Hierarchical mechanistic modelling of clinical pharmacokinetic data

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

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List of abbreviations

AD: adipose
AFT: accelerated failure time
ALB: albumin level
ALP: alkaline phosphatase level
AUC: area under the concentration–time curve
AUROC: area under a receiver operating characteristic curve
BILI: bilirubin level
BO: bones
BQL: below the quantification limit
BR: brain
BSA: body surface area
BW: body weight
CA19-9: carbohydrate antigen 19-9 level
CAT: compartmental absorption and transit
CEN: central compartment
CI: confidence interval
CL: systemic clearance
CLOC: cancer location
CPH: Cox proportional hazards
CV: coefficient of variation
CYP: cytochrome P450
DDI: drug-drug interaction
DIAB: diabetes status
ECOG: eastern cooperative oncology group
ENT: small intestine enterocytes
FaSSIF: fasted-state simulated intestinal fluid
FO: first-order maximum likelihood estimation method
FOCE-I: first-order conditional with interaction maximum likelihood estimation method
GI: gastro-intestinal
GU: gut
HT: heart
IG: inverse Gaussian
IIV: inter-individual variability
IMPMAP: Monte Carlo importance sampling method assisted by mode a posteriori with interaction
IR: immediate-release
ISEF: inter-system extrapolation factor
ISV: inter-subject variability
IV: intravenous
KI: kidneys
LASSO: least absolute shrinkage and selection operator
LI: liver
LOO: approximate leave-one-out cross-validation
LU: lungs
LUM: lumped compartment
MCMC: Markov chain Monte Carlo
mGluR5: metabotropic glutamate receptor 5
MPC: metastatic pancreatic cancer
MR: modified-release
MU: muscles
MVG: mavoglurant
NEUT: neutrophil count
ODE: ordinary differential equation
OFV: objective function minimum value
PA: pancreas
PBPK: physiologically-based pharmacokinetic(s)
PD: pharmacodynamic(s)
PK: pharmacokinetic(s)
POS: powder for oral suspension
PTR: percentage change from baseline tumour size
Q: inter-compartmental clearance
RB: rest-of-body
RECIST: response evaluation criteria in solid tumours
ROC: receiver operating characteristic
SD: standard deviation
SI: small intestine
SK: skin
SP: spleen
SPL: splanchnic compartment
ST: stomach
TS: tumour size
Vc: volume of distribution of the central compartment
Vp: volume of distribution of the peripheral compartment
WBPBPK: whole-body physiologically-based pharmacokinetic
Abstract

THE UNIVERSITY OF MANCHESTER
Abstract of thesis submitted by Thierry Wendling for the degree of Doctor of Philosophy, entitled: “Hierarchical mechanistic modelling of clinical pharmacokinetic data”
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Pharmacokinetic and pharmacodynamic models can be applied to clinical study data using various modelling approaches depending on the aim of the analysis. In population pharmacokinetics for instance, simple compartmental models can be employed to describe concentration-time data, identify prognostic factors and interpolate within well-defined experimental conditions. The first objective of this thesis was to illustrate such a ‘semi-mechanistic’ pharmacokinetic modelling approach using mavoglurant as an example of a compound under clinical development. In particular, methods to accurately characterise complex oral pharmacokinetic profiles and evaluate the impact of absorption factors were investigated.

When the purpose of the model-based analysis is to further extrapolate beyond the experimental conditions in order to guide the design of subsequent clinical trials, physiologically-based pharmacokinetic (PBPK) models are more valuable as they incorporate information not only on the drug but also on the system, i.e. on mammillary anatomy and physiology. The combination of such mechanistic models with statistical modelling techniques in order to analysis clinical data has been widely applied in toxicokinetics but has only recently received increasing interest in pharmacokinetics. This is probably because, due to the higher complexity of PBPK models compared to conventional pharmacokinetic models, additional efforts are required for adequate population data analysis. Hence, the second objective of this thesis was to explore methods to allow the application of PBPK models to clinical study data, such as the Bayesian approach or model order reduction techniques, and propose a general mechanistic modelling workflow for population data analysis.

In pharmacodynamics, mechanistic modelling of clinical data is even less common than in pharmacokinetics. This is probably because our understanding of the interaction between therapeutic drugs and biological processes is limited and also because the types of data to analyse are often more complex than pharmacokinetic data. In oncology for instance, the most widely used clinical endpoint to evaluate the benefit of an experimental treatment is survival of patients. Survival data are typically censored due to logistic constraints associated with patient follow-up. Hence, the analysis of survival data requires specific statistical techniques. Longitudinal tumour size data have been increasingly used to assess treatment response for solid tumours. In particular, the survival prognostic value of measures derived from such data has been recently evaluated for various types of cancer although not for pancreatic cancer. The last objective of this thesis was therefore to investigate different modelling approaches to analyse survival data of pancreatic cancer patients treated with gemcitabine, and compare tumour burden measures with other patient clinical characteristics and established risk factors, in terms of predictive value for survival.
Declaration

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Chapter 1: General introduction
1.1. Modelling and simulation in drug development

1.1.1. The drug development process

The drug development process is a sequence of information-gathering activities that starts from the selection of the most promising chemical entity and ends when market-access of the medicine is approved by a regulatory agency. Each phase of the process (pre-clinical and Phase I, II, III and post-marketing) has its own specific goals and requirements, and is designed to accrue the required information for assessment of the probability of technical success of the chemical entity. Ultimately, safety and efficacy in the targeted patient population must be proved before any market-access decision is undertaken. Even after approval, the drug undergoes a continuous post-marketing surveillance to further refine both the safety and efficacy profile of the novel therapeutic agent in a more diverse patient population.

In 1997, Lewis Sheiner pointed out a new paradigm, conceptualizing the drug development process as two successive ‘learn-confirm’ cycles [1]. The first cycle (Phase I and Phase IIa) aims at showing that the drug offers a benefit over former therapies, i.e. learning about the maximum dose tolerated in humans (usually healthy volunteers) without causing serious side effects and testing the short-term benefit of that dose in intended-to-treat patients. Positive outcomes from this first cycle generally lead to the second one (Phase IIb and III) which aims at learning about the optimal dose regimen that would provide a reasonable benefit/risk ratio, and testing that regimen against a comparator in further more elaborate clinical trials. Following this paradigm, it is clear that investigation of pharmacokinetics and pharmacodynamics is a key point in the development process in order to define the optimal conditions of use of a new drug for clinical practice.

Although the ‘learn-confirm’ paradigm applies to most therapeutic areas, traditional clinical development strategies are not adequate for some more serious diseases such as cancers, especially at early development phases (Phase I and II). In oncology, early clinical development usually aims at defining the maximum tolerated dose in patients with advanced incurable malignancy rather than in healthy volunteers [2]. For standard chemotherapeutic agents, this dose is recommended for exploratory Phase II trials under the assumption that the higher the dose the higher the antitumor
response. However, this strategy might not be appropriate for molecularly targeted agents (e.g. targeting growth factor receptors) which are generally less toxic and might provide a desirable effect at doses much lower than the maximum tolerated one. Hence, approaches to early development of such agents have been considerably changing, shifting from toxicity-based dose finding to the determination of an optimal dose for target inhibition, and from standard efficacy endpoints (e.g. tumour shrinkage) to markers based on the mechanism of antitumor activity [2,3].

Since the 1990s, there has been an increased focus on modelling and simulation as decision making and development risk management tool at each phase of the drug development process [4]. Theoretically, modelling and simulation is useful in activities that do not require a great objectivity, i.e. in explanatory trials and analyses (learning steps), which are often viewed as the task to determine the input-outcome relationship. Confirmatory trials are associated with a strong objectivity which is required for market-access decisions [5]. Therefore, modelling and simulation are not systematically recommended for the confirming steps although many practical cases have shown that if applied in appropriate conditions, it can help confirm prior findings and significantly reduce the development time.

1.1.2. The role of pharmacokinetic and pharmacodynamic modelling in drug development

Pharmacokinetics is in simple terms what the body does to the drug. It is the relationship between drug inflow (single or repeated administration) and the resulting concentrations at relevant sites with respect to the drug action. This involves investigating absorption, distribution and elimination (metabolism and/or excretion) processes using blood or plasma concentrations as a surrogate. Pharmacokinetic models describe drug concentrations as a function of both the administered dose and time.

Pharmacodynamics is basically what the drug does to the body, i.e. the relationship between drug concentrations and pharmacological effects (also called pharmacological surrogates or bioresponses or biomarkers). Different levels of biomarkers can be considered as an indicator of the pharmacological effect. It can be the pharmacological response itself or indicators of normal biological or pathogenic
processes. Figure 1.1 shows a classification of biomarkers. Ultimately, the relationships between biomarkers and clinical (efficacy or safety) outcomes should be established. Pharmacokinetic and pharmacodynamic models can be combined to describe an overall dose-exposure-response relationship.

