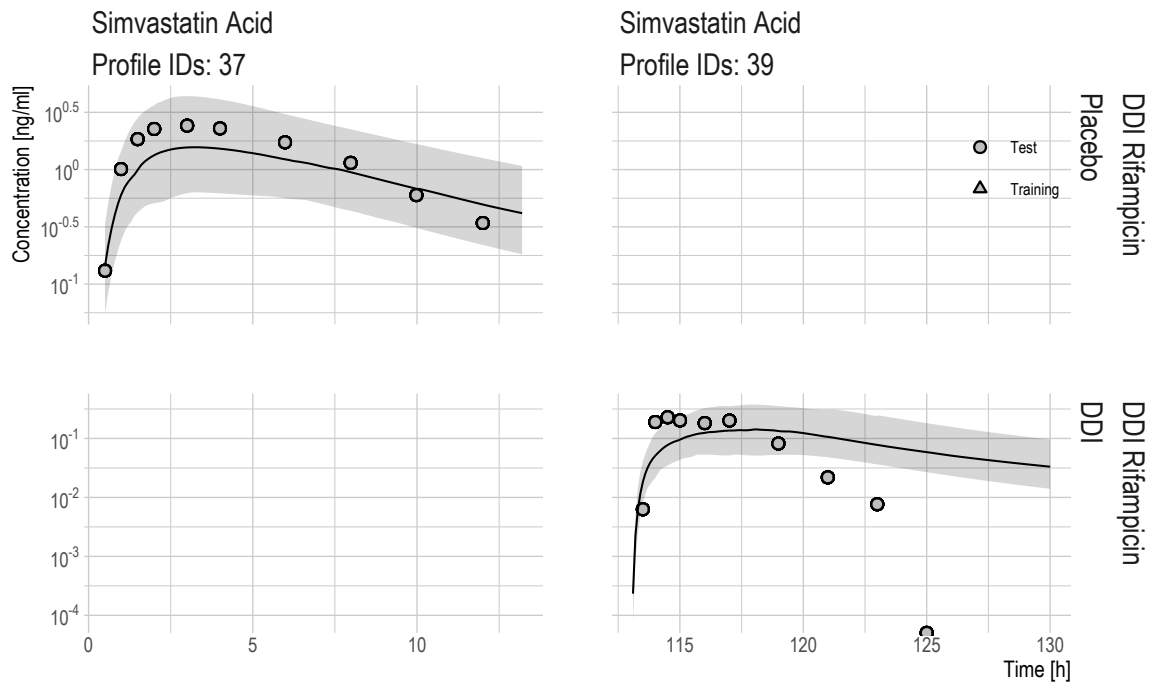


Figure S3.8: Semilogarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Rifampicin Simvastatin Acid. Solid line and shaded area are predicted median and 90 % CI



### 3.4 Itraconazole

The antifungal substance itraconazole and its four metabolites hydroxy-itraconazole, keto-itraconazole and N-desalkyl-itraconazole are inhibitors of CYP3A4, OATP1B1, OATP1B3, P-gp and BCRP [146]. A previously developed itraconazole model was extended and subsequently used to describe the interactions [16]. Unfortunately, as already described by Tsamandouras et al. [39], due to limitation of the assay in the interaction study performed by Neuvonen et al. [145] SL and SA profiles could not be directly compared with model predicted values. However, the authors reportet an at least approximate 10-fold increase in SL AUC and  $C_{\max}$ . Following, this value was used for evaluating the model performance. The parameters of the extended parent-metabolite itraconazole model are given in Tables S3.6 - S3.13.

#### 3.4.1 Drug-dependent parameters

Table S3.6: Drug-dependent parameters in the itraconazole model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	0.6	1.1 (0.2–3.6)	Literature	Fraction unbound plasma
Lipophilicity	-	4.62	5.14 (4.62–5.66)	Literature	Lipophilicity
MW	g mol <sup>-1</sup>	705.6	705.6	Literature	Molecular weight
pKa	-	3.7	3.7	Literature	Acid dissociation constant (basic)
Solubility	mg l <sup>-1</sup>	14.5	1.58 (0.69–14.5)	Literature	Solubility
<b>Enzymes</b>					
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.04	-	Literature	CYP3A4 catalytic rate constant
CYP3A4 K <sub>m</sub>	μmol l <sup>-1</sup>	0.00207	-	Literature	CYP3A4 Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	10	2 (1.9–10)	Literature	Concentration for half-maximal BCRP competitive inhibition
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.0013	0.042 (0.0013–7)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.008	1.7 (0.008–9.5)	Literature	Concentration for half-maximal P-gp competitive inhibition
<b>Formulation</b>					
Dissolution shape	-	1.1	0.96 (0.82–1.1)	Optimized	Weibull function dissolution shape
Dissolution time (50% dissolved)	min	407	273 (139–407)	Optimized	Weibull function dissolution time (50% dissolved)
<b>System</b>					

Table S3.6: Drug-dependent parameters in the itraconazole model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
Specific intest. perm.	cm min <sup>-1</sup>	0.000533	0.000533	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.0144	0.0144	Literature	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim Standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland; formulation parameter values were used for solid oral dosage forms only

Table S3.7: Extracted drug-dependent literature values for itraconazole

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	0.6	-	-	[2]
fu	%	0.2	-	-	[2]
fu	%	1.6	-	-	[2]
fu	%	3.6	-	-	[2]
Lipophilicity	-	4.62	-	-	[2]
Lipophilicity	-	5.66	-	-	[2]
MW	g mol <sup>-1</sup>	705.6	-	-	[2]
pKa	-	3.7	-	-	[2]
Solubility	mg l <sup>-1</sup>	8	-	FaSSIF	[2]
Solubility	mg l <sup>-1</sup>	1.58	-	capsule fasted	[2]
Solubility	mg l <sup>-1</sup>	14.5	-	capsule fasted	[2]
Solubility	mg l <sup>-1</sup>	0.69	-	capsule fed	[2]
Solubility	mg l <sup>-1</sup>	0.78	-	-	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.04	-	-	[2]
CYP3A4 K <sub>M</sub>	-	3.09	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00207	-	-	[2]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	2	-	-	[242]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	10	-	-	[150]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	1.9	-	-	[146]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.076	-	-	[243]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.7	0.2	-	[244]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.044	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.016	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.045	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.012	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.013	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.016	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.016	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.052	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.013	-	-	[116]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.5	-	-	[245]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	3.12	-	-	[245]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.32	-	-	[245]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.23	-	-	[245]

Table S3.7: Extracted drug-dependent literature values for itraconazole (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0031	-	-	[105]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0038	-	-	[105]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0014	-	-	[105]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0047	-	-	[105]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0013	-	-	[246]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.04	-	-	[247]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	7	-	-	[248]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	2	-	-	[248]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.49	-	-	[249]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	1	-	-	[249]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.0013	-	-	[2]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	2.3	-	-	[250]
K <sub>i</sub> CYP3A4	μmol <sup>-1</sup>	0.03	-	-	[251]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	6	-	-	[153]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	2	-	-	[153]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.16	-	-	[252]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	1.25	-	-	[122]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.41	-	-	[154]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.46	-	-	[154]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	2	-	-	[242]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	1.8	-	-	[123]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	9.5	-	-	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	6.7	-	-	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.45	-	-	[124]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.008	-	-	[2]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	0.048	-	-	[146]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	1.7	-	-	[43]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	2.4	-	-	[43]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol <sup>-1</sup>	1.7	-	-	[253]
Dissolution shape	-	0.82	-	capsule fed	[2]
Dissolution shape	-	1.1	-	capsule fasted	[2]
Dissolution time (50 % dissolved)	min	139	-	capsule fed	[2]

Table S3.7: Extracted drug-dependent literature values for itraconazole (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
Dissolution time (50 % dissolved)	min	407	-	capsule fasted	[2]
Specific intest. perm.	cm min <sup>-1</sup>	0.000533	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.0144	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

Table S3.8: Drug-dependent parameters in the hydroxy-itraconazole model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
$f_u$	%	1.7	1.7	Literature	Fraction unbound plasma
Lipophilicity	-	3.72	4.11 (3.72–4.5)	Literature	Lipophilicity
MW	$\text{g mol}^{-1}$	721.6	721.6	Literature	Molecular weight
pKa	-	3.7	3.7	Literature	Acid dissociation constant (basic)
<b>Enzymes</b>					
CYP3A4 $k_{\text{cat}}$	$\text{min}^{-1}$	0.02	0.02	Literature	CYP3A4 catalytic rate constant
CYP3A4 $K_M$	$\mu\text{mol l}^{-1}$	0.00417	0.01558 (0.00417–0.027)	Literature	CYP3A4 Michaelis-Menten constant
<b>Inhibition</b>					
$K_i$ BCRP ( <i>ABCG2</i> )	$\mu\text{mol l}^{-1}$	12	5.7 (0.44–12)	Literature	Concentration for half-maximal BCRP competitive inhibition
$K_i$ CYP3A4	$\mu\text{mol l}^{-1}$	0.0144	0.0378 (0.0144–6.3)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
$K_i$ OATP1B1 ( <i>SLCO1B1</i> )	$\mu\text{mol l}^{-1}$	0.23	0.23 (0.018–5.9)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
$K_i$ OATP1B3 ( <i>SLCO1B3</i> )	$\mu\text{mol l}^{-1}$	0.1	0.1	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
$K_i$ P-gp ( <i>ABCB1</i> )	$\mu\text{mol l}^{-1}$	0.49	3.3 (0.49–7)	Literature	Concentration for half-maximal P-gp competitive inhibition
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder

Table S3.8: Drug-dependent parameters in the hydroxy-itraconazole model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	1.52e-05	1.52e-05	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.00155	0.00155	Literature	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland



Table S3.9: Extracted drug-dependent literature values for hydroxy-itraconazole

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	1.7	-	-	[2]
Lipophilicity	-	3.72	-	-	[2]
Lipophilicity	-	4.5	-	-	[2]
MW	g mol <sup>-1</sup>	721.6	-	-	[2]
pKa	-	3.7	-	(basic)	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.02	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00417	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.027	-	-	[2]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	12	-	IC50	[242]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	5.7	-	IC50	[150]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	0.44	-	IC50	[146]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	6.3	-	K <sub>i</sub>	[246]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.0144	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.0378	-	K <sub>i</sub>	[250]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	5.9	-	IC50	[150]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	0.018	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	0.23	-	IC50	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	0.23	-	IC50	[146]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	0.1	-	-	[146]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	5	-	IC50	[242]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	7	-	IC50	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	1.6	-	IC50	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.49	-	IC50	[146]
Specific intest. perm.	cm min <sup>-1</sup>	1.52e-05	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.00155	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

