General Introduction

Pharmaceutical drugs are essential for modern medical care, being used as sole agents or coadjuvant therapy in the treatment and prevention of most human diseases. However, despite their benefits, drugs carry the potential to induce toxic side effects. Such adverse drug reactions (ADRs) can be fatal and, therefore, require a thorough analysis prior to drug administration to patients. Approximately 5-10 % of hospitalised patients experience at least one ADR associated with their hospitalisation [1]. This does not include accidental or intentional overdoses but rather the side effects related to the therapeutic dosing according to the drug label. Thus, the threat posed to patients' health, along with the monetary pressure on the healthcare system and drug developers, render ADRs a research area of high value to the medical community.

ADRs can affect various organ systems in the body, such as the central nervous system and the immune system, or single organs, e.g., the heart or the liver. Common ADRs are diarrhoea, allergic reactions, and dizziness, depending on the affected system [2]. The symptoms of ADRs can range from elevated biomarkers in plasma with only minor signs of illness up to acute organ failure with possibly fatal consequences. Since the liver is the primary site of detoxification in the body, the chance of experiencing enlarged exposure to toxic substances, such as a drug or its metabolites, is substantially higher in the liver, making it a high-risk organ for ADRs [3]. ADRs in the liver show a vast spectrum of injuries such as cholestasis (perturbation of bile flow), steatosis (accumulation of fat), fibrosis and cirrhosis (tissue scarring), liver failure, and hepatic cancer [4, 5]. Thus, due to the incidence and potentially high severity of ADRs, substantial efforts are made during drug development and patient care to prevent them.

The prediction and prevention of ADRs is a key effort in the pharmaceutical industry and clinical care. The different categories of ADRs, intrinsic or idiosyncratic, directly impact the chance of successful prediction. Intrinsic ADRs are dose-related and can be identified in the late stages of drug development, often leading to the discontinuation of the development program of the respective compound. With the substantial attrition rates entailing a significant subsequent depreciation of financial investments, dose-related ADRs constitute a high economic burden to the industry [6]. Idiosyncratic ADRs are not dose-related but rather patient-specific, posing an intricate problem to drug development and a direct danger to patients. Since the underlying mechanisms for idiosyncratic ADRs are often unclear, and their incidence rates are frequently too low to be discovered in clinical studies, their prediction is very difficult. Although idiosyncratic events can be related to predisposing patient-specific factors such as metabolic phenotype, co-medication, or state of the immune system, they mostly become apparent only after drug launching. The detection of an ADR in the post-marketing phase may lead to additional restrictions on the drug's usage or even market withdrawal, implying fewer benefits for patients as well as the marketing company [7]. Hence, the early identification or prediction of idiosyncratic ADR risks and their underlying mechanism is as important as predicting intrinsic ADR risks but much more complex.

The identification of the underlying mechanisms of ADRs requires two main contributors to be present: overall systemic knowledge and patient-specific considerations. On one hand, a systemic approach comprising the whole-body perspective with the interaction of several organs down to the cellular level is necessary to understand the complex interplay and reactions of a body to a drug. This entails the understanding of the drug-organism interactions at different levels, including comprehensive physiological processes, *in vitro* knowledge of metabolism and drug effects, and detailed information on the substance's chemical and pharmacological properties. On the other hand, the effect of patient-specific factors on the drug-organism interactions, e.g., due to metabolic capacity or diseases, needs to be unravelled to identify those patients who are at particular risk of experiencing

toxic effects. The knowledge of both aspects must be combined in an integrative model that generates a quantifiable biomarker indicating an ADR risk [8]. Ideally, such a biomarker would be non- or minimally invasively accessible in the clinical routine, e.g., by blood samples, and would allow identifying patients on the bedside predisposed to an ADR when administering a specific drug.
Model-based approaches and studies addressing the identification of ADRs are deployed on several levels of pharmaceutical development programmes. The most reliable ADR studies are *in vivo* clinical trials or even post-marketing pharmacovigilance records since they are conducted in the target species and, thus, cannot suffer from translational biases. However, these studies are also the most expensive ones, not only with respect to patient safety but also in terms of financial expenses for the development process. Due to the *post hoc* nature of pharmacovigilance reports, they are not suited for predictions before launching a drug. However, they may still help prevent further harm to patients by investigating the ADR potential and the subsequent labelling or market withdrawal of the drug [9].