**Figure 1.1** Schematic representation of a classification of biomarkers [6]

The role of pharmacokinetic / pharmacodynamic modelling and simulation in drug development is to enhance primarily the learning steps to improve information-gathering, hence label documentation, and guide confirmatory clinical trials [5]. Figure 1.2 illustrates the drug development process with the use of modelling and simulation in parallel. The integration of pharmacokinetic and pharmacodynamic information through modelling and simulation is used to accelerate the evaluation of new entities in human and to optimize the post-marketing surveillance of the newly marketed drug [7]. Unlike basic models describing pharmacokinetic and/or pharmacodynamic data, the use of system biology and disease models for analysis of exposure-response relationships and clinical trial simulations is more recent [8].
In the pre-clinical phase, modelling and simulation is especially important to screen lead compounds, and to translate the pharmacological knowledge gained from in vitro and in vivo animal experiments to human in order to select the most promising candidate [10] and guide the design of Phase I studies [11-13]. For instance, clinical trial simulations are commonly performed to explore the impact of drug-drug interactions and rationally select which clinical drug-drug interaction studies to conduct [14].

Although used for a long time in Phase I clinical trials for many different purposes, modelling and simulation applications occurred for many years mainly in patient studies using the population approach for sparse-data analysis. However, since the use of biomarkers and trial simulation increased and since the learn-confirm paradigm has been adopted, the role of modelling and simulation in Phase I clinical trials has dramatically increased [15]. Currently, Phase I studies are tantamount to all the ‘learning’ studies having non-therapeutic objectives, and include trials carried out in healthy volunteers as well as in patients. The core studies typically aim at evaluating the initial safety and tolerability of the drug as well as its pharmacokinetics and pharmacodynamics in human. Moreover, investigation of the food effect on oral bioavailability, of pharmacokinetics in sub-populations (e.g. patients with renal or hepatic impairment, elderly and children, pregnant women, and ethnic sub-groups) and of drug-drug interactions, are highly recommended by guidelines from the International Conference on Harmonization [16]. The benefits of
modelling and simulation during Phase I programs have been pointed out through many examples which can be classified into three categories based on the frequency of their value. Firstly, there are situations that appear to be solvable only through the use of modelling and simulation, such as characterising nonlinear pharmacokinetics, handling of censored data (e.g. data below the limit of quantification of the assay) and of sparse data by hierarchical modelling [17], establishing the relationship between exposure and a time-dependent biomarker [18], and quantifying the time-course of metabolites and their relationship to the parent drug [19]. In addition, it has been recognized that combining or comparing pre-clinical results with those of early human studies can be highly valuable to further design of Phase II clinical trials [20], and that pooling rich data of Phase I clinical studies allows investigation of relevant prognostic factors for the therapeutic response to a drug (e.g. gender, age and genotype) [15]. There are others situations where modelling and simulation tools are not invaluable but are nonetheless very helpful: prediction of exposure-response following a multiple dose regimen based on single dose data [21], and use of a model-based deconvolution method to determine the optimal input profiles that provide the desired systemic exposure for new formulations [22]. To summarize, the use of modelling and simulation in Phase I allows pharmaceutical industries to move faster but safely into patient studies.

Following the ‘learn-confirm’ paradigm, Phase IIa studies are conducted to confirm that the clinical dose range predicted from Phase I data provides a positive benefit/risk ratio in the target patient population. At this stage, the role of the population modelling approach is very important to investigate and predict the dose-exposure-response relationship in patients, from both a safety and an efficacy perspective [4]. The predictive potential of pharmacokinetic and pharmacodynamic models represents a key element for rational decision-making, especially for the design of subsequent clinical trials. In that perspective, developing a drug-disease model may help to understand the time course of disease progression and thus dose-response in patients. Positive findings at the end of this phase generally justify the subsequent larger and more costly ‘learn-confirm’ cycle (traditionally Phase IIb and III).

The second ‘learn-confirm’ cycle is intended to provide information about the optimal use of the drug that provides an acceptable benefit/risk ratio, in a more
representative group of patients (Phase IIb), and to confirm the benefit achieved under this therapeutic regimen in a larger patient population (Phase III). Overall, modelling and simulation have been successfully applied to Phase II and Phase III trials by using the population approach [23]. The main task during this cycle is to investigate the exposure-response relationship in patient groups with known relevant prognostic factors (e.g. genotype) (Phase IIb) and to elucidate whether some groups need adjusted dosing regimens (Phase III) [4]. In addition to a group-specific dosage approach, population analyses from previous studies can help to individualise the dose in order to achieve the target window (if known) in most patients [24]. Such an individual-specific dosage approach requires patients from groups at risk (e.g. elderly) to be included at an early stage, so that the full range of population characteristics can be obtained. However, in order to avoid decreasing the power of clinical trials by including patients with different pharmacokinetic and/or pharmacodynamic profiles (additional variability in clinical response), those patients should be included as a satellite group. This group would be excluded from the efficacy assessment database but the obtained pharmacokinetic and pharmacodynamic results would be compared with those of the control group [4]. It should be emphasise that the use of simulation and optimization tools in Phase IIb is critical as it guides the design of dosing and sampling schemes, and to evaluate the probability of success of this design [25].

Post-marketing surveillance (Phase IV) studies are part of the confirming stage of the last ‘learn-confirm’ cycle. Assessment of the long term safety is the primary goal of this surveillance. Moreover, it also intends to further refine the dosage guidelines established during development, enrich the drug-drug interactions and drug-disease information base, and re-evaluate the safety and efficacy of the new medicine in a more diverse patient population. Population analyses are well suited for the assessment and design of Phase IV studies as concentration monitoring during post-marketing surveillance is uncommon [26]. Hence, sample collection from outpatients is usually associated with sparse data which are likely to be best analysed using the population approach. Also, a thorough understanding of the variability in the pharmacokinetics and pharmacodynamics of a newly marketed drug is essential to review its safety profile [27]. Nevertheless, successful population analysis requires a good quality of the database, i.e. a rigorous administration accounting history,
compliance monitoring, and transparency about any other factors known to affect the pharmacokinetics and pharmacodynamics of the drug [28].

1.2. Mechanistic modelling of clinical pharmacokinetic data

1.2.1. Physiologically-based pharmacokinetic models

The complexity of pharmacokinetic or pharmacodynamic models can range from ‘empirical’ to ‘mechanistic’ or ‘semi-mechanistic’. An empirical model is driven by the analysis of collected data and cannot be fully specified prior to the experiment (e.g. sum of exponentials). On the other hand, a mechanistic model includes parameters reflecting physical or conceptual entities in the subject-matter domain of the model. Hence, the values of such parameters can be found in the relevant literature or based on experiments and can be used for exploratory simulations. Semi-mechanistic models, in which parameters are related to biological or physiological processes (e.g. clearance related to renal function through creatinine clearance), are an intermediate level between empirical and mechanistic models. For instance, a two-compartment disposition model with a central and a peripheral compartment that are parameterised in terms of systemic clearance and volume of distribution can be considered as a semi-mechanistic model. However, for both empirical and semi-mechanistic models, compartments do not represent real physical spaces and their meanings are thus often limited. In general, a model can be ‘descriptive’, i.e. applicable only to restricted circumstances, or ‘predictive’, i.e. includes design-specific variables as well as baseline features allowing the prediction of outcomes in different conditions [5]. A mechanistic model is naturally predictive as it incorporates information on the system of interest (e.g. mammillary physiology).

In drug development, the aim of pharmacokinetic and pharmacodynamic modelling is primarily to enhance the learning steps. Sheiner emphasised that as opposed to empirical models, mechanistic models are more likely to suit learning, i.e. to extrapolate beyond the bounds of the design on which they are defined (the bounds must be expressed in the models) and provide credible extrapolation [5]. Indeed, the parameters of an empirical pharmacokinetic or pharmacodynamic model have no
biological or physiological significance as the structural model is strictly defined by the data. Therefore predictions in new clinical conditions are difficult. Overall, the more complete/complex a model is the better extrapolation it provides.