Table S3.10: Drug-dependent parameters in the keto-itraconazole model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	1	1	Literature	Fraction unbound plasma
Lipophilicity	-	4.21	4.355 (4.21–4.5)	Literature	Lipophilicity
MW	g mol <sup>-1</sup>	719.6	719.6	Literature	Molecular weight
pKa	-	3.7	3.7	Literature	Acid dissociation constant (basic)
<b>Enzymes</b>					
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.393	0.393	Literature	CYP3A4 catalytic rate constant
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00222	0.00222	Literature	CYP3A4 Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	2.1	1.1 (0.1–2.1)	Literature	Concentration for half-maximal BCRP competitive inhibition
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.00512	0.03 (0.00512–0.098)	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	0.29	5.195 (0.29–10.1)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	0.088	0.088	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.49	2.2 (0.12–2.5)	Literature	Concentration for half-maximal P-gp competitive inhibition
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder

Table S3.10: Drug-dependent parameters in the keto-itraconazole model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	4.79e-05	4.79e-05	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.00492	0.00492	Literature	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland

Table S3.11: Extracted drug-dependent literature values for keto-itraconazole

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	1	-	-	[2]
Lipophilicity	-	4.21	-	-	[2]
Lipophilicity	-	4.5	-	-	[2]
MW	g mol <sup>-1</sup>	719.6	-	-	[2]
pKa	-	3.7	-	(basic)	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.393	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00222	-	-	[2]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	2.1	-	IC50	[150]
K <sub>i</sub> BCRP ( <i>ABCG2</i> )	μmol l <sup>-1</sup>	0.1	-	IC50	[146]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.098	-	IC50	[254]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.053	-	IC50	[246]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.007	-	IC50	[246]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.00512	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	10.1	-	IC50	[150]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	0.29	-	IC50	[146]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	0.088	-	-	[146]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	2.5	-	IC50	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	2.2	-	IC50	[150]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.12	-	IC50	[146]
Specific intest. perm.	cm min <sup>-1</sup>	4.79e-05	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.00492	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

Table S3.12: Drug-dependent parameters in the n-desalkyl-itraconazole model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	1.1	1.1	Literature	Fraction unbound plasma
Lipophilicity	-	5.18	4.69 (4.2–5.18)	Literature	Lipophilicity
MW	g mol <sup>-1</sup>	649.5	649.5	Literature	Molecular weight
pKa	-	3.7	3.7	Literature	Acid dissociation constant (basic)
<b>Enzymes</b>					
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.061	0.061	Literature	CYP3A4 catalytic rate constant
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00063	0.00063	Literature	CYP3A4 Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.00032	0.00032	Literature	Concentration for half-maximal CYP3A4 competitive inhibition
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	7.5	7.5	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	2.1	2.1	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.26	0.26	Literature	Concentration for half-maximal P-gp competitive inhibition
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine

Table S3.12: Drug-dependent parameters in the n-desalkyl-itraconazole model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
Specific intest. perm.	cm min <sup>-1</sup>	0.000737	0.000737	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.0891	0.0891	Literature	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permeabilities calculation method: PK-Sim standard; organ-plasma partition coefficient calculation method: Rodgers and Rowland

Table S3.13: Extracted drug-dependent literature values for n-desalkyl-itraconazole

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	1.1	-	-	[2]
Lipophilicity	-	5.18	-	-	[2]
Lipophilicity	-	4.2	-	-	[2]
MW	g mol <sup>-1</sup>	649.5	-	-	[2]
pKa	-	3.7	-	(basic)	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	0.061	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	0.00063	-	-	[2]
K <sub>i</sub> CYP3A4	μmol l <sup>-1</sup>	0.00032	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	7.5	-	IC50	[150]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	2.1	-	-	[146]
K <sub>i</sub> P-gp ( <i>ABCB1</i> )	μmol l <sup>-1</sup>	0.26	0.05	IC50	[146]
Specific intest. perm.	cm min <sup>-1</sup>	0.000737	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.0891	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

### 3.5 Gemfibrozil

Gemfibrozil is an antihyperlipidaemic drug and together with its metabolite gemfibrozil 1-O- $\beta$ -glucuronide they are strong inhibitors of CYP2C8 and OATP1B1 [255, 2]. It has to be noted that at the beginning, the parent-metabolite gemfibrozil model was not capable to describe the observed decrease of SL and increase of SA exposure under gemfibrozil co-treatment. This effect is visible in humans as well as in animals [142, 128, 256]. In a study performed by Prueksaritanont et al. [256] in dogs, SA AUC under gemfibrozil treatment is increased by 160 % accompanied with an SL AUC decrease of 51 %. For this reason, it was assumed that gemfibrozil additionally induces PON3. Although not investigated for PON3, at least this has previously been shown for paraoxonase 1 (PON1) [257]. Following, for the simvastatin co-treatment with gemfibrozil an increase of the PON3 activity by 59 % was assumed which reflects the increase observed for PON1. The parameters of the extended gemfibrozil model are given in Tables S3.14 - S3.17. Results of the simvastatin-gemfibrozil DDI interaction are shown in Figs. S3.9 - S3.12.

#### 3.5.1 Drug-dependent parameters



Table S3.14: Drug-dependent parameters of the final gemfibrozil model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	0.648	2.1 (0.648-3)	Literature	Fraction unbound plasma
Lipophilicity	-	2.8	4.3 (2.8-4.77)	Literature	Lipophilicity
MW	-	250.3	250.3	Literature	Molecular weight
pKa	-	4.7	4.7	Literature	Acid dissociation constant
Solubility	mg l <sup>-1</sup>	170	170	Literature	Solubility at pH=5.9
<b>Enzymes</b>					
UGT2B7 k <sub>cat</sub>	min <sup>-1</sup>	51.98	51.98	Literature	UGT2B7 catalytic rate constant
UGT2B7 K <sub>M</sub>	μmol l <sup>-1</sup>	2.2	2.2	Literature	UGT2B7 Michaelis-Menten constant
<b>Transporters</b>					
Active hepatic uptake k <sub>cat</sub>	min <sup>-1</sup>	59.42	59.42	Literature	Active hepatic uptake catalytic rate constant
Active hepatic uptake K <sub>M</sub>	μmol l <sup>-1</sup>	2.39	2.39	Literature	Active hepatic uptake Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> CYP2C8	μmol l <sup>-1</sup>	9.3	19.85 (9.3-30.4)	Literature	Concentration for half-maximal CYP2C8 competitive inhibition
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	4	35.8 (4-381)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	10	10	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>i</sub> UGT1A1	μmol l <sup>-1</sup>	36	36	Literature	Concentration for half-maximal UGT1A1 competitive inhibition

Table S3.14: Drug-dependent parameters of the final gemfibrozil model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
K <sub>i</sub> UGT1A3	μmol <sup>-1</sup>	37.6	37.6	Literature	Concentration for half-maximal UGT1A3 competitive inhibition
<b>Induction</b>					
PON3 factor	-	1.59	1.59	Literature	Assumed PON3 induction under gemfibrozil co-treatment
<b>Formulation</b>					
Dissolution shape	-	1.56	1.56	Literature	Weibull function dissolution shape
Dissolution time (50 % dissolved)	min	24.45	24.45	Literature	Weibull function dissolution time (50% dissolved)
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	0.00662	0.00662	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.07	0.07	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permeabilities calculation method: Charge-dependent Schmitt; organ-plasma partition coefficient calculation method: Berezhkovskiy; formulation parameter values were used for solid oral dosage forms only

Table S3.15: Extracted drug-dependent literature values for gemfibrozil

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	0.648	-	-	[2]
fu	%	2.1	-	-	[2]
fu	%	3	-	-	[2]
Lipophilicity	-	2.8	-	-	[2]
Lipophilicity	-	4.3	-	-	[2]
Lipophilicity	-	4.77	-	-	[2]
MW	-	250.3	-	-	[2]
pKa	-	4.7	-	-	[2]
Solubility	mg l <sup>-1</sup>	170	-	pH 5.9	[2]
UGT2B7 k <sub>cat</sub>	min <sup>-1</sup>	51.98	-	-	[2]
UGT2B7 K <sub>M</sub>	μmol l <sup>-1</sup>	2.2	-	-	[2]
Active hepatic uptake k <sub>cat</sub>	min <sup>-1</sup>	59.42	-	-	[2]
Active hepatic uptake K <sub>M</sub>	μmol l <sup>-1</sup>	2.39	-	-	[2]
K <sub>i</sub> CYP2C8	μmol l <sup>-1</sup>	30.4	-	K <sub>i</sub>	[2]
K <sub>i</sub> CYP2C8	μmol l <sup>-1</sup>	9.3	-	K <sub>i</sub>	[114]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	7.4	-	IC50	[258]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	25.2	-	K <sub>i</sub>	[147]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	25	-	IC50	[259]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	173	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	26.4	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	381	-	IC50	[177]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	26.4	2.1	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	381	60	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	173	34	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	58.8	10.7	K <sub>i</sub> Pitavastatin	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	46	8.9	K <sub>i</sub> Atorvastatin	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	72.7	8.7	K <sub>i</sub> Fluvastatin	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	63.6	8.4	K <sub>i</sub> Rosuvastatin	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	9.65	2.79	K <sub>i</sub> Pravastatin	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	48.3	18.6	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	252	100	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	29.6	5.2	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	36.6	5.8	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	13.4	0.3	K <sub>i</sub>	[178]

Table S3.15: Extracted drug-dependent literature values for gemfibrozil (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	49.5	10.8	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	31.4	4.3	K <sub>i</sub>	[178]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	18.1	-	K <sub>i</sub>	[181]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	68.05	-	K <sub>i</sub>	[181]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	31.5	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	89.5	-	K <sub>i</sub>	[148]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	35.8	-	K <sub>i</sub>	[260]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	15.5	-	K <sub>i</sub>	[260]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	75	-	IC50	[149]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	4	-	IC50	[261]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	27	-	IC50	[262]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	32	-	IC50	[263]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	42	-	IC50	[263]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	100	-	IC50	[263]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	18	-	IC50	[263]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	19	-	IC50	[263]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	41.4	-	IC50	[193]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	72.4	-	IC50	[264]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	27.5	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	200	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	38	-	IC50	[121]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	33.7	-	IC50	[198]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	32.9	-	IC50	[198]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	25.2	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	58	-	K <sub>i</sub>	[204]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol <sup>-1</sup>	12.5	-	K <sub>i</sub>	[265]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol <sup>-1</sup>	10	-	-	[266]
K <sub>i</sub> UGT1A1	μmol <sup>-1</sup>	36	-	-	[267]
K <sub>i</sub> UGT1A3	μmol <sup>-1</sup>	37.6	-	-	[268]
PON3 factor	-	1.59	-	-	[257]
Dissolution shape	-	1.56	-	-	[2]
Dissolution time (50 % dissolved)	min	24.45	-	-	[2]
Specific intest. perm.	cm min <sup>-1</sup>	0.00662	-	-	[2]