Preferably, the ADR risk should be detected before a drug enters the market or even the clinical phase of development. During preclinical testing, animal models can be used to identify harmful drugs, but since translation to humans is not straightforward, their reliability is limited [10]. *In vitro* models with human cell cultures like Hep3G sandwich or spheroids are good surrogates to help qualify or quantify the ADR potential in the human system. Unfortunately, *in vitro* cell cultures can be biased because of the lack of the proper physiological environment and systemic influence, e.g., the pharmacodynamic adaptation of other organs or co-factor supply [11]. In addition to wet-lab experiments, *in silico* approaches have been used to establish quantitative structure-activity relationship (QSAR) models, which can be applied to predict a drug's interaction with a protein based on chemical properties [12]. The advantage of *in silico* models is that they are cost-efficient since they do not involve living animals or cells, which are expensive to acquire, keep and analyse. QSAR results, though, are often imprecise and may rather serve as rough guidance [13, 14]. However, all these approaches, i.e., *in vitro*, preclinical, and *in silico*, produce valuable pieces of information that help fill specific knowledge gaps and that, once consolidated in a bigger picture, may help better predict ADR risks in humans.
Computational models such as physiologically-based pharmacokinetic (PBPK) or Quantitative Systems Pharmacology (QSP) models are suitable tools for contextualising such manifold information by combining models focusing on single aspects, like QSAR or *in vitro* metabolism and effect models, to comprising models. The general approach of QSP and (PB)PK- pharmacodynamic models is to mathematically describe the drug exposure in the body or relate this exposure to drug effects following the principle of a dose-response relationship. The body drug exposure, which is key for the prediction of ADRs, is governed by the drug's pharmacokinetics, comprising the processes of absorption, distribution, metabolism, and excretion (ADME). These ADME processes can be modelled with PBPK models, which are a mechanistic description of a drug's distribution in the body, incorporating physiological and anatomical information, such as organ volumes or blood flow rates, as well as compound-specific information such as physico-chemical and biochemical properties. Metabolic processes can be informed by *in vitro* measurements or QSAR predictions and validated by (pre-)clinical PK data. By their mechanistic nature, PBPK models are well-suited to assess drug interactions [15] or to test hypotheses on the mechanistic processes underlying observed phenomena [16].
While PK describes the effect that the body has on the drug, pharmacodynamics (PD) describes the effects of a drug on the body or on its specific target, e.g., a receptor [17]. PD models translate a drug concentration to a quantifiable effect, for example, the percentage of surviving tumour cells after exposure to a specific drug concentration; thus, the concentration of the drug which reaches the target site needs to be known to quantify the drug action after drug administration accurately. However, measurements of drug concentrations on the site of action often imply invasive sampling

approaches that are, in most cases, unethical to perform in humans. An alternative to direct drug concentration measurements is the use of computational models to estimate drug concentrations in tissues which can subsequently be provided as an input for effect models. Such a prediction of time-drug concentration profiles for different organs can be accomplished by PBPK models, which have already been integrated as part of the drug-development process [18, 19]. Apart from describing drug PK, PBPK models can also be used to represent endogenous physiological molecules like glucose, insulin, or bile acids and their interplay on the whole-body level. By coupling them to a drug-specific PBPK model, endogenous models can function as the PD part in the resulting QSP model [20]. Computational approaches linking drug exposure to drug effects are called PK-PD models, e.g., non-compartmental models with covariate analyses, dose-effects, signal transduction models, stoichiometric models, or sophisticated QSP models [21, 22].

PBPK or QSP models are deployed on different levels of risk assessment. The integration of different types of data, such as a reasonable representation of the PK processes and detailed information about the treated disease and the drug effect, is necessary for accurate ADR prediction. On the drug development level, *in vitro* testing of candidate compounds can be performed in a reasonable and clinically relevant setup. Thus, a beforehand prediction of physiologically relevant drug exposures can be made using PBPK or QSP models to inform *in vitro* assays, yielding more meaningful results [23]. On the clinical level, the readout from such a model-informed *in vitro* assay can be incorporated with physiological knowledge to identify interactions with organs at risk, like the liver [24]. On the patient level, personalised information can be included in the model to simulate individual ADME kinetics and, by that, drug exposure, even in organs where physical sampling is not feasible. Such a model can be used to evaluate and improve a clinical study design, e.g., by the consideration of the pharmacogenomic phenotype [25].

Existing approaches for the identification of predisposed patients by assessing the metabolic phenotype are accompanied by several shortcomings. After administering a probe drug and measuring the resulting drug levels, conclusions are drawn on the individual capacity of metabolising enzymes by calculating metabolite ratios. However, such non-model-based approaches, e.g., for caffeine or dextromethorphan [26, 27], do not account for patients' individualities. Since these methods are purely data-driven and not mechanistic, the true metabolic capacity and other drug-specific influences are hard to separate, hampering the translatability of the results to different cohorts or drugs. Additionally, only a small subset of drug-metabolising enzymes can be tested by the use of a single drug, while it would be beneficial to gather information on a broad spectrum of enzymes when conducting such a phenotyping test.