In pharmacokinetics, comprehensive mechanistic models are based on subject physiology [29]. In a physiologically-based pharmacokinetic (PBPK) model, compartments represent actual tissues and organ spaces that are characterised by their structure, volume, composition and associated blood flows [30]. This system-specific information is independent of the pharmacokinetic data and can usually be found in the anatomy and physiology literature. Figure 1.3 shows an example of a whole-body PBPK model in which the tissues and organs of the body are arranged anatomically and are perfused by and connected via the vascular system [31]. Basically, the drug enters compartments in the arterial blood, returns to the heart in the venous blood, and is eliminated in specific organs such as the liver and the kidney. Generally, it is assumed that the drug uptake by a tissue is limited by the blood flow (especially for lipophilic drugs which are likely to cross membranes by passive diffusion). However, for some specific drugs and tissues, the uptake can be limited by the permeability of the drug. The model can be even more complex if information about lower level processes is available (e.g. cellular level). PBPK models are highly mechanistic as drug-specific data on tissue affinity, plasma protein binding, membrane permeability, and enzymatic and transporter activity, reflect biological/physiological ‘entities’. As a consequence, in addition to providing a better understanding of the pharmacokinetics of therapeutic drugs, it helps to predict plasma and tissues concentration-time profiles under more complex conditions [32].
The PBPK modelling approach truly emerged in the 60s [29,30]. Traditionally, semi-mechanistic models were more frequently used to characterise pharmacokinetics than PBPK models which were considered too complex and applied essentially in the academic field rather than in industries and in regulatory submissions [31]. Nevertheless, there has been a growing focus on the use of PBPK in drug discovery and development especially for lead compound selection and early studies in human [33]. In some instances, PBPK modelling can significantly enhance the clinical candidate optimisation and selection process before entering the clinical development. Indeed, unlike the allometric approach which assumes that differences across species are essentially driven by body size, PBPK models can account for interspecies differences in active processes (e.g. metabolism and membrane transport) thereby providing meaningful in vivo predictions of pharmacokinetics in both plasma and tissues, and thus a more rational strategy for first-in-human clinical trials [19]. The incorporation of active processes is feasible due to readily available in silico computation results combined with relevant in vitro data (e.g. plasma
protein binding, microsomal or hepatocyte intrinsic clearance and cell membrane permeability) [31]. More importantly, PBPK simulations applied from early clinical trials help understand and extrapolate likely pharmacokinetic profiles and dose across patient populations and disease states that are typically associated with variable physiological conditions (e.g. body weight and composition, hepatic and renal function, and cardiovascular function) [34,35]. Finally, it should be emphasized that PBPK modelling is the only approach that can address safety concerns about tissue exposure to a drug and potential risks [36], and explore efficacy differences between active site(s) and plasma [37].

So far, semi-mechanistic pharmacodynamic models have been used successfully in clinical practice. However, just as with PBPK models, there is an increasing interest in the so-called ‘systems biology’ approach in which drug-specific data such as receptor affinity and intrinsic efficacy, are separated from system data such as receptor density, the stimulus-response relationship and homeostatic control mechanisms [38]. The use of PBPK models linked with mechanism-based pharmacodynamic models may provide a powerful mechanistic framework that aids the prediction of in vivo dose-exposure-response relationship across species, and understanding the variability in drug response. In addition, reliable anatomical databases which include physiological, biological and pharmacological information are more and more available to inform and give partial guidance for both PBPK and systems biology models [39].

1.2.2. Oral absorption models

Most medicines are administered by the oral route as it is more practical and less invasive for patients than parenteral administration routes. Using oral formulations, therapeutic drugs can be self-administered so that patients do not have to go to specialised medical centres to receive their treatment. This is particularly important to reduce the health care costs associated with chronic diseases. The oral administration route has been widely adopted as most therapeutic drugs have physicochemical properties that enable permeation through the small intestine epithelium (sometimes also through the gastric epithelium) either by passive diffusion or active transport. However, oral absorption is a complex process as it is governed by a number of mechanisms and factors including gastric pH and emptying.
rate, drug solubility, dissolution of the dosage form, intestinal transit and stability, gut wall efflux and metabolism, disease state, and other environmental factors such as the food diet and smoking status. A biopharmaceutical classification system has been proposed to correlate in vitro drug solubility and permeability properties to the extent of absorption following oral administration in human [40]. However, this classification is a qualitative tool that takes into account only a few properties of pharmaceuticals. For instance, it does not account for gut wall metabolism characteristics and hence cannot provide information on the extent of bioavailability. Quantitative compartmental models are more valuable to describe and predict the entire input process from the administration of a drug to when the drug reaches the systemic circulation.

Complex mechanistic absorption models that account for dissolution of the dosage form, gastrointestinal transit, absorption into the intestinal enterocytes, enterocytic metabolism and efflux, and transport to the liver, have been proposed [41-43] and successfully applied to predict pharmacokinetics of various drugs after oral administration [44-48]. Clearly the advantage of physiologically-based absorption models over more empirical models is that a better understanding of the first-pass effect can be gained, human oral bioavailability and its components (fraction absorbed, fraction escaping enterocytic metabolism and fraction escaping hepatic extraction) can be predicted based solely on in vitro and animal data, and absorption profiles can be extrapolated across different formulations and physiological conditions. In addition, relationships between system-specific parameters and demographics (e.g. between gastric pH and age) have been studied and can be incorporated in the model to account for inter-individual variation in the absorption pattern observed in vivo [43]. Nevertheless, implementing a mechanistic absorption model is time consuming and requires a sufficient amount of system-, drug- and formulation-specific information from the literature and from in vitro and animal experiments. In addition, scientists sometimes simply want to describe the oral pharmacokinetic profile of a drug in human and possibly estimate the systemic bioavailability based on clinical data. For that purpose, less complex absorption models may be used.

Traditionally, the rate and extent of drug input into the blood stream is described using empirical or semi-mechanistic (compartmental) absorption models. A review
of these models has been presented in [49]. Since these models are data-driven, it is recommended to plot concentration-time profiles prior to any model-based analysis. The complexity of the absorption model required often depends on the number of blood samples collected during the initial input phase of the pharmacokinetic process. When linked to a compartmental disposition model, these models can describe a concentration-time curve following oral administration. However, the blood/plasma output is a result of both the input and disposition processes that occur simultaneously. In mathematical terms, a concentration-time function (output function) is a result of the convolution (a mathematical operation) of an input and a disposition function. When both oral and intravenous data are available the input and disposition processes can be distinguished as intravenous data informs drug disposition only. In these conditions, the model can be globally identifiable (the parameters can be uniquely determined given perfect input-output data) and the systemic bioavailability of an orally administered drug can be estimated [50,51]. If intravenous data are not available, local identifiability (there is a finite number of sets of parameter values that equally well describe the input-output data) can be achieved by fixing the bioavailability to one and estimating ‘apparent’ disposition parameters, e.g. the ratio of clearance to bioavailability [52]. For most drugs and formulations, conventional absorption models that assume a zero- or first-order input rate for a fixed period can adequately describe pharmacokinetic profiles following oral administration. However, more flexible input models are required to describe complex oral concentration-time curves. Flexible input models have been implemented in the past by using the probability density function of a flexible statistical distribution (e.g. gamma, Weibull and inverse Gaussian) as an input function for the pharmacokinetic model [53-55]. In more complex situations, the input process is multiphasic, meaning that the distribution of the input transit times is possibly multimodal. This is often observed for extended-release oral formulations [56]. Flexible nonparametric or semiparametric methods have been proposed to describe such a complex input process [57,58]. An accurate characterisation of the input process is particularly important in drug development to compare the pharmacokinetic properties of two or more formulations and advise on the optimal conditions of use of the selected formulation. An adequate formulation should provide a desirable systemic exposure as well as a safe and convenient dosing for patients.
1.2.3. **Hierarchical pharmacokinetic modelling**

Traditionally, clinical pharmacokinetic studies were conducted in small homogeneous groups, frequently healthy volunteers, with a dense sampling scheme for each individual thereby allowing the characterisation of individual pharmacokinetic parameters [59]. Longitudinal data typically collected during clinical trials are often sparse due to logistic and ethical constraints. For instance, a dense sampling scheme is often not appropriate in special populations such as neonates or elderly patients. The population approach allows characterisation of pharmacokinetics using sparse sampling data from a group of individuals that is treated as a random sample from a larger population [60]. To satisfy the statistical requirements, a relatively large number of subjects is required with at least two samples per individual [61]. If the study design is appropriate and the information on sample collection sufficient, the population approach enables the quantification of the variability in the data and the identification of factors that partially explain this variability. It was primarily applied to therapeutic drug monitoring in order to individualise patient care. Information about dose individualisation is relevant for the clinicians and usually leads to a safer and more effective use of the therapeutic drug. It then received widespread attention in the clinical pharmacology area, as it helps understand the difference of drug response between patient groups given their clinical characteristics and the dosing regimen. In drug development, even though population analyses cause additional costs for the assessment of potential prognostic factors, database support and time consumed, the population approach is thought to be ultimately cost effective [27].