## 3 Simvastatin DDIs

Table S3.15: Extracted drug-dependent literature values for gemfibrozil (*continued*)

Parameter	Unit	Literature value	Standard deviation	Note	Reference
Specific organ perm.	cm min <sup>-1</sup>	0.07	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed

Table S3.16: Drug-dependent parameters of the final gemfibrozil glucuronide model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	11.5	14.3 (11.5–17.1)	Literature	Fraction unbound plasma
Lipophilicity	-	1.41	1.83 (1.22–2.44)	Literature	Lipophilicity
MW	-	426.5	426.5	Literature	Molecular weight
pKa	-	2.68	2.68	Literature	Acid dissociation constant
Solubility	mg l <sup>-1</sup>	789	789	Literature	Solubility at pH=7
<b>Transporters</b>					
MRP2 ( <i>ABCC2</i> ) k <sub>cat</sub>	min <sup>-1</sup>	7.13	7.13	Literature	MRP2 ( <i>ABCC2</i> ) catalytic rate constant
MRP2 ( <i>ABCC2</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	21.49	21.49	Literature	MRP2 ( <i>ABCC2</i> ) Michaelis-Menten constant
OATP1B1 ( <i>SLCO1B1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	15.36	15.36	Literature	OATP1B1 ( <i>SLCO1B1</i> ) catalytic rate constant
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	0.43	0.43	Literature	OATP1B1 ( <i>SLCO1B1</i> ) Michaelis-Menten constant
<b>Inhibition</b>					
K <sub>i</sub> CYP2C8	μmol l <sup>-1</sup>	20	20	Literature	Concentration for half-maximal CYP2C8 inactivation (MBI)
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	7.9	22.6 (7.9–24.3)	Literature	Concentration for half-maximal OATP1B1 competitive inhibition
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	74	74	Literature	Concentration for half-maximal OATP1B3 competitive inhibition
K <sub>inact</sub> CYP2C8	min <sup>-1</sup>	0.21	0.21	Literature	Maximum inactivation rate constant (MBI)
<b>System</b>					
EHC	-	1	-	Assumed	Fraction of bile continually released from the gallbladder

Table S3.16: Drug-dependent parameters of the final gemfibrozil glucuronide model compared to literature values (*continued*)

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
GFR	-	1	-	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	5.98e-07	5.98e-07	Calculated	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.000122	0.000122	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: PK-Sim Standard; organ-plasma partition coefficient calculation method: PK-Sim Standard

Table S3.17: Extracted drug-dependent literature values for gemfibrozil glucuronide

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	11.5	-	-	[2]
fu	%	17.1	-	-	[2]
Lipophilicity	-	1.22	-	-	[2]
Lipophilicity	-	2.44	-	-	[2]
MW	-	426.5	-	-	[2]
pKa	-	2.68	-	-	[2]
Solubility	mg l <sup>-1</sup>	789	-	pH 7	[2]
MRP2 ( <i>ABCC2</i> ) k <sub>cat</sub>	min <sup>-1</sup>	7.13	-	-	[2]
MRP2 ( <i>ABCC2</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	21.49	-	-	[2]
OATP1B1 ( <i>SLCO1B1</i> ) k <sub>cat</sub>	min <sup>-1</sup>	15.36	-	-	[2]
OATP1B1 ( <i>SLCO1B1</i> ) K <sub>M</sub>	μmol l <sup>-1</sup>	0.43	-	-	[2]
K <sub>i</sub> CYP2C8	μmol l <sup>-1</sup>	20	-	-	[2]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	22.6	-	K <sub>i</sub>	[147]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	9.3	-	K <sub>i</sub>	[260]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	7.9	2.1	K <sub>i</sub>	[260]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	24.3	-	IC50	[264]
K <sub>i</sub> OATP1B1 ( <i>SLCO1B1</i> )	μmol l <sup>-1</sup>	22.6	-	-	[2]
K <sub>i</sub> OATP1B3 ( <i>SLCO1B3</i> )	μmol l <sup>-1</sup>	74	-	-	[266]
K <sub>i</sub> UGT1A1	μmol l <sup>-1</sup>	69	-	-	[269]
K <sub>inact</sub> CYP2C8	min <sup>-1</sup>	0.21	-	-	[2]
Specific intest. perm.	cm min <sup>-1</sup>	5.98e-07	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.000122	-	-	[2]

*Note:*

If IC50 values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC50$  was assumed



## 3 Simvastatin DDIs

## 3.5.2 Profiles

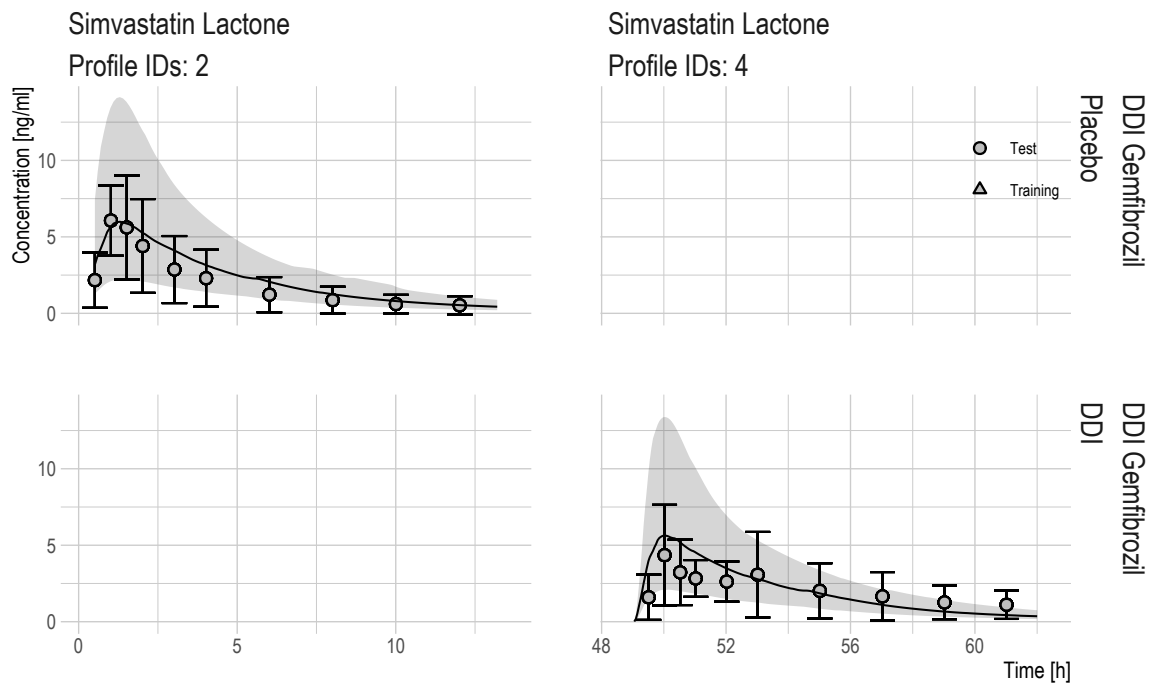


Figure S3.9: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Gemfibrozil - Simvastatin Lactone. Solid line and shaded area are predicted median and 90 % CI

3 Simvastatin DDIs

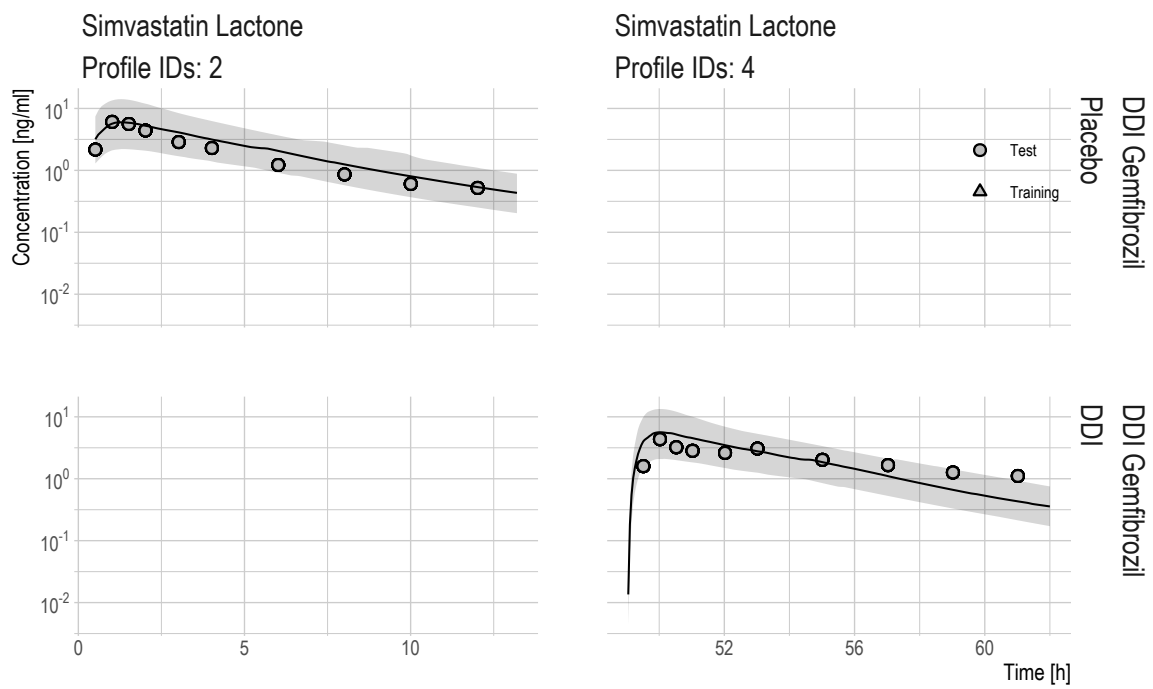


Figure S3.10: Semilogarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Gemfibrozil - Simvastatin Lactone. Solid line and shaded area are predicted median and 90% CI