For the prediction of drug-induced liver injury (DILI), *in silico* PBPK model-based approaches trying to predict different types of hepatotoxicity exist [28] but are not freely available, obscuring their level of mechanistic detail utilized. For the prediction of drug-induced cholestasis, computational models need a proper representation of the endogenous bile acid (BA) metabolism but also the possibility of simulating drug PK on the whole-body scale. Although there are some BA models available [29], they neither represent the whole body nor do they focus on the prediction of DILI; thus, their integration into drug PK or PD models is not straightforward. Other models described BA metabolism in detail and were used to simulate DILIs. However, the nature of the mechanistic description of cholestasis development and the possibility of integrating data reflecting such an onset remains unclear [30].

Thus, despite the existing approaches aiming to predict ADRs, freely available, tailor-made model-based methods for risk assessment are still scarce. This work presents several integrative model-based approaches for risk assessment related to idiosyncratic ADRs. Besides a PBPK model-guided test strategy for a metabolic phenotyping test for the identification of ADR risked patients, a PBPK model representing the BA circulation was developed. This model was used to identify genetic

predispositions and drugs, potentially compromising BA metabolism and, thus, increasing the risk for DILI events. The outlined structure was followed:

- General Introduction: Overall motivation
- Part I: Background (Chapters 1-6)
- Part II: Materials and methods of the research chapters (Chapters 7-10)
- Part III: Results of the research chapters (Chapters 11, 12, and 13) followed by the
- General Conclusion and Outlook
- Part IV: Appendices containing additional and supportive information

First, in an introduction to drug-induced toxic events, the importance of ADRs in drug development and clinical care is explained. A particular focus is set on drug-induced liver injuries since they are very frequent and were investigated in the research chapters (Chapters 11 and 12). The basics of pharmacokinetics, which are crucial for the identification of ADRs, are elucidated along with the respective modelling approaches. In particular, this part is focused on PBPK models, which are sophisticated tools to simulate the fate of drugs within the body and can be coupled with PD models to generate QSP models able to describe specific drug effects. This, in turn, is needed for the modelling of ADRs. Individual factors that influence the PK and PD of a drug, e.g., interindividual variability or pharmacogenomics, are last outlined in the background section, providing the scientific foundation for the following research chapters.
The first research chapter introduces a mechanistic model describing the enterohepatic circulation of BA as a physiological system of bile acid homeostasis. The coupling of this physiology-based bile acid (PBBA) model with a drug-specific PBPK model of cyclosporine A (CsA) allowed the assessment of potential interactions that this drug exhibits on BA metabolism, possibly leading to an ADR. Further, by combining the developed model with patient-specific information on transporter proteins, the predisposition towards BA homeostasis disturbances of clinically known phenotypes like progressive familial intrahepatic cholestasis (PFIC) patients was demonstrated. The results of Chapter 11 were published as an original article in *Frontiers of Physiology* [31].
The second systematic workflow developed in this work was the integration of *in vitro* expression data into the PBBA model in order to benchmark the cholestatic potential of drugs. Thus, Chapter 12 focuses on the application of the developed PBBA model in the context of assessing the cholestatic risk of drugs by integrating *in vitro* expression data. The expression data were generated with a model-based approach, aiming at replicating *in-vivo*-like drug exposures over 14 days of repeated administration in the *in vitro* model. This yielded a realistic representation of the effect of ten different drugs on liver cells. The generated time-resolved expression data was integrated into the QSP-PBBA model to assess changes in BA levels. This re-integration of the system's reaction by means of gene expression data into a PBPK model allowed for a comparative analysis of the cholestatic potential of the ten assessed drugs. The results of this chapter were published as an original article in *Clinical Pharmacology & Therapeutics* [32].
In the third research chapter (Chapter 13), the potential of PBPK model-guided metabolic phenotyping to support personalised medicine decisions and prevent ADRs was investigated. To establish a clinically applicable test for the metabolic phenotype of patients, four drug-specific PBPK models were built. Then, the drug PK was modelled after a single intake of an over-the-counter drug cocktail. Following the generation of a reference PK profile for standard patient phenotypes, deviating metabolisers with different enzyme activities were simulated, allowing the comparison with the reference PK profile. The differences in PK profiles were analysed to give recommendations for an optimised sampling strategy within the test. The model-based approaches established in this chapter

can be used to help identify individuals at risk of experiencing ADRs due to an uncommon metabolic capacity and may hence assist in the risk assessment with minimally invasive techniques.

To conclude and summarize the thesis, the General Conclusion and Outlook recaps the research chapters and embeds the work into the current scientific context. Preceded by the bibliography used for this work, appendices with supplementary information on the research chapters are provided (Appendices A-C).