In population pharmacokinetic model-based analysis, intra- and inter-individual variations are accommodated within the framework of a two-stage hierarchical model. At the first stage, intra-individual variation is usually characterised by a compartmental model with a random error term reflecting uncertainty in the predicted response. This uncertainty can arise from unexplained intra-individual variability, model misspecification, bioassay measurement error, dosing history error etc.. At the second stage, inter-individual variability is taken into account by assuming a distribution of the individual-specific model parameters. In addition, factors that partially explain the differences across individuals can be included in the model to decrease the random component of the variability. These explanatory
variables are also known as covariates and encompass demographics (e.g. age and body weight), genetics (e.g. polymorphism of metabolic enzymes), environmental factors (e.g. smoking) and pathophysiological conditions (e.g. hepatic impairment and hypertension). Relationships between covariates and parameters can either be tested during the statistical analysis or directly incorporated in the model based on biological and physiological evidence. Finally, another level of variation can be incorporated in population pharmacokinetic models to account for inter-occasion variability arising from the unexplained change over time in the individual parameters [62].

The parameters of a population pharmacokinetic model are commonly estimated using maximum likelihood methods. These methods can be thought of as a frequentist interpretation of probabilities as the conditional probability of the data (or model likelihood) is viewed as what might happen in the limit as the experiment is infinitely repeated. Sometimes a scientist has prior information on the model and its parameters coming from previous studies. The Bayesian modelling approach is fundamentally different to the frequentist approach in the sense that it takes into account the information coming from both the observed data and prior studies to estimate the model parameters. Also, as opposed to maximum likelihood techniques, Bayesian methods assume that parameters are random variables with probability distributions rather than unknown fixed variables. Hence, while maximum likelihood analysis produces point estimates of the pharmacokinetic parameters with some degree of confidence, Bayesian analysis produces the uncertainty distribution of the parameter values from which meaningful probabilistic inferences can be made. The Bayesian approach can be considered as an alternative to the maximum likelihood approach that may fail to provide adequate estimates when the data are too sparse, the underlying distributional assumptions are violated or the model is too complex [63].

As in a frequentist analysis, a Bayesian population pharmacokinetic model is a multilevel hierarchical model that accounts for the prior distributions of the parameters in addition to intra- and inter-individual variations. For example, a three-stage hierarchical model can be implemented by making distributional assumptions on the model likelihood at the first stage, on the inter-individual variability at the second stage and on the parameter priors at the last stage. Bayesian population
analysis produces statistical distributions not only for the population parameters (means and variances) but also for the individual-specific parameters. A posterior distribution is a compromise between the model likelihood and the prior distribution and can be used to derive clinically relevant probabilities. For instance, clinicians might be interested in the probability that peak concentration for a particular patient or group of patients is below a certain toxicity limit. For a multivariate nonlinear model, the posterior distribution cannot be computed analytically in closed form. Instead, efficient numerical integration techniques such as Markov chain Monte Carlo simulation might be employed to approximate the target distribution. For example, the Gibbs sampler has been widely used in population pharmacokinetics [64], mainly because it is implemented in the software WinBUGS popular for Bayesian analysis. However, an inherent inefficiency of the Gibbs sampler and other samplers based on Markov chain simulation is their random walk behaviour. To suppress this behaviour, the Hamiltonian Monte Carlo sampling algorithm has recently been proposed and implemented in the computer program Stan [65]. Yet, this new sampling technique has not been widely applied to population pharmacokinetics and pharmacodynamics.

The mechanistic pharmacokinetic modelling approach inherently belongs to the Bayesian philosophy as the idea is to include in the model prior knowledge on the parameters that represent biological or physiological entities. In drug development, such a translational tool is used to bridge preclinical and clinical studies. If the model is highly mechanistic, e.g. a whole-body PBPK model, prior information on all parameters can be gathered and used for exploratory simulations of human studies. Even when all parameters can be informed from the literature and preclinical experiments, it is often desirable to then fit the model to clinical data in order to update the current pharmacokinetic knowledge and propagate the information along the development process. The use of priors in PBPK model-based analysis is essential not only to produce biologically plausible estimates of the parameters but also to stabilise the analysis of clinical data that are typically sparse given the complexity of the models [66]. In other words, the information in the data is usually insufficient to support the estimation of all the model parameters with reasonable accuracy. This is because clinical data generally include only blood samples due to ethical constraints. Assessment of the sensitivity of the blood or plasma response to
the parameters of a PBPK model is recommended prior to any data analysis in order to identify which parameters particularly need prior information to support their estimation. The Bayesian approach has been widely used in population toxicokinetics and pharmacokinetics [67-71]. When the model is complex and prior information on the system and its parameters too vague, it might be desirable to simplify the model to possibly stabilise the data analysis. Methods to formally reduce pharmacokinetic models and retain their mechanistic interpretation have been proposed and successfully applied in the past [72-74].

1.3. Modelling survival data in cancer research

1.3.1. Pharmacodynamics in oncology

As seen on Figure 1.1, several levels of pharmacological response surrogates can be used in clinical research. In oncology, pharmacodynamic markers range from the characteristics of solid tumour lesions (number, size, appearance of new lesions etc.) and cancer biomarkers (e.g. prostate specific antigen), to the survival probability of patients. Improvement in patients’ survival and quality of life are considered as reliable measures to show direct evidence of clinical benefit of a cancer drug [75]. Hence, establishing the relationship between drug pharmacokinetics, pharmacodynamic markers and survival is essential to evaluate the effect of an experimental agent and classify patients for treatment optimisation or individualisation.

Assessment of the change in tumour burden is important for clinical evaluation of cancer therapeutics for patients with solid tumours. The main clinical endpoints used in cancer clinical trials are tumour shrinkage (objective response) and time to disease progression or time to death from any cause (overall survival) [76]. These endpoints are traditionally applied based on standard anatomical tumour burden criteria known as Response Evaluation Criteria in Solid Tumours (RECIST) [77]. According to RECIST, progressive disease corresponds to a relative increase of at least 20% in the sum of diameters of target lesions (relative to the smallest sum on study) as well as an absolute increase of at least 5 mm. The appearance of new lesions is also considered as disease progression. The use of RECIST as a surrogate of tumour burden has been recently criticised mainly because the information is summarised as
a categorical variable rather than a continuous variable [78]. Joint pharmacokinetic-
pharmacodynamic modelling of treatment exposure and longitudinal tumour size
data is believed to be a useful quantitative tool to investigate antitumor response as a
continuous variable and investigate the impact of different dosing regimens [3].

The clinical evaluation of response to cancer drugs using RECIST or other tumour
imaging data is expensive and not practical in clinical research as it requires
radiological tests and physical examination of patients typically done every six or
eight weeks. In addition, for drugs that are cytostatic rather than cytotoxic, change in
tumour size might not be an adequate surrogate of pharmacological efficacy. A
number of biomarkers are used in clinical practice to diagnose cancer, monitor and
optimise the treatment response, and detect disease progression. For instance,
carcinoembryonic antigen is used in colorectal cancer, cancer antigen 125 in ovarian
cancer and M-protein in myeloma [3]. These circulating biomarkers are readily
measurable in blood and hence associated with less economic and logistic constraints
than imaging assessments of tumour burden. Consequently, their predictive value for
the risk of disease progression or mortality has been increasingly assessed through
statistical analyses of clinical trial data. The dose-exposure-response relationship for
many of these biomarkers has been explored using population pharmacokinetic-
pharmacodynamic modelling in order to guide dose selection for the corresponding
anticancer agents [79-81].

Finally, monitoring adverse events during treatment cycles is also an important task
in cancer research. Myelosuppression for instance, is a frequent side effect of
cytotoxic drugs, which results in reduced levels of red and white blood cells,
including neutrophils and platelets that play an essential role in the innate immune
system. Hence myelosuppression is often a dose-limiting factor for cancer
therapeutics. A model of myelosuppression has been proposed [82] and applied to
describe white blood cell response to various cancer drugs [83-86]. Other examples
of common cancer drugs’ side effects are anaemia, elevated levels of liver enzymes
and elevated diastolic blood pressure.

1.3.2. Survival analysis

In many cancer studies, the main outcome under assessment is the time to an event
of interest which ranges from disease progression to death from cancer or any cause,
and is usually named ‘survival time’. Investigating the effect of cancer therapeutics on patient survival time is a major task in cancer research to assess the clinical benefit of a treatment and monitor the progress of cancer programs. In addition, it is important to identify prognostic or risk factors to individualise patient care and hence provide a more effective treatment. These factors can also be used as stratification variables in large Phase III randomised trials in order to account for known source of variability in the data. The appropriate use of data from cancer registries or oncology clinical trials for outcome analysis requires a good understanding of the quality of the data and the degree to which they represent the target patient population, and of the appropriate quantitative tools and their limitations.