3 Simvastatin DDIs

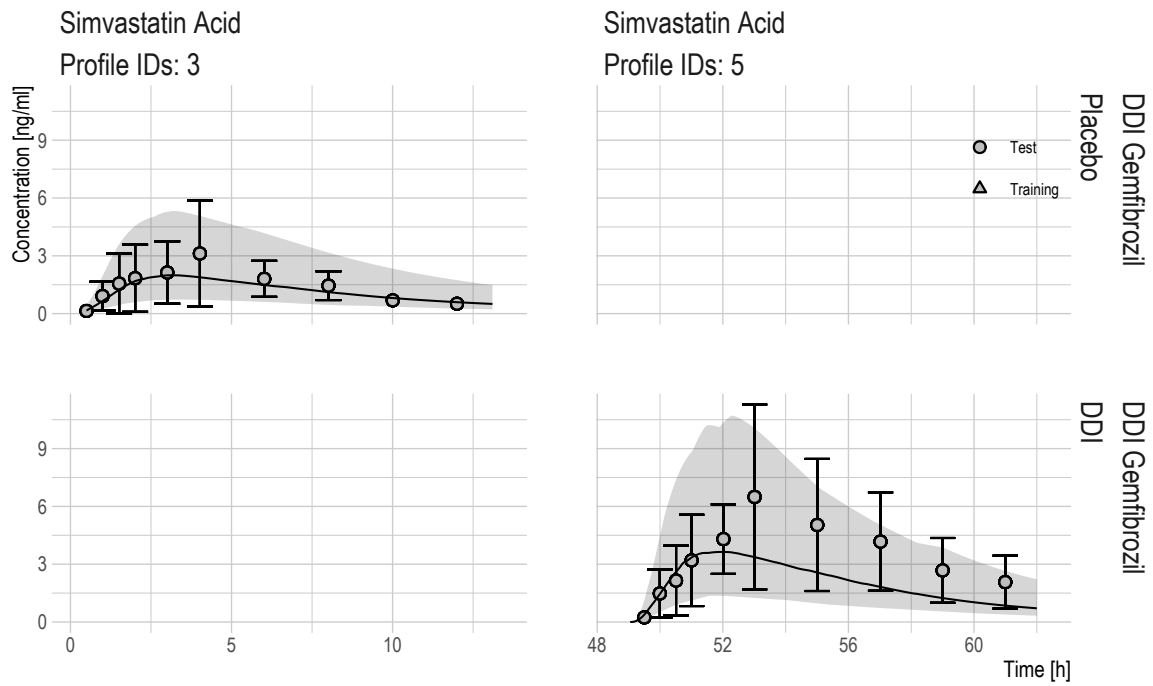


Figure S3.11: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Gemfibrozil - Simvastatin Acid. Solid line and shaded area are predicted median and 90% CI

3 Simvastatin DDIs

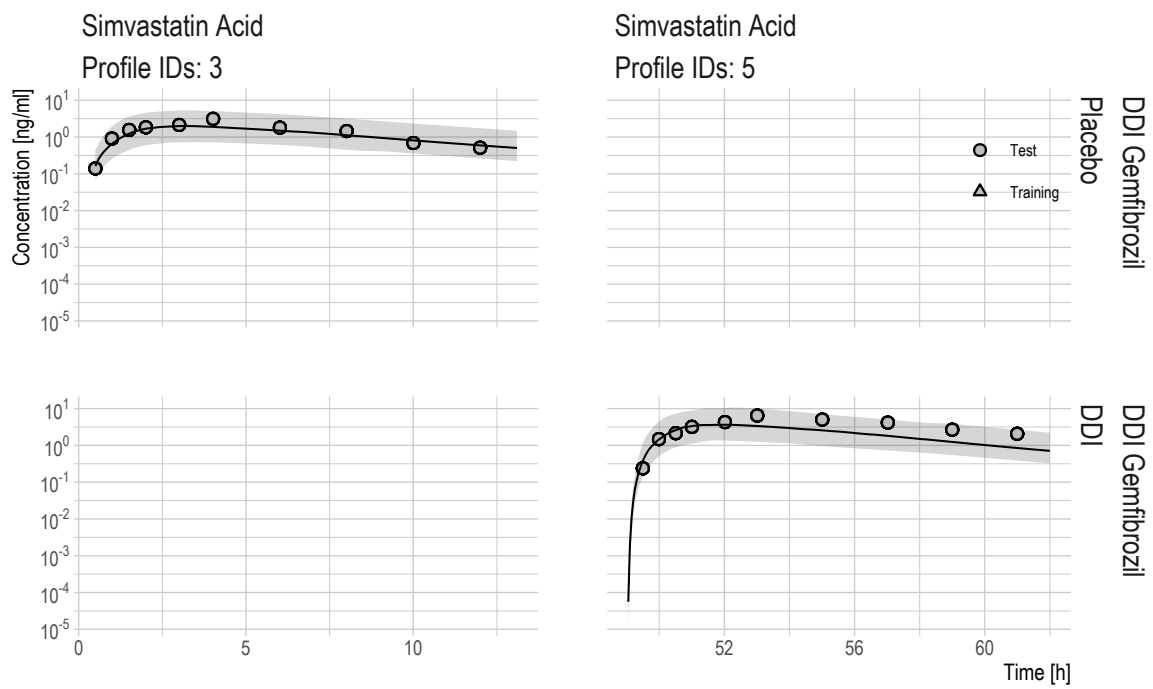


Figure S3.12: Semilogarithmic VPCs of plasma concentration-time profiles for investigated DDIs: Gemfibrozil - Simvastatin Acid. Solid line and shaded area are predicted median and 90 % CI

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### 3 Simvastatin DDIs

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## 3.6 Midazolam

Midazolam is a CYP3A4 probe drug. In a study performed by Prueksaritanont et al. [141] it was investigated if simvastatin would affect the PK of midazolam. For this reason a previously established midazolam model was used and coupled with the newly developed simvastatin model. The parameters of the midazolam model are given in Tables S3.18 and S3.19. Results of the midazolam-simvastatin DDI interaction are shown in Fig. S3.13.

### 3.6.1 Drug-dependent parameters

Table S3.18: Drug-dependent parameters in the midazolam model compared to literature values

Parameter	Unit	Model value	Median (range) literature values	Origin	Description
<b>Molecule</b>					
fu	%	1.6	1.6	Literature	Fraction unbound plasma
Lipophilicity	-	3.13	3.13	Literature	Lipophilicity
MW	-	325.8	325.8	Literature	Molecular weight
pKa	-	6.15	6.15	Literature	Acid dissociation constant (basic)
Solubility	mg l <sup>-1</sup>	49	49	Literature	Solubility at pH=6.5
<b>Enzymes</b>					
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	13	13	Literature	CYP3A4 catalytic rate constant
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	2.73	2.73	Literature	CYP3A4 Michaelis-Menten constant
<b>System</b>					
CL <sub>Ren</sub>	ml min <sup>-1</sup>	100	-	Literature	Renal plasma clearance
EHC	-	1	1	Assumed	Fraction of bile continually released from the gallbladder
GFR	-	1	1	Assumed	Fraction of filtered drug reaching the urine
Specific intest. perm.	cm min <sup>-1</sup>	2e-06	2e-06	Literature	Permeation across intestinal mucosa normalized to surface area
Specific organ perm.	cm min <sup>-1</sup>	0.007	0.007	Calculated	Permeation across cell membranes normalized to surface area

*Note:*

Cellular permabilites calculation method: Charge-dependent Schmitt; organ-plasma partition coefficient calculation method: Rodgers and Rowland

Table S3.19: Extracted drug-dependent literature values for midazolam

Parameter	Unit	Literature value	Standard deviation	Note	Reference
fu	%	1.6	-	-	[2]
Lipophilicity	-	3.13	-	-	[2]
MW	-	325.8	-	-	[2]
pKa	-	6.15	-	-	[2]
Solubility	mg l <sup>-1</sup>	49	-	-	[2]
CYP3A4 k <sub>cat</sub>	min <sup>-1</sup>	13	-	-	[2]
CYP3A4 K <sub>M</sub>	μmol l <sup>-1</sup>	2.73	-	-	[2]
EHC	-	1	-	-	[2]
GFR	-	1	-	-	[2]
Specific intest. perm.	cm min <sup>-1</sup>	2e-06	-	-	[2]
Specific organ perm.	cm min <sup>-1</sup>	0.007	-	-	[2]

*Note:*

If IC<sub>50</sub> values could not be used for  $K_i$  value estimation utilizing Cheng Prusoff Equation (e.g. due to missing substrate affinities)  $K_i = IC_{50}$  was assumed

### 3.6.2 Profiles

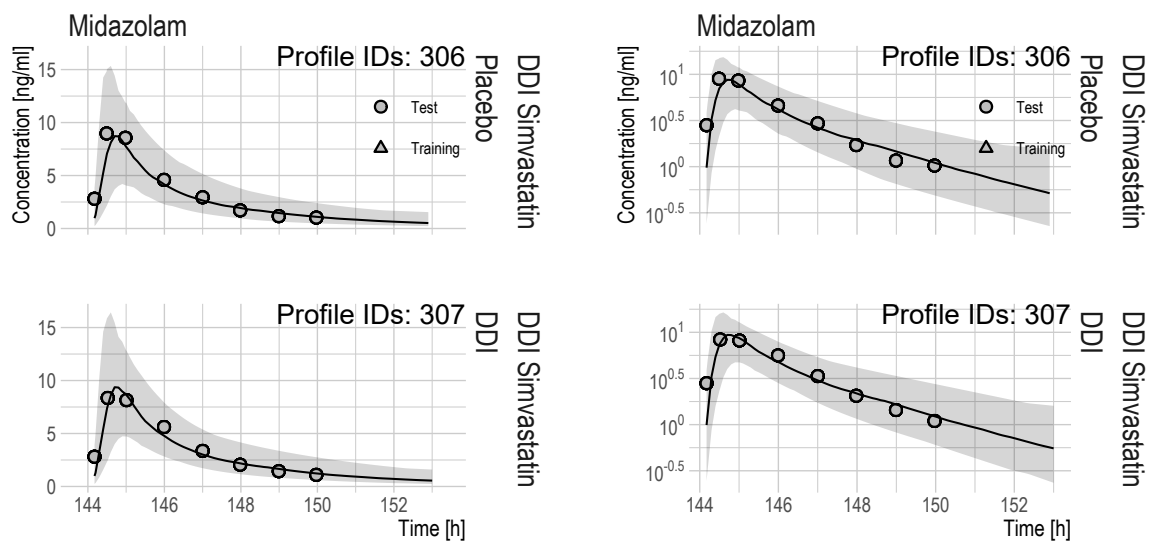


Figure S3.13: Linear VPCs of plasma concentration-time profiles for investigated DDIs: Simvastatin - Midazolam. Solid line and shaded area are predicted median and 90 % CI



## 3 Simvastatin DDIs

## 3.7 DDI evaluation

DDI prediction performance was assessed using the previously described graphical and statistical measures. Figures S3.14–S3.16 show the GOF plots as well as the predicted versus observed NCA ratios for SL and SA, respectively. Tables S3.20 and S3.21 summarise the statistical prediction performance.