It is usual in cancer research that at the end of the follow-up period some patients have not had the event of interest and their true time to event is thus unknown or ‘censored’. Further, survival data typically have a skewed distribution rather than a Gaussian one as there are usually many early events and few late ones. Hence, specific quantitative tools have to be used in survival analysis. It is important to define the observation period that determines survival time prior to the survival analysis as it conditions the type of censored data to be analysed. For example, survival time is commonly defined as the time from the start of the study to the time of death, in which case some of the data are right censored. In general, right censoring may arise from a patient who has not experienced the event of interest, a patient lost to follow-up, or patient who has experienced a different event that makes follow-up impossible. Scientists may also be interested in the time period between a confirmed response to treatment and cancer relapse, which can yield left censored data if the response time is missing for some patients, or interval censored data if the event is also not observed for these patients. Most survival data include observed and right censored data only.

The purpose of analysing survival data is to make inference on two probabilities, namely the ‘hazard’ and ‘survival’, which are related as follows:

\[
h(t) = -\frac{dS(t)/dt}{S(t)} = \frac{f(t)}{1 - F(t)} = \frac{p(T = t)}{p(T > t)}
\]

(1.1)
where $T$ is the survival time, $h(t)$ and $S(t)$ are the hazard and survival functions, respectively, $f(t)$ is the probability density function of the survival times and $F(t)$ is the cumulative distribution function for $f(t)$. While the survival function simply describes the probability that a patient survives from the time origin to a specified time $t$ (cumulative non-occurrence), the interpretation of the hazard function is less trivial as it represents the instantaneous event rate for an individual who has already survived to time $t$. Several statistical methods have been proposed to infer these probabilities from survival data, including the Kaplan-Meier estimates, logrank test, Cox regression and parametric survival models. Although these methods are described in detail in many survival analysis textbooks, such as in [87], or in tutorial articles [88-91], we briefly present their principles and limitations in the following paragraphs.

In 1958, Kaplan and Meier proposed a nonparametric method to estimate the survival probability at a time $t$, based on the probability of being alive at the previous time, the number of patients alive just before time $t$ and the number of events occurring at time $t$ [92]. The resulting survival function is a step function that changes values only at the time of an event. If there is no censoring in the data, the estimator reduces to the proportion of studied patients still alive at the end of the observation period. This is not true when some patients do not experience the event as their data still contribute to the calculation of the Kaplan-Meier estimates (number of patients alive just before time $t$). A plot of the Kaplan-Meier survival probability estimates over time provides a useful summary of the data from which measures like the median survival time can be estimated.

To compare the survival time between two or more groups of patients, the logrank test is the most widely used method as it is a nonparametric statistical test that can be easily applied to survival data [93]. Briefly, the aim of this test is to calculate a statistic based on the total expected number of events in each group and the observed number of events, and compare it to a $\chi^2$ distribution. $P$-values are then calculated to assess the statistical significance of the differences between the group-specific survival curves. When only two groups are compared, the logrank test is testing the null hypothesis that the ratio of hazard rates (hazard ratio) is equal to 1. The hazard ratio is a measure of the relative instantaneous risk in two groups and is routinely
used to identify risk factors. A hazard ratio of 1 means that there is no statistically significant difference in survival times between the two groups. In practice, it is better to estimate the hazard ratio using a regression technique. In addition, it is often desirable to adjust for the effect of patient clinical characteristics (cofounding factors or covariates) on survival time when comparing the benefit of treatments in terms of survival prolongation. Also, clinicians may want to determine the prognostic value of these covariates for patient survival. Therefore, a multivariate regression is recommended to estimate treatment effect on survival while accounting for cofounding factors [89].

The Cox proportional hazards model is the most common multivariate regression method used for survival analysis [94]. A Cox model describes the relationship between the hazard function $h(t)$ and a set of $p$ covariates $(x_1, x_2, ..., x_p)$ as follows:

$$h(t) = h_0(t) \cdot \exp \left( \sum_{i=1}^{p} \beta_i x_i \right)$$  \hspace{1cm} (1.2)

where $h_0(t)$ is the baseline hazard, and the covariate effects are measured by the regression coefficients $(\beta_1, \beta_2, ..., \beta_p)$. It is essentially a multiple linear regression of the natural logarithm of the hazard and some covariates, with the intercept being the baseline hazard. Since the baseline hazard is estimated nonparametrically, no distribution of survival times is assumed. The main assumption made in Cox regression is that the hazards between two or more groups are proportional over time. In other words, the group-specific hazard curves should not cross. The proportionality assumption implies that $\exp(\beta_i)$ are the hazard ratios. Hence, a value of $\beta_i$ greater than zero means that as the value of the $i^{th}$ covariate increases, the instantaneous event rate increases and the survival time decreases. The proportionality assumption is often appropriate for survival data but should always be verified.

An alternative to multivariate Cox regression is to use a parametric survival model. Two types of parametric models can be used for survival analysis: parametric proportional hazards models and accelerated failure time models. A parametric proportional hazards model is similar in concept and interpretation to the Cox model.
with the only difference being that the hazard is assumed to follow a specific statistical distribution (e.g. exponential, Weibull or lognormal). When the proportional hazards assumption does not hold, accelerated failure time models can be used and are expressed as follows:

\[ S(t) = S_0(\varphi t) \]  

(1.3)

where \( S_0(t) \) is the baseline survival function and \( \varphi \) is an ‘acceleration factor’ that depends on covariates as follows:

\[ \varphi = \exp \left( \sum_{i=1}^{n} \beta_i x_i \right) \]  

(1.4)

Commonly used statistical distributions for accelerated failure time models are lognormal, Weibull, log-logistic and generalised gamma [95]. It is obvious from Eq. 1.3 and Eq. 1.4 that the covariates act multiplicatively on the survival time rather than on the hazard rate as in proportional hazards models. Therefore, as opposed to the Cox and parametric proportional hazards models, a value of \( \beta_i \) higher than zero indicates that as the value of the \( i^{th} \) covariate increases, the time ratio \( \exp(\beta_i) \) is higher than 1 and survival is prolonged.

In general, statisticians prefer the Cox model over parametric models as no distributional assumption needs to be made for survival times. Although, the parametric approach is more attractive if simulations are intended to be performed following the data analysis. Regardless of the approach applied, efforts should be made to assess whether the specified model is an appropriate representation of the survival data using goodness-of-fit tests. For instance, it is recommended to compare the Kaplan-Meier survival curve with the model-predicted survival curve. More specific tests for proportional hazards models and accelerated failure time models should also be considered [90]. Finally, potential prognostic factors should be selected carefully, that is with regards to the aim of the analysis and the data sample size, using robust variable selection methods [91].
1.4. Project objectives

The general aim of this thesis was to propose modelling strategies to facilitate the application of mechanistic pharmacokinetic models and cancer survival models to clinical study data. To illustrate these strategies the work was divided into two parts. The first part (pharmacokinetic scenario) was a project developed in collaboration with Novartis Pharma AG (Basel, Switzerland) and aimed at gaining insight into the population pharmacokinetics of mavoglurant (Figure 1.4), a compound that was under clinical development for the treatment of central nervous system diseases at the time of the work. The second unrelated part (pharmacodynamic scenario) aimed at applying existing modelling approaches to the analysis of survival data from patients with metastatic pancreatic cancer undergoing gemcitabine chemotherapy.

![Figure 1.4 Chemical structure of mavoglurant](image)

The research on mavoglurant population pharmacokinetics was first carried out with the focus on mathematical and statistical techniques to be able to adequately describe the complex absorption pattern of the drug (multiple-peak profiles) and further evaluate the impact of factors like the type of oral formulation or the intake of food. This work is published and presented in Chapter 2 entitled ‘Model-based evaluation of the impact of formulation and food intake on the complex oral absorption of mavoglurant in healthy subjects’.

The focus was then re-oriented toward the use of Bayesian hierarchical modelling techniques to enable the analysis of the clinical pharmacokinetic data with a complex mechanistic or physiologically-based model, in order to understand better the
absorption, distribution and elimination of mavoglurant and be able to extrapolate this knowledge to different clinical scenarios, such as different experimental conditions (e.g. co-administration of the drug) or sub-populations (e.g. children). This study is published and presented in Chapter 3 entitled ‘Application of a Bayesian approach to physiological modelling of mavoglurant population pharmacokinetics’.

We also considered applying an existing mathematical method to simplify the physiologically-based pharmacokinetic model originally developed for mavoglurant and facilitate the analysis of clinical data while possibly preserving the extrapolation ability of the original model. This research is published and presented in Chapter 4 entitled ‘Reduction of a whole-body physiologically-based pharmacokinetic model to stabilise the Bayesian analysis of clinical data’.

Finally, the investigation of the survival pattern of patients with metastatic pancreatic cancer was carried out with the intention of comparing existing modelling approaches as well as possibly providing new findings with regards to factors known to influence survival of patients treated with gemcitabine. This is work is under consideration for publication and presented in Chapter 5 entitled ‘Predicting survival of pancreatic cancer patients treated with gemcitabine using longitudinal tumour size data’.

1.5. **List of manuscripts and author contribution statement**

In accordance with the University of Manchester guidance on the alternative thesis format for the Doctor of Philosophy degree, the contributions of the candidate and co-authors to the herein published manuscripts and the manuscript in preparation are explained below. Please note that since Chapter 2-4 are already published, only the offprints of the journal articles are provided in this thesis.
Chapter 2: Model-based evaluation of the impact of formulation and food intake on the complex oral absorption of mavoglurant in healthy subjects

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*T. Wendling*: Literature search, extraction and analysis of clinical study data, simulation of mavoglurant pharmacokinetics, design and management of the research, main preparation of the manuscript.

*K. Ogungbenro*: Supervision of research, input on modelling complex absorption patterns and population pharmacokinetic data, input on the manuscript.

*E. Pigeolet*: Supervision of research, input on modelling population pharmacokinetic data, expert opinion on the pharmacokinetics of mavoglurant, input on manuscript.

*S. Dumitras*: Supervision of research, expert opinion on the pharmacokinetics of mavoglurant, input on the manuscript.

*R. Woessner*: Supervision and design of research, expert opinion on the pharmacokinetics of mavoglurant, input on the manuscript.

*L. Aarons*: Supervision and design of research, expert opinion on the population modelling approach, input on the manuscript.

Chapter 3: Application of a Bayesian approach to physiological modelling of mavoglurant population pharmacokinetics

*J Pharmacokinet Pharmacodyn.* 2015, 42(6):639-57

*T. Wendling*: Literature search, extraction and analysis of preclinical data, mechanistic model development, statistical analysis of clinical data, simulation of mavoglurant pharmacokinetics, design and management of the research, main preparation of the manuscript.

*S. Dumitras*: Supervision of research, extraction of preclinical data, expert opinion on the pharmacokinetics of mavoglurant, input on the manuscript.

*K. Ogungbenro*: Supervision of research, input on physiologically-based pharmacokinetic modelling, on pharmacokinetics in paediatrics and on the manuscript.

*L. Aarons*: Supervision and design of research, expert opinion on hierarchical mechanistic pharmacokinetic modelling, input on the manuscript.
Chapter 4: Reduction of a whole-body physiologically-based pharmacokinetic model to stabilise the Bayesian analysis of clinical data

*AAPS J.* 2015. 18(1):196-209

**T. Wendling:** Literature search, simplification of a complex physiological pharmacokinetic model, statistical analysis of clinical data, simulation of mavoglurant pharmacokinetics, design and management of the research, main preparation of the manuscript.

**N. Tsamandouras:** Design of the research, input on hierarchical mechanistic pharmacokinetic modelling and on the manuscript.

**S. Dumitras:** Supervision of research, expert opinion on the pharmacokinetics of mavoglurant, input on the manuscript.

**E. Pigeolet:** Supervision of research, input on the simplification of a complex physiological pharmacokinetic model, expert opinion on the pharmacokinetics of mavoglurant, input on manuscript.

**K. Ogungbenro:** Supervision of research, input on the simplification of a complex physiological pharmacokinetic model, on pharmacokinetics in paediatrics and on the manuscript.

**L. Aarons:** Supervision and design of research, expert opinion on the simplification of a complex physiological pharmacokinetic model and on scaling pharmacokinetic models within or between species, input on the manuscript.

Chapter 5: Predicting survival of pancreatic cancer patients treated with gemcitabine using longitudinal tumour size data

*In preparation*

**T. Wendling:** Literature search, extraction and statistical analysis of clinical study data, evaluation of survival modelling approaches, design and management of the research, main preparation of the manuscript.

**H. Mistry:** Design and supervision of the research, expert opinion on survival analysis, input on the manuscript.

**K. Ogungbenro:** Supervision of research, input on modelling longitudinal tumour size data and on the manuscript.

**L. Aarons:** Supervision and design of research, expert opinion on modelling longitudinal tumour size data, input on survival modelling strategy and on the manuscript.
1.6. References


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Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. Journal of the National Cancer Institute 92 (3):205-216


Chapter 2: Model-based evaluation of the impact of formulation and food intake on the complex oral absorption of mavoglurant in healthy subjects

T. Wendling, K. Ogungbenro, E. Pigeolet, S. Dumitras, R. Woessner and L. Aarons

*Pharm Res.* 2015, 32(5):1764-78
ABSTRACT

Purpose To compare the pharmacokinetics of intravenous (IV), oral immediate-release (IR) and oral modified-release (MR) formulations of mavoglurant in healthy subjects, and to assess the food effect on the MR formulation’s input characteristics.

Methods Plasma concentration-time data from two clinical studies in healthy volunteers were pooled and analysed using NONMEM®. Drug entry into the systemic circulation was modelled using a sum of inverse Gaussian (IG) functions as an input rate function, which was estimated specifically for each formulation and food state.

Results Mavoglurant pharmacokinetics was best described by a two-compartment model with a sum of either two or three IG functions as input function. The mean absolute bioavailability from the MR formulation (0.387) was less than from the IR formulation (0.436). The MR formulation pharmacokinetics were significantly impacted by food: bioavailability was higher (0.508) and the input process was shorter (complete in approximately 36 versus 12 h for the fasted and fed states, respectively).

Conclusions Modelling and simulation of mavoglurant pharmacokinetics indicate that the MR formulation might provide a slightly lower steady-state concentration range with lower peaks (possibly better drug tolerance) than the IR formulation, and that the MR formulation’s input properties strongly depend on the food conditions at drug administration.

KEY WORDS dose superimposition · food effect · input rate function · mavoglurant population pharmacokinetics · modified-release formulation

ABBREVIATIONS

BQL Below the quantification limit
BW Actual bodyweight
CL Plasma clearance
IG Inverse Gaussian
IMPMAP Monte Carlo importance sampling method assisted by mode a posteriori with interaction
IR Immediate-release
ISV Intersubject variability
IV Intravenous
mGluR5 Metabotropic glutamate receptor 5
MR Modified-release
OFV Objective function minimum value
Q Inter-compartmental clearance
Vc Volume of distribution of the central compartment
Vp Volume of distribution of the peripheral compartment

INTRODUCTION

Mavoglurant is a structurally novel, subtype-selective, non-competitive antagonist at the metabotropic glutamate
receptor 5 (mGluR5). It is currently under clinical development (Novartis Pharma AG, Basel, Switzerland) for the treatment of fragile X syndrome, which is the most common hereditary form of mental retardation (1) and the most common single genetic cause of autism (2–5) in humans. The typical phenotype includes intellectual disability, developmental delays and behavioural disorders, and is usually diagnosed in young children (6). By blocking the glutamatergic signalling through mGluR5, mavoglurant is thought to have the potential to rescue the disease state (7–10). A recent study in adult males showed that behavioural symptoms of fragile X syndrome can be improved by mavoglurant treatment (11), but efficacy needs to be confirmed in lager clinical studies. Also, a study of the effect of mavoglurant on obsessive-compulsive disorder in patients resistant to selective serotonin reuptake inhibitor therapy, has been conducted (ClinicalTrials.gov identifiers NCT01813019). A good understanding of mavoglurant pharmacokinetics in healthy subjects is required to determine its optimal conditions of use in the target populations. In this view, it is important to select a formulation that produces steady-state plasma concentration resulting in a desirable therapeutic effect while providing a safe and convenient dosing for patients.

Mavoglurant is intended to be administered by the oral route. The pharmacokinetics of mavoglurant after oral administration of a single 200 mg $^{14}$C-radiolabeled dose in four healthy males, was described recently: the extent of absorption was ≥50% of the total dose; it is a neutral and very lipophilic compound (logP of 4.7) and was therefore extensively distributed to organs and tissues (the mean apparent terminal volume of distribution was estimated to be 38.7 l/kg); the blood-to-plasma concentration ratio and unbound fraction to plasma proteins were estimated to 0.61 and 0.028, respectively; its elimination occurred primarily by oxidative metabolism leading to the formation of seven metabolites which accounted for 60% of the systemic exposure to the total radioactivity over 72 h; the estimate of mean apparent plasma clearance was 2.07 l/h/kg; based on the metabolites observed in human excreta and prior in vitro metabolism results, the major biotransformation pathway was thought to involve cytochromes P450 2Cs, 3A4, 1A1 and 2D6 and accounted for 56% of total metabolism (12). An immediate-release (IR) hard gelatine capsule formulation that requires a twice-daily dosing regimen was first used in human clinical studies. Since in these studies, the majority of adverse events attributed to mavoglurant treatment (dizziness, dyskinesia, hallucination and fatigue) were assumed to be peak plasma concentrations related (13,14), a modified-release (MR) matrix tablet was developed in order to reduce peak plasma concentrations without substantial change in the plasma exposure to mavoglurant. The pharmacokinetics of the IR and MR formulations were compared in a Phase I study in healthy volunteers in order to guide formulation selection for further clinical development. Since mavoglurant is poorly soluble in aqueous solution (pH independent solubility of 0.025 mg/ml) but well absorbed in vivo (highly permeable) (12), it is considered as a Class II compound in the Biopharmaceutics Classification System. Furthermore, since it is extensively metabolised, it is assumed to be also a Class II compound in the Biopharmaceutics Drug Disposition Classification System (15). Therefore, an increase in the extent of bioavailability was suspected for concomitant administration with a high fat meal, and was investigated for the MR formulation.