## 3.7.1 Predicted concentrations versus observed concentrations GOF plots

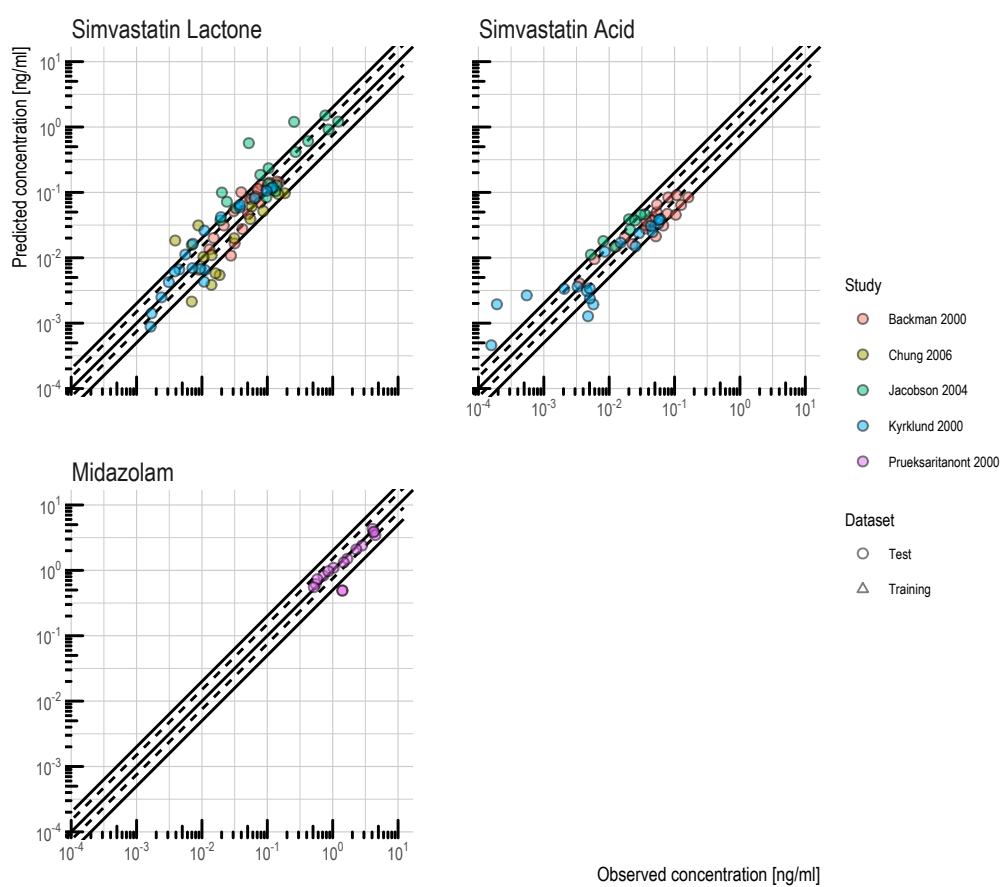


Figure S3.14: Goodness of fit plots - Observed versus predicted plasma concentration-time values of the DDI test dataset. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations

3 Simvastatin DDIs

3.7.2 NCA GOF plots

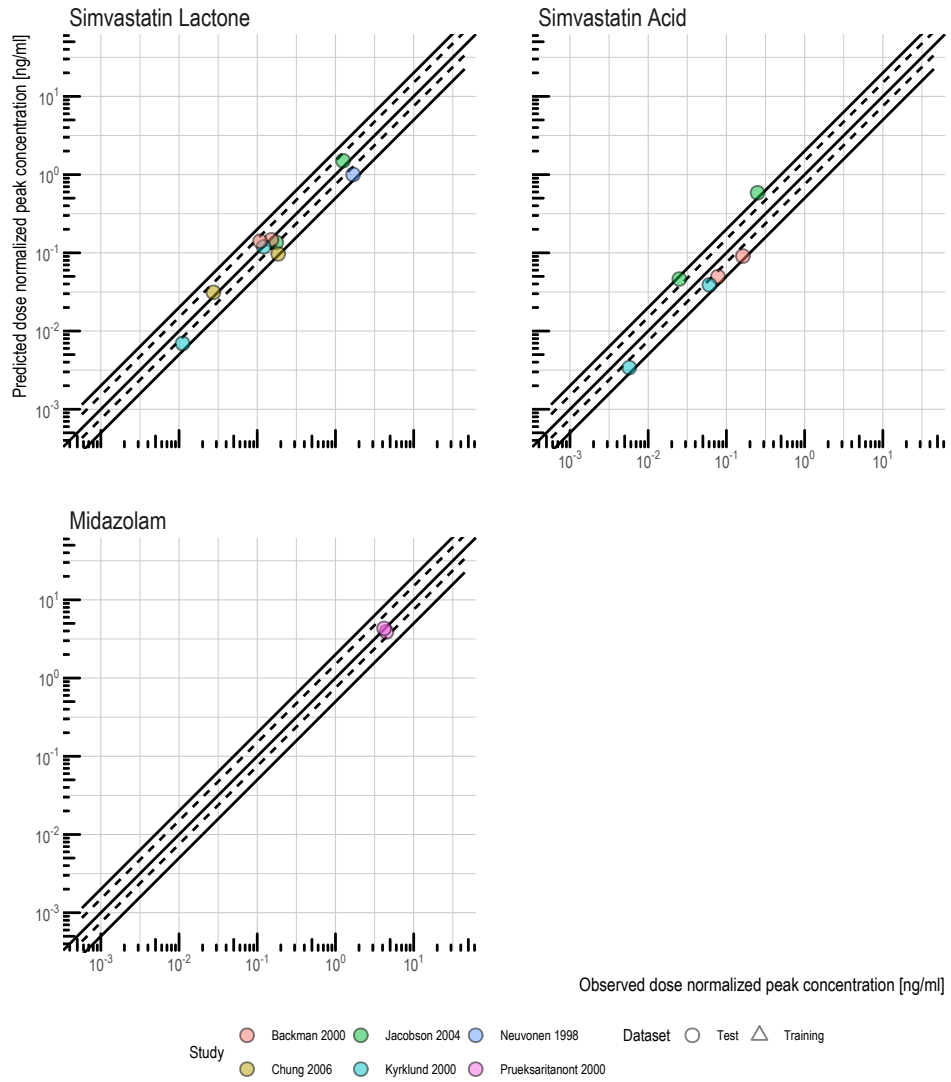


Figure S3.15: NCA ratios of the DDIs. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations.: Test  $C_{max}$

3 Simvastatin DDIs

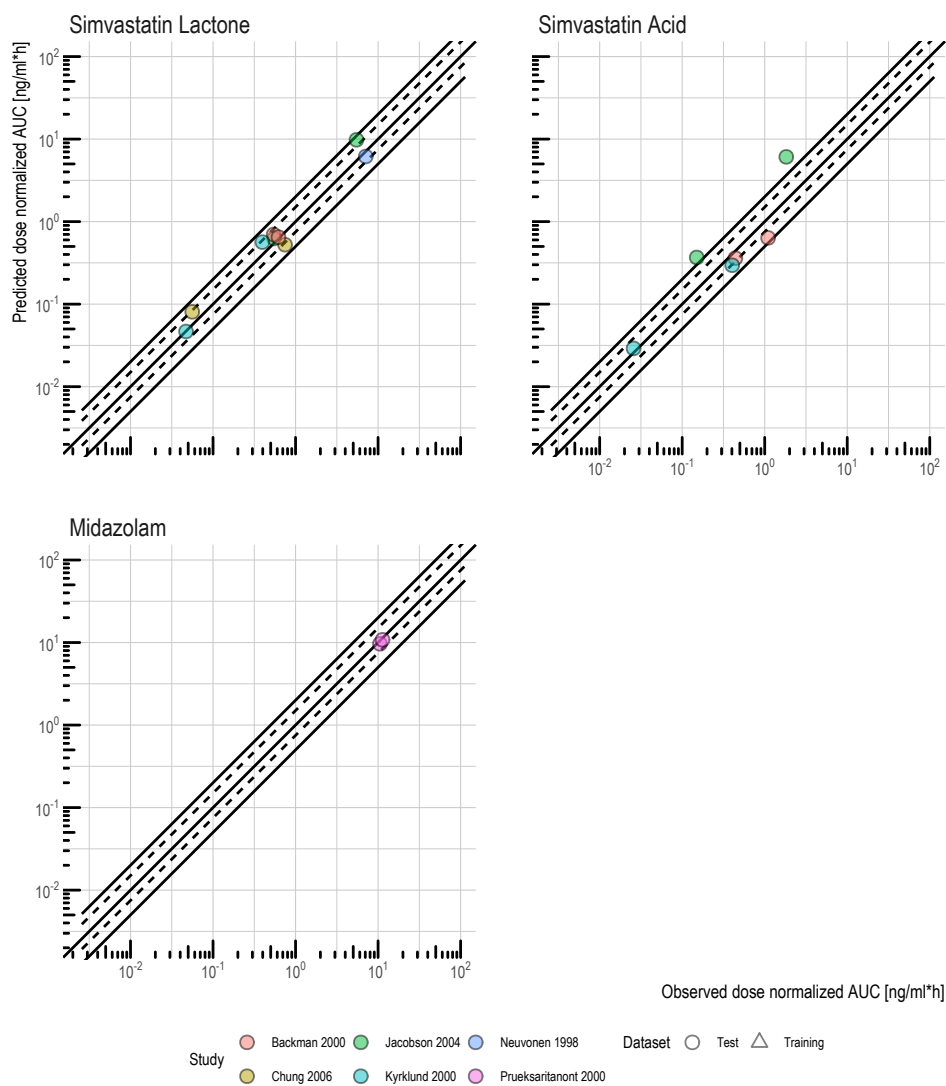


Figure S3.16: NCA ratios of the DDIs. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations.: Test AUC

## 3 Simvastatin DDIs

## 3.7.3 MRD and MSA of plasma concentration predictions

Table S3.20: Summary of the statistical DDI model evaluation (MSA and MRD)

Molecule	MRD mean (sd)	MSA mean (sd)
Simvastatin Lactone	1.98 (0.674) N = 8 (N MRD > 2 = 2)	78.3 (67.3) N = 8 (N MSA > 100 = 2)
Simvastatin Acid	1.91 (0.815) N = 5 (N MRD > 2 = 1)	77 (67.4) N = 5 (N MSA > 100 = 1)
Midazolam	1.48 (0.019) N = 2 (N MRD > 2 = 0)	12.2 (0.38) N = 2 (N MSA > 100 = 0)

## 3.7.4 NCA ratios and GMFE of NCA values

Table S3.21: Summary of the statistical DDI model evaluation (NCA ratio and GMFE)

Parameter	NCA ratio mean (sd)	GMFE
<b>Simvastatin Lactone</b>		
AUC	1.19 (0.334) N = 9 (N ratio > 2   ratio < 0.5 = 0)	1.28
$C_{max}$	0.902 (0.283) N = 9 (N ratio > 2   ratio < 0.5 = 0)	1.32
<b>Simvastatin Acid</b>		
AUC	1.51 (1.12) N = 6 (N ratio > 2   ratio < 0.5 = 2)	1.73
$C_{max}$	1.1 (0.784) N = 6 (N ratio > 2   ratio < 0.5 = 1)	1.78
<b>Midazolam</b>		
AUC	0.934 (0.0263) N = 2 (N ratio > 2   ratio < 0.5 = 0)	1.07
$C_{max}$	0.951 (0.114) N = 2 (N ratio > 2   ratio < 0.5 = 0)	1.09

## 4 Drug-drug-gene interaction network

For the final DDGI evaluation, effect ratios of all included DDIs and DGIs were calculated and compared. Figures S4.1 and S4.2 display the  $C_{\max}$  and AUC effect ratios. Table S4.1 shows the effect ratio values and Table S4.2 the summary of the effect ratio values.

## 4 Drug-drug-gene interaction network

## 4.1 Effect ratios

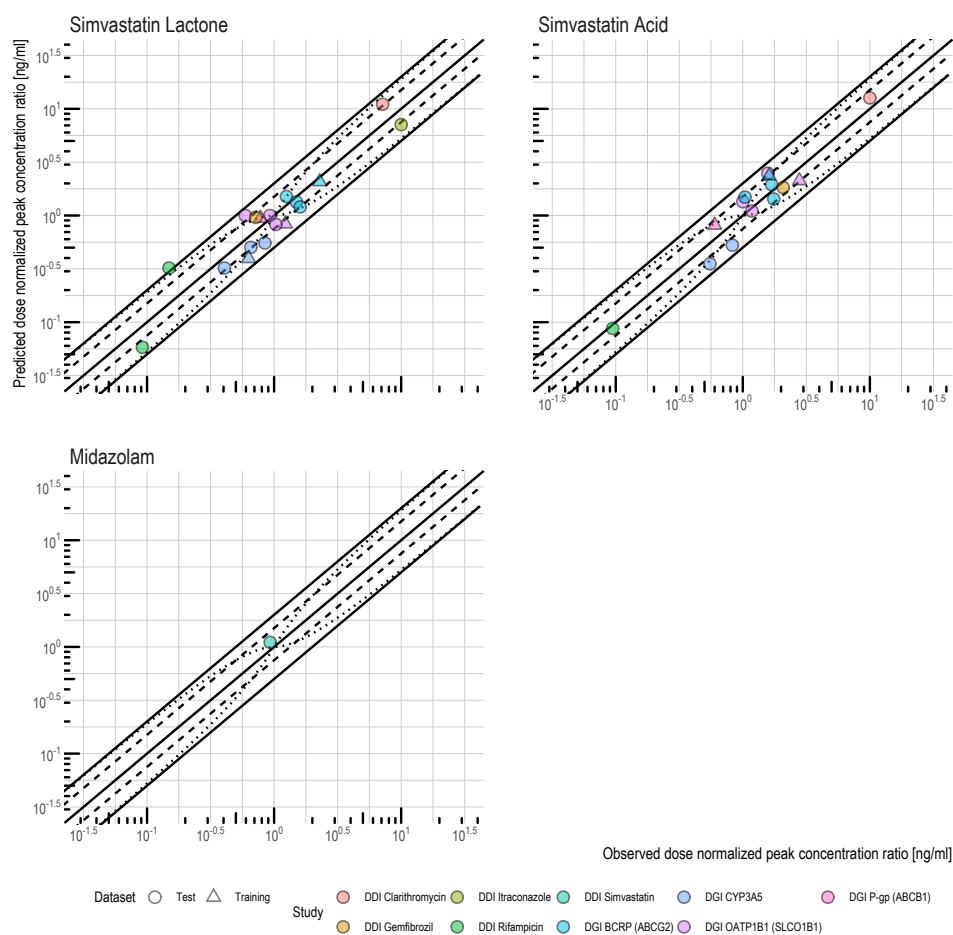


Figure S4.1: NCA effect ratios ( $C_{\max}$ ) of the DDGI network. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations. Dotted lines show the limits proposed by Guest et al. [17]

## 4 Drug-drug-gene interaction network

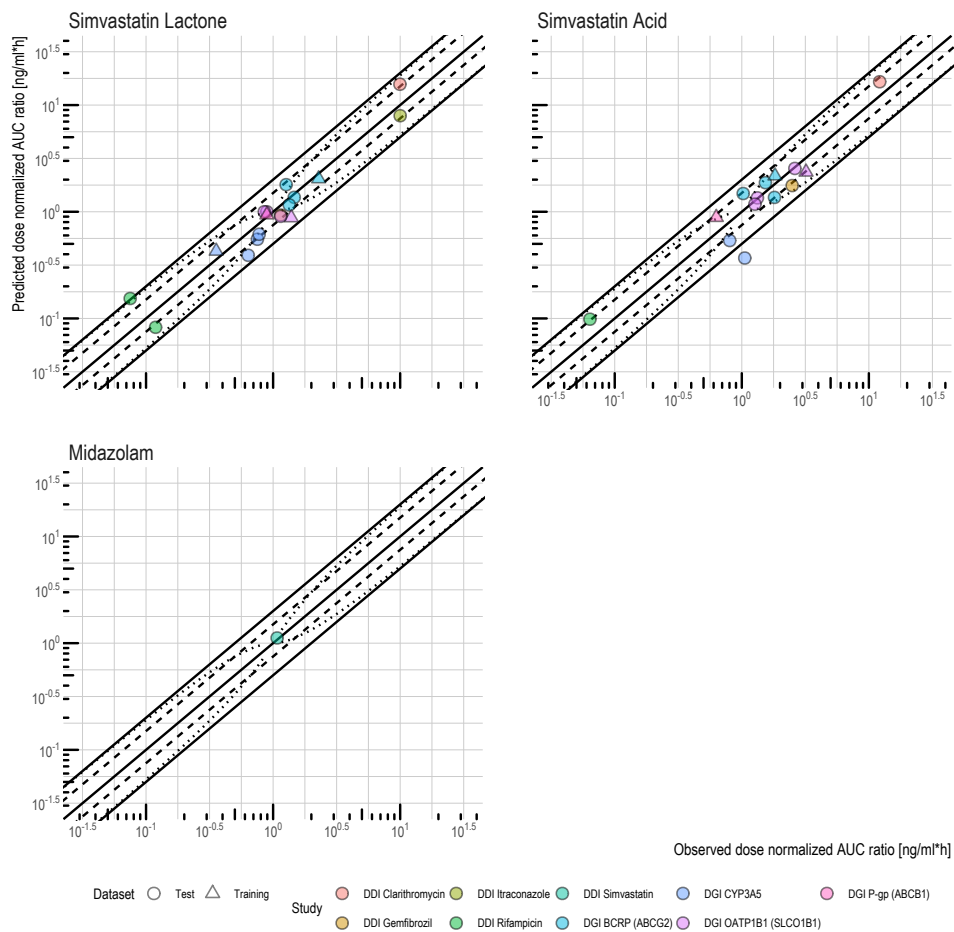


Figure S4.2: NCA effect ratios (AUC) of the DDGI network. The solid lines mark the line of identity as well as the 2-fold deviations. Dashed lines indicate the 1.5-fold deviations. Dotted lines show the limits proposed by Guest et al. [17]

## 4 Drug-drug-gene interaction network

Table S4.1: Predicted and observed DDGI AUC and Cmax effect ratios

Parameter DDI / DGI	Observed effect ratio	Predicted effect ratio	Predicted / Observed	Reference
<b>Simvastatin Lactone</b>				
AUC	DDI Clarithromycin	9.95	15.7	1.58 [138]
	DDI Gemfibrozil	1.15	0.931	0.813 [142]
	DDI Itraconazole	10	7.95	0.795 [145]
	DDI Rifampicin	0.075	0.154	2.05 [143]
	DDI Rifampicin	0.119	0.0826	0.695 [144]
	DGI BCRP (ABCG2) c.421AA	1.27	1.79	1.42 [96]
	DGI BCRP (ABCG2) c.421AA	2.28	2.04	0.894 [94]
	DGI BCRP (ABCG2) c.421CA	1.47	1.37	0.931 [96]
	DGI BCRP (ABCG2) c.421CA	1.34	1.16	0.864 [94]
	DGI CYP3A5 *1/*1	0.637	0.391	0.613 [96]
	DGI CYP3A5 *1/*1	0.357	0.427	1.2 [93]
	DGI CYP3A5 *3/*1	0.752	0.552	0.734 [96]
	DGI CYP3A5 *3/*1	0.777	0.615	0.793 [93]
	DGI OATP1B1 (SLCO1B1) c.521CC	0.853	1	1.17 [96]
	DGI OATP1B1 (SLCO1B1) c.521CC	1.39	0.88	0.633 [95]
	DGI OATP1B1 (SLCO1B1) c.521TC	0.892	1	1.12 [96]
	DGI OATP1B1 (SLCO1B1) c.521TC	1.15	0.906	0.79 [95]
	DGI P-gp (ABCB1) c.1236C-c.2677G-c.3435C	0.893	0.936	1.05 [41]
C <sub>max</sub>	DDI Clarithromycin	7.14	11	1.54 [138]
	DDI Gemfibrozil	0.717	0.96	1.34 [142]
	DDI Itraconazole	10	7.12	0.712 [145]
	DDI Rifampicin	0.149	0.322	2.17 [143]
	DDI Rifampicin	0.0919	0.0582	0.633 [144]
	DGI BCRP (ABCG2) c.421AA	1.25	1.51	1.21 [96]
	DGI BCRP (ABCG2) c.421AA	2.29	2.08	0.908 [94]
	DGI BCRP (ABCG2) c.421CA	1.5	1.34	0.894 [96]
	DGI BCRP (ABCG2) c.421CA	1.6	1.2	0.751 [94]