The goals of this analysis were to develop and evaluate a nonlinear mixed-effects model to (i) describe mavoglurant disposition in a healthy population and identify any contributing demographic covariate factors, (ii) characterise and compare mavoglurant input rate and bioavailability from the IR and MR formulations, (iii) quantify the effect of a high fat meal on the bioavailability and input rate of the MR dosage form, and (iv) predict the impact of mavoglurant release-rate and of concomitant food intake on the concentration range provided by a twice-daily repeated administration. To overcome potential identifiability issues when mathematically characterising the input kinetics of a prolonged-release formulation, it is necessary to inform drug disposition with data provided by the intravenous (IV) route. Therefore, pharmacokinetic data following IV administration of mavoglurant in healthy volunteers were extracted and included in the analysis. Following oral administration, mavoglurant plasma concentration-time profiles appeared to be complex and highly variable across the studied subjects. For instance, an erratic multiple-peak phenomenon was observed in individual profiles after administration of both the IR and MR formulations under fasted conditions. Since plasma concentrations following IV administration clearly revealed two exponential phases of drug disposition and did not exhibit multiple peaks, it was assumed that the complexity of the pharmacokinetics arose from the absorption process. Complex input profiles are difficult to characterise using conventional absorption models that assume a first-order or zero-order input rate for a fixed period. For orally administered drugs, more mechanistic models that incorporate, to a certain extent, physiological factors involved in the absorption process are difficult to implement when the underlying mechanism is unknown (16). Therefore, a flexible empirical model describing the erratic input transit time of orally administered mavoglurant was sought in this analysis.

DATA AND METHODS

Clinical Data

Frequently sampled pharmacokinetic data from two clinical studies in healthy volunteers were pooled to form the data set
used in the analysis (Table I). The majority of subjects were young (median age of 28 years) Caucasian (90.2%) males (72.0%) with a median weight of 80.3 kg.

Study A2121 was conducted to quantify the effect of single IV doses of mavoglurant on baseline- and placebo-corrected QTc intervals in healthy subjects, as well as to characterise the pharmacokinetics of mavoglurant following a 10-min IV infusion. The first part of the study (Part A) was a single-ascending dose phase during which three parallel groups of 12 subjects received either a dose of 25, 37.5 or 50 mg. In the second part (Part B), 84 subjects received two doses of 25 and 50 mg in a randomized crossover design. Plasma concentration-time data from 120 subjects in total were available.

The objective of Study A2167 was to compare the pharmacokinetic properties of three different oral prolonged-release formulations of mavoglurant after a single 100-mg dose in healthy subjects, with reference to a 50-mg single dose in two IR capsules (25 mg/capsule), and to evaluate the effect of a high-fat (~50% of the meal’s total calories) and high-caloric (~800–1000 calories) breakfast on the pharmacokinetics of the prolonged-release forms. Medication was thus given either under fasted conditions or within 5 min of completion of a high fat meal. 44 young Caucasian male volunteers were to receive a total of five single doses of mavoglurant out of the seven treatments tested. The three prolonged-release forms differed by the time window within which the drug was intended to be released in the gastrointestinal tract (6, 7 and 8 h). Since the formulation developed to release mavoglurant over 8 h (denoted MR in the present work) was selected for further clinical development, data for the other prolonged-release formulations were excluded from this analysis. Hence, three treatments were retained for the herein model-based analysis: a 50-mg dose in IR capsules under fasted conditions (IR-fasted), a 100-mg dose in the MR tablet under fasted conditions (MR-fasted) and a 100-mg dose in the MR tablet under fed conditions (MR-fed). A total of 16 subjects discontinued the study due to adverse events, or withdrew consent. While all subjects completed the IR-fasted period, only 29 subjects were administered a dose as per the MR-fasted treatment and 28 subjects received the MR-fed treatment.

Additional pharmacokinetic data from a study of the effect of three different meal compositions and three different timing of food intake on the pharmacokinetics of mavoglurant following a single oral administration of the 100-mg MR tablet

<table>
<thead>
<tr>
<th>Table I</th>
<th>Subjects Demographics and Designs of the Clinical Studies of Mavoglurant Pharmacokinetics in the Healthy Subjects Included in the Population Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Analysed data set</td>
</tr>
<tr>
<td>Design</td>
<td>Randomized, partially-blinded, active-comparator controlled, crossover, two sequential parts: pilot single-ascending dose phase (Part A) and core thorough QTc phase (Part B)</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>Part A: 36, Part B: 84</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Gender (%)</td>
<td>Male = 86.7, Female = 13.3</td>
</tr>
<tr>
<td>Race (%)</td>
<td>Caucasian (61.7), Black (30), Native American (1.7) and Other (6.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.8 (56.6–115.3)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>26.5 (18.7–32.2)</td>
</tr>
<tr>
<td>Route / formulation</td>
<td>IV / 10 min infusion</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>Part A: 25, 37.5 or 50, Part B (crossover): 25 and 50</td>
</tr>
<tr>
<td>Food conditions</td>
<td>Fasted</td>
</tr>
<tr>
<td>PK sampling schedule</td>
<td>Part A: predose and at 0.25, 0.33, 0.5, 0.67, 1, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h postdose, Part B: predose and at 0.33, 0.5, 0.67, 1, 2, 3, 4, 6, 8, 12 and 24 h postdose</td>
</tr>
<tr>
<td>Typical no. of samples/subject</td>
<td>Part A: 14, Part B: 24</td>
</tr>
</tbody>
</table>

Continuous variables are given as median (range) and were reported for all subjects

a Total number of samples
in healthy subjects (Study A2171), were used for cross-validation of the final model rather than in the analysis (Table II). Thirty-eight healthy volunteers were administered a total of five single doses of mavoglurant: one dose under fasted conditions and four doses under fed conditions. Since the meal compositions were substantially different from the composition of the high fat breakfast assessed in Study A2167, data from the fed periods were excluded for external validation of mavoglurant pharmacokinetic model.

All studies were conducted according to the ethical principles of the Declaration of Helsinki and all protocols were approved by the Institutional Review Board for the study centers. The study participants were males and non-pregnant females over the age of 18 years and all provided full written informed consent prior to inclusion in the studies.

In all studies, mavoglurant concentrations in plasma were determined by a validated liquid chromatography-tandem mass spectrometry method (17). The lower limit of quantification was 2 ng/ml. Concentrations below this limit were labeled as zero.

**Pharmacokinetic Data Analysis and Modelling Methods**

A total of 3483 concentration-time observations of 164 healthy subjects were available for the model-based analysis. Since the data were of population type, a nonlinear mixed-effects modelling approach was applied using the software NONMEM® (version VII, level 2.0) (18). NONMEM runs were conducted using the software tool Pearl-speaks-NONMEM 3.5.3 (19). The first-order conditional estimation method with interaction was first used for parameter estimation during model building. Since numerical issues were experienced with this method, we resorted to a Monte Carlo importance sampling method assisted by mode a posteriori with interaction (IMPMAP) to estimate the standard value of the pharmacokinetic parameters in the population, random intersubject variability (ISV) and random interoccasion variability in these parameters, and residual variability between model predictions and observed plasma concentrations. The random residual variability may arise from unexplained within-subject variability, model misspecification and experimental error. Correlations between variability components were tested. Using the IMPMAP estimation method, the number of iterations (NITER option in NONMEM) and the number of random samples per individual (ISAMPLE) were set to 3000 and 1000, respectively. Convergence was tested on the objective function, fixed-effects, random-effects (diagonal elements of the variance-covariance matrix only, i.e. option CTYPE=2) and the residual error. To evaluate the convergence, a linear regression test was performed on the 10 (CITER=10) most recent, consecutive (CINTERVAL=1) iterations with an alpha error rate of 5% (CALPHA=0.05). Observations below the lower limit of quantification were discarded during the analysis. The statistical package R (version 2.15.1) (20) was used for exploratory data analysis and to produce descriptive statistics of demographics prior to the population analysis; for graphical assessment of NONMEM outputs during model building.