## 4 Drug-drug-gene interaction network

Table S4.1: Predicted and observed DDGI AUC and Cmax effect ratios (*continued*)

Parameter DDI / DGI	Observed effect ratio	Predicted effect ratio	Predicted / Observed	Reference	
DGI CYP3A5 *1/*1	0.406	0.322	0.792	[96]	
DGI CYP3A5 *1/*1	0.631	0.395	0.627	[93]	
DGI CYP3A5 *3/*1	0.656	0.503	0.767	[96]	
DGI CYP3A5 *3/*1	0.846	0.552	0.653	[93]	
DGI OATP1B1 (SLCO1B1) c.521CC	0.593	1	1.69	[96]	
DGI OATP1B1 (SLCO1B1) c.521CC	1.23	0.835	0.681	[95]	
DGI OATP1B1 (SLCO1B1) c.521TC	0.926	1	1.08	[96]	
DGI OATP1B1 (SLCO1B1) c.521TC	1.03	0.824	0.798	[95]	
DGI P-gp (ABCB1) c.1236C-c.2677G-c.3435C	0.782	0.945	1.21	[41]	
<b>Simvastatin Acid</b>					
AUC	DDI Clarithromycin	12.2	16.5	1.36	[138]
	DDI Gemfibrozil	2.49	1.76	0.706	[142]
	DDI Rifampicin	0.0638	0.0984	1.54	[144]
	DGI BCRP (ABCG2) c.421AA	1.53	1.87	1.22	[96]
	DGI BCRP (ABCG2) c.421AA	1.83	2.17	1.19	[94]
	DGI BCRP (ABCG2) c.421CA	1.81	1.36	0.754	[96]
	DGI BCRP (ABCG2) c.421CA	1.03	1.48	1.45	[94]
	DGI CYP3A5 *1/*1	1.06	0.368	0.349	[96]
	DGI CYP3A5 *3/*1	0.803	0.538	0.669	[96]
	DGI OATP1B1 (SLCO1B1) c.521CC	2.62	2.55	0.974	[96]
	DGI OATP1B1 (SLCO1B1) c.521CC	3.2	2.36	0.736	[95]
	DGI OATP1B1 (SLCO1B1) c.521TC	1.33	1.35	1.02	[96]
	DGI OATP1B1 (SLCO1B1) c.521TC	1.27	1.18	0.928	[95]
	DGI P-gp (ABCB1) c.1236C-c.2677G-c.3435C	0.63	0.886	1.41	[41]
C <sub>max</sub>	DDI Clarithromycin	10	12.7	1.27	[138]
	DDI Gemfibrozil	2.08	1.83	0.88	[142]

## 4 Drug-drug-gene interaction network

Table S4.1: Predicted and observed DDGI AUC and Cmax effect ratios (*continued*)

Parameter DDI / DGI	Observed effect ratio	Predicted effect ratio	Predicted / Observed	Reference
DDI Rifampicin	0.0946	0.0874	0.924	[144]
DGI BCRP (ABCG2) c.421AA	1.69	1.95	1.15	[96]
DGI BCRP (ABCG2) c.421AA	1.61	2.36	1.46	[94]
DGI BCRP (ABCG2) c.421CA	1.75	1.43	0.817	[96]
DGI BCRP (ABCG2) c.421CA	1.04	1.49	1.43	[94]
DGI CYP3A5 *1/*1	0.552	0.353	0.64	[96]
DGI CYP3A5 *3/*1	0.828	0.527	0.637	[96]
DGI OATP1B1 (SLCO1B1) c.521CC	1.58	2.49	1.58	[96]
DGI OATP1B1 (SLCO1B1) c.521CC	2.79	2.12	0.76	[95]
DGI OATP1B1 (SLCO1B1) c.521TC	1	1.35	1.35	[96]
DGI OATP1B1 (SLCO1B1) c.521TC	1.18	1.1	0.934	[95]
DGI P-gp (ABCB1) c.1236C-c.2677G-c.3435C	0.601	0.812	1.35	[41]
<b>Midazolam</b>				
AUC DDI Simvastatin	1.08	1.12	1.04	[141]
C <sub>max</sub> DDI Simvastatin	0.931	1.1	1.18	[141]

Table S4.2: Summary of the predicted and observed DDGI AUC and Cmax effect ratios

Parameter	NCA effect ratio mean (sd)	GMFE
<b>Simvastatin Lactone</b>		
AUC	1.01 (0.372) N = 18 (N ratio > 2   ratio < 0.5 = 1)	1.31
C <sub>max</sub>	1.03 (0.429) N = 18 (N ratio > 2   ratio < 0.5 = 1)	1.38
<b>Simvastatin Acid</b>		
AUC	1.02 (0.354) N = 14 (N ratio > 2   ratio < 0.5 = 1)	1.36
C <sub>max</sub>	1.08 (0.322) N = 14 (N ratio > 2   ratio < 0.5 = 0)	1.31
<b>Midazolam</b>		
AUC	1.04 (NA) N = 1 (N ratio > 2   ratio < 0.5 = 0)	1.04
C <sub>max</sub>	1.18 (NA) N = 1 (N ratio > 2   ratio < 0.5 = 0)	1.18

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## CREDIT CATEGORIES

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**CONCEPTUALIZATION:** Ideas; formulation or evolution of overarching research goals and aims

**DATA CURATION:** Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse

**FORMAL ANALYSIS:** Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data

**FUNDING ACQUISITION:** Acquisition of the financial support for the project leading to this publication

**INVESTIGATION:** Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection

**METHODOLOGY:** Development or design of methodology; creation of models

**PROJECT ADMINISTRATION:** Management and coordination responsibility for the research activity planning and execution

**RESOURCES:** Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools

**SOFTWARE:** Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components

**SUPERVISION:** Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team

**VALIDATION:** Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs

**VISUALIZATION:** Preparation, creation and/or presentation of the published work, specifically visualization/data presentation

**WRITING ORIGINAL DRAFT:** Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)

WRITING REVIEW AND EDITING: Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision - including pre- or post-publication stages

## PUBLICATIONS

## C.1 ORIGINAL ARTICLES

- 1 Nadine Schaefer, **Jan-Georg Wojtyniak**, Mattias Kettner, Julia Schlote, Matthias W. Laschke, Andreas H. Ewald, Thorsten Lehr, Michael D. Menger, Hans H. Maurer, and Peter H. Schmidt. "Pharmacokinetics of (synthetic) cannabinoids in pigs and their relevance for clinical and forensic toxicology." In: *Toxicology letters* 253 (June 2016), pp. 7–16. ISSN: 1879-3169. DOI: 10.1016/j.toxlet.2016.04.021.
- 2 Nina Hanke, Michael Teifel, Daniel Moj, **Jan-Georg Wojtyniak**, Hannah Britz, Babette Aicher, Herbert Sindermann, Nicola Ammer, and Thorsten Lehr. "A physiologically based pharmacokinetic (PBPK) parent-metabolite model of the chemotherapeutic zoletarelin doxorubicin — integration of in vitro results, Phase I and Phase II data and model application for drug-drug interaction potential analysis." In: *Cancer Chemotherapy and Pharmacology* 81.2 (Feb. 2018), pp. 291–304. DOI: 10.1007/s00280-017-3495-2.
- 3 Robert Miller, **Jan-Georg Wojtyniak**, Lisa J. Weckesser, Nina C. Alexander, Veronika Engert, and Thorsten Lehr. "How to disentangle psychobiological stress reactivity and recovery: a comparison of model-based and non-compartmental analyses of cortisol concentrations." In: *Psychoneuroendocrinology* 90 (Apr. 2018), pp. 194–210. ISSN: 1873-3360. DOI: 10.1016/j.psyneuen.2017.12.019.
- 4 Nadine Schaefer, **Jan-Georg Wojtyniak**, Ann-Katrin Kroell, Christina Koerbel, Matthias W. Laschke, Thorsten Lehr, Michael D. Menger, Hans H. Maurer, Markus R. Meyer, and Peter H. Schmidt. "Can toxicokinetics of (synthetic) cannabinoids in pigs after pulmonary administration be upscaled to humans by allometric techniques?" In: *Biochemical Pharmacology* 155 (Sept. 2018), pp. 403–418. ISSN: 1873-2968. DOI: 10.1016/j.bcp.2018.07.029.
- 5 Maximilian A. Ardelt, Thomas Fröhlich, Emanuele Martini, Martin Müller, Veronika Kanitz, Carina Atzberger, Petra Cantonati, Martina Meßner, Laura Posselt, Thorsten Lehr, **Jan-Georg Wojtyniak**, Melanie Ulrich, Georg J. Arnold, Lars König, Dario Parazzoli, Stefan Zahler, Simon Rothenfuß, Doris Mayr, Alexander Gerbes, Giorgio Scita, Angelika M. Vollmar, and Johanna Pachmayr. "Inhibition of cyclin-dependent kinase 5: a strategy

- to improve sorafenib response in hepatocellular carcinoma therapy." In: *Hepatology* 69.1 (Jan. 2019), pp. 376–393. ISSN: 1527-3350. DOI: 10.1002/hep.30190.
- 6 Michael Ramharter, Matthias Schwab, Ghyslain Mombo-Ngoma, Rella Zoleko Manego, Daisy Akerey-Diop, Arti Basra, Jean-Rodolphe Mackanga, Heike Würbel, **Jan-Georg Wojtyniak**, Raquel Gonzalez, Ute Hofmann, Mirjam Geditz, Pierre-Blaise Matsiegui, Peter G. Kremsner, Clara Menendez, Reinhold Kerb, and Thorsten Lehr. "Population pharmacokinetics of mefloquine intermittent preventive treatment for malaria in pregnancy in gabon." In: *Antimicrobial Agents and Chemotherapy* 63.2 (Nov. 2019), e01113–18. ISSN: 1098-6596. DOI: 10.1128/AAC.01113-18.
  - 7 Simeon Rüdeshheim, **Jan-Georg Wojtyniak**, Dominik Selzer, Nina Hanke, Felix Mahfoud, Matthias Schwab, and Thorsten Lehr. "Physiologically based pharmacokinetic modeling of metoprolol enantiomers and  $\alpha$ -hydroxymetoprolol to describe CYP2D6 drug-gene interactions." In: *Pharmaceutics* 12.12 (Dec. 2020), p. 1200. DOI: 10.3390/pharmaceutics12121200.
  - 8 **Jan-Georg Wojtyniak**, Hannah Britz, Dominik Selzer, Matthias Schwab, and Thorsten Lehr. "Data digitizing: accurate and precise data extraction for quantitative systems pharmacology and physiologically-based pharmacokinetic modeling." In: *CPT: Pharmacometrics & Systems Pharmacology* 9.6 (June 2020), pp. 322–331. DOI: 10.1002/psp4.12511.
  - 9 **Jan-Georg Wojtyniak**, Dominik Selzer, Matthias Schwab, and Thorsten Lehr. "Physiologically based precision dosing approach for drug-drug-gene interactions: a simvastatin network analysis." In: *Clinical Pharmacology & Therapeutics* (Dec. 2020). DOI: 10.1002/cpt.2111.

## C.2 CONFERENCE ABSTRACTS

- 1 **Jan-Georg Wojtyniak**, Johanna Pachmayr, and Thorsten Lehr. "A cancer cell cycle model to predict effects of combination therapy and different dosing schedules on cell cycle, tumor growth and therapy outcome." In: PAGE Meeting (June 7, 2016). Lisboa, June 7, 2016.
- 2 Katharina Martha Götz, **Jan-Georg Wojtyniak**, and Thorsten Lehr. "Package information leaflets (PILs) fail to inform patients – a study on the readability of PILs." In: DPhG Annual Meeting (2017). 2017.
- 3 **Jan-Georg Wojtyniak**, Katharina Martha Götz, and Thorsten Lehr. "The benefits of a one-page summary sheet (OPSS) compared to the patient information leaflet (PIL) to enhance health



- literacy – a randomized crossover trial.” In: DPhG Annual Meeting (2017). 2017.
- 4 **Jan-Georg Wojtyniak**, Roman Tremmel, Elke Schaeffeler, Matthias Schwab, and Thorsten Lehr. “Application of population pharmacokinetic (popPK) modeling to improve physiologically-based pharmacokinetic (PBPK) modeling decision making.” In: Uppsala Pharmacometric Summer School (2017). 2017.
  - 5 **Jan-Georg Wojtyniak**, Nina Hanke, Roman Trellem, Elke Schaeffeler, Matthias Schwab, and Thorsten Lehr. “Physiologically-based pharmacokinetic (PBPK) modeling of simvastatin drug-gene interaction with ABCG2 and drug-drug interactions with rifampicin and clarithromycin.” In: PAGE Meeting (June 6, 2017). Budapest, June 6, 2017.
  - 6 Fatima Zahra Marok, **Jan-Georg Wojtyniak**, Matthias Schwab, and Thorsten Lehr. “Optimizing 5-fluorouracil chemotherapy with regard to DPD drug-gene interactions and circadian effects utilizing a physiologically based pharmacokinetic (PBPK) modeling approach.” In: DPhG annual meeting (2019). 2019.
  - 7 Fatima Zahra Marok, **Jan-Georg Wojtyniak**, Matthias Schwab, and Thorsten Lehr. “Physiologically-based pharmacokinetic modeling of DPYD substrate 5-fluorouracil and its prodrug capecitabine.” In: PAGE (2019). 2019.
  - 8 **Jan-Georg Wojtyniak**, Simeon Rüdeshheim, Roman Tremmel, Matthias Schwab, and Thorsten Lehr. “Physiologically-based pharmacokinetic modelling of metoprolol drug-drug-gene interactions with paroxetine and CYP2D6.” In: PAGE Meeting (June 11, 2019). Stockholm, June 11, 2019.
  - 9 **Jan-Georg Wojtyniak**, Hannah Britz, Fatima Zahra Marok, Denise Türk, Laura Fuhr, Lukas Kovar, Nina Hanke, Matthias Schwab, and Thorsten Lehr. “Physiologically-based pharmacokinetic (PBPK) modelling of a CYP3A4/P-gp ddi network with ketoconazole, midazolam, alfentanil, repaglinide and digoxin.” In: DPhG Doktorandentagung (2019). 2019.

### C.3 ORAL PRESENTATIONS

- 1 **Jan-Georg Wojtyniak**, Thorsten Lehr, Roman Tremmel, and Matthias Schwab. *Physiologically Based Pharmacokinetic (PBPK) Modeling Approaches in WP9 - Systems Pharmacology and Gene-Drug-Drug Interactions*. 3rd U-PGx Consortium Meeting. Liverpool, Sept. 20, 2018.

- 2 **Jan-Georg Wojtyniak.** *Physiologically-Based Pharmacokinetic (PBPK) Modeling Approaches for the Prediction of Drug-Drug and Drug-Gene Interactions.* Boehringer Ingelheim guest lecture. Germany, May 14, 2019.

#### C.4 BOOK CHAPTERS

- 1 **Jan-Georg Wojtyniak,** Christiane Dings, and Thorsten Lehr. "Kardiologie." In: *Pharmakogenetik und Therapeutisches Drug Monitoring.* Ed. by Hanns-Georg Klein. Ed. by Ekkehard Haen. 1st ed. Walter de Gruyter GmbH, Dec. 18, 2017. Chap. Kardiologie. ISBN: 3110352907. URL: [https://www.ebook.de/de/product/31386633/pharmakogenetik\\_und\\_therapeutisches\\_drug\\_monitoring.html](https://www.ebook.de/de/product/31386633/pharmakogenetik_und_therapeutisches_drug_monitoring.html).

#### C.5 OTHERS

- 1 Lukas Kovar, **Jan-Georg Wojtyniak,** Christina Schräpel, and Thorsten Lehr. "Pharmakometrie erklärt - Wie verändert sie die moderne Pharmazie?" In: *PZ PRISMA* 4 (2019). ISSN: 0945-5566.

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CURRICULUM VITAE

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# Curriculum Vitae

## Jan-Georg Wojtyniak

### Pharmacist

Focus of research: Physiologically based pharmacokinetic (PBPK) modeling, population pharmacokinetic (popPK) modeling, reproducible analysis and report creation



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### Profile

**Creative, reliable, highly motivated** – these are my value propositions. I am a trained pharmacist with expertise in a broad range of PK/PD related topics working in the field of pharmacometrics

### Skills

#### Modeling and Markup:

R	○○○○○
RMarkdown	○○○○○
PK-Sim/MoBi	○○○○○
NONMEM	○○○○○
R-Shiny	○○○○○
LaTeX	○○○○○
HTML/CSS	○○○○○

#### Languages:

English:	
Reading	C1
Speaking	C1
Writing	C1
French	
Reading	A2
Speaking	A2
Writing	A2
Russian	
Reading	A1
Speaking	A1
Writing	A1

## Work experience

- since 01/2020 **Pharmacometrician**  
Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein (DE)  
Focus:
- Clinical drug development (oncology)
- 05/2016 – 12/2019 **Doctoral researcher**  
Universität des Saarlandes, Saarbrücken (DE)  
Robert Bosch Gesellschaft für medizinische Forschung mbH, Stuttgart (DE)  
Focus:
- PBPK modeling of pharmacogenetic effects and drug-drug interactions in the context of the European Ubiquitous Pharmacogenomics (U-PGx) projects
- 07/2014 – 12/2019 **Student research assistant**  
Universität des Saarlandes, Saarbrücken (DE)  
Focus:
- Shiny web applications for PK/PD
  - PopPK-analysis and allometric scaling
  - NCA-analysis
  - PBPK video tutorials
  - Method evaluation – data digitization and PBPK-modeling
  - Healthcare research – study design and result evaluation
- 02/2011 – 05/2017 **Freelance first aid trainer**  
Gesellschaft für Notfallmedizin, Saarbrücken (DE)
- 07/2010 – 03/2011 **Civil service as paramedic**  
DRK-LV Saarland e.V., Saarbrücken (DE)

## Education

- since 05/2016 **Ph.D. student**  
Universität des Saarlandes, Saarbrücken (DE)  
Robert Bosch Gesellschaft für medizinische Forschung mbH, Stuttgart (DE)  
Topic:
- Model Informed Drug Development And Precision Dosing for Drug-Drug-Gene-Interactions

# CV – Jan-Georg Wojtyniak

## Education

- 04/2011 – 09/2016 **Approbation as pharmacist**  
Landesamt für Soziales, Saarbrücken (DE)
- 05/2015 – 03/2016 **Diploma pharmacist**  
Universität des Saarlandes, Saarbrücken (DE)  
Topic:
  - A mathematical semi-mechanistic cancer cell cycle model to predict effects of combination therapy and different dosing schedules on cell cycle, tumor growth and therapy outcome
- 02/2011 **First aid trainer**  
Sanitätsschule Nord, Hutzfeld (DE)
- 01/2011 **Paramedic**  
Rettungsdienstschule Saar, St.Ingbert (DE)
- 09/2002 – 06/2010 **Abitur (including Latinum)**  
Gymnasium am Rotenbühl, Saarbrücken (DE)

## Personal information

- Date of birth • 24.02.1992
- Place of birth • Saarbrücken, Saarland (DE)
- Certificates • Leadership  
• Entrepreneurship  
• Uppsala Pharmacometric Summer School
- Organisations • **President** Deutsche Herzwacht e.V. (since 2014)  
• **Vice president** Alumni und Freunde der Fachrichtung Pharmazie an der Universität des Saarlandes e.V. (Universität des Saarlandes, 2014-2017)  
• **Voluntary member** of the Fachschaft Pharmazie (Universität des Saarlandes, 2013-2015)
- Attended conferences • **PK/PD Expert Meeting** (2019 – active participation)  
• **DPhG-Doktorandentagung** (2019 – poster presentation)  
• **Basel M&S Seminar** (2018/2016 – poster presentation)  
• **Uppsala Pharmacometric Summer School** (2017 – poster presentation)  
• **DPhG-Jahrestagung** (2017 – poster presentation)  
• **Population Approach Group Europe (PAGE) meeting** (2019/2017/2016 – poster presentation)
- Hobbies • Go bouldering  
• Learning new technologies  
• Meeting with friend  
• Managing non-profit projects in the health sector

## References

Prof. Dr. Thorsten Lehr  
Klinische Pharmazie  
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