Model selection was achieved by use of the objective function minimum value (OFV) as goodness-of-fit statistic, as well as by examination of the NONMEM-provided asymptotic standard errors on each parameter estimate and goodness-of-fit plots. The OFV is minus twice the logarithm of the maximum likelihood of the model. Differences between objective functions of two fits of hierarchical models to the same data are approximately chi-squared distributed with degrees of freedom equal to the difference in the number of parameters between models. A significance level of 0.05 was considered for the likelihood ratio test during model building, meaning that, a drop of >3.84 in the objective function after addition of a single model parameter, was deemed a statistically significant improvement of the model.

In the preliminary stage of model building, one- and two-compartment disposition models, with linear and non-linear elimination from the central compartment, were fitted to IV data alone although graphical inspection of the concentration-time curves revealed a clear bi-exponential decrease in plasma concentrations and no signs of nonlinearity with the dose. Thereafter, concentration-time data provided by both the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Validation data set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomized, open-label, crossover, single dose</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>38</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26 (19–44)</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>Male = 80.5, Female = 10.5</td>
</tr>
<tr>
<td>Race (%) of total no. of subjects</td>
<td>Caucasian (55.3) and Black (44.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.7 (61.6–105.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.0 (21.2–29.8)</td>
</tr>
<tr>
<td>Route / formulation</td>
<td>Oral / MR tablet</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Food conditions</td>
<td>Fasted</td>
</tr>
<tr>
<td>PK sampling schedule</td>
<td>Predose and at 1, 2, 3, 4, 5, 6, 8, 10, 14, 24, 36 and 48 h postdose</td>
</tr>
<tr>
<td>Typical no. of samples/subject</td>
<td>12</td>
</tr>
</tbody>
</table>

Continuous variables are given as median (range) and were reported for all subjects.
IV and oral routes were modelled simultaneously in order to characterize the input and disposition kinetics during the same analysis. Conventional absorption models, as well as a transit compartment model (21) and models assuming parallel or sequential zero- and first-order absorption rates (22), were initially used to describe drug input after oral administration of mavogulant. The absorption component of the model was subsequently modified as described in the next paragraph. ISV was assessed on all structural model parameters as exponential variance and was defined as being normally distributed with mean zero and variance $\sigma^2$. Intercorrelation variability was assessed following the method proposed by Karlsson and Sheiner (23). The residual error was modelled as additive to the logarithmically-transformed observed concentrations and was defined as being normally distributed with mean zero and a homogenous variance $\sigma^2$. Another error model for logistically-transformed observations, described by Beal, was assessed (24).

Because of the irregular complexity of individual concentration-time profiles following oral administration of mavogulant, drug entry into the systemic circulation was eventually modelled using a flexible input rate function. The method proposed by Csajka et al., which uses a weighted sum of $n$ inverse Gaussian (IG) density functions as an analytical solution of the input transit time model, was considered (25). The input function was hence expressed as follows:

$$I(t) = F \cdot D \cdot \sum_{j=1}^{n} f_j \cdot IG_j(t)$$  \hspace{1cm} (1)

where $F$ is the bioavailability from the drug formulation, $D$ is the administered dose (in mg), $f_j$ is a weight parameter attached to the $j^{th}$ IG density function, $IG_j(t)$, such that $\sum f_j = 1$. $IG_j(t)$ is given by the following equation:

$$IG_j(t) = \frac{\sqrt{MIT_j}}{2 \pi \cdot CV_j^2 \cdot t^3} \cdot \exp \left[ -\frac{(t-MIT_j)^2}{2CV_j^2 \cdot MIT_j \cdot t} \right]$$  \hspace{1cm} (2)

In Eq. (2), $t$ is the time after dose administration (in h), $MIT_j$ is the mean of the $j^{th}$ IG distribution, and $CV_j$ is the coefficient of variation of $MIT_j$ (normalised variance of the $j^{th}$ IG distribution). $MIT_j$ was calculated from an estimate of the mode of the $j^{th}$ density, $t_{max_j}$, as follows (26):

$$MIT_j = t_{max_j} \left[ \sqrt{1 + \frac{9}{4} CV_j^4} - \frac{3}{2} CV_j^2 \right]$$  \hspace{1cm} (3)

$t_{max_j}$ is in fact the time at which the $j^{th}$ input rate reaches its maximum. Estimating $t_{max_j}$ rather than $MIT_j$ allows the information on each of the $n$ IG functions to be reduced to a single measure, as $t_{max_j}$ depends on both $MIT_j$ and $CV_j$ (26). The model was expressed using the following ordinary differential equations (using NONMEM subroutine ADVAN13):

$$\frac{dA_1}{dt} = I(t) - A_1 \cdot (k_{10} + k_{12}) + A_2 \cdot k_{21}$$  \hspace{1cm} (4)

$$\frac{dA_2}{dt} = A_1 \cdot k_{12} - A_2 \cdot k_{21}$$  \hspace{1cm} (5)

Where $I(t)$ is a single IG function or a sum of two or three IG functions (Eqs. (1) and (2)); $A_1$ and $A_2$ are the amounts of drug (in mg) in the central and peripheral compartments, respectively; $k_{10}$ (h$^{-1}$) is the first-order rate constant accounting for the elimination of drug from the body; and $k_{12}$ and $k_{21}$ (h$^{-1}$) are the first-order rate constants associated with drug transfer from the central to the peripheral compartment and vice versa, respectively.

A “saturated” stochastic model, in which random effects were assigned to all parameters of each IG function, was used in the analysis (25). However, to avoid potential random-effect identifiability issues, the same variances $\omega^2_{t_{max}}$ and $\omega^2_{CV}$ were estimated for all $t_{max}$ and $CV_j$ (for $j=1,...,n$) of the $n$ IG functions, respectively. This can be done by using the “SAME” option when defining the variance-covariance matrix in NONMEM (see NONMEM guide VIII). Considering the example of a sum of two IG functions, this means that the random effects on $t_{max}$ and $CV$, as well as on $CV_1$ and $CV_2$, can be different at the individual levels since they are sampled from different distributions that have the same variance. To avoid flip-flop between the IG densities and allow a natural ordering of the $n$ input rates, the constraint $t_{max_j} \geq t_{max_{j-1}}$ was imposed as follows (25):

$$t_{max_j,i} = t_{max_{j-1,i}} + \theta_{t_{max,j}} \cdot e^{\eta_{t_{max,j}}}$$  \hspace{1cm} (6)

for $j=2,...,n$, and where $\theta_{t_{max}}$ is the standard value of $t_{max_j}$ in the population and $\eta_{t_{max,j}}$ is the ISV in $t_{max_j}$ for the $i^{th}$ individual. At the individual levels, the constraint $0 \leq F_i \leq 1$ was imposed by defining the parameter as logit-normally distributed in the population, using the following equation:

$$F_i = \frac{e^{\theta_F + \eta_F}}{1 + e^{\theta_F + \eta_F}}$$  \hspace{1cm} (7)

where $\theta_F$ is the logit-transportation of the standard value of $F$ in the population and $\eta_F$ is the ISV in the logit-transformed $F$ for the $i^{th}$ individual. For a sum of two IG functions ($n=2$), the constraint $\sum f_j = 1$ was simply imposed by defining the first weight parameter $f_1$ as logit-normally distributed such that $0 \leq f_1 \leq 1$ for individual $i$, and deriving the second weight parameter as $f_2 = 1 - f_1$. However, for a sum of more than two IG functions ($n>2$), constraining the joint distribution of the $f_j$ parameters such that $\sum f_j = 1$...
while ensuring that $0 \leq f_j \leq 1$ for $j = 1, ..., n$, was performed by assigning a multivariate logistic-normal distribution to the individual $f_j$ parameters. The details about the implementation and application of the logistic-normal distribution in nonlinear mixed-effects models were provided by Nikolaos Tsamandouras (personal communication, June 20, 2014). In the present work, since $f_j$ can be interpreted as the fraction of bioavailable-dose reaching the systemic circulation as per the $j^{th}$ IG density, imposing the constraint $0 \leq f_j \leq 1$ allows consistency with physiology.

From the graphical assessment of the raw data, it was evident that the absorption process exhibited different patterns depending on the formulation and food state at drug administration. To increase the flexibility of the absorption model, the input function was defined specifically for each oral formulation and food status, i.e. specifically for each treatment of Study A2167. Three different input functions were thus optimised rather than testing the formulation and food-status variables as categorical covariates for the input parameters. Both fixed- and random-effects were allowed to be different across the three input functions. To ease the determination of the number of IG functions that would best describe mavoglurant input under each condition (IR-fasted, MR-fasted and MR-fed), each subset of Study A2167 data was first analysed separately with IV data. Furthermore, given the complexity of the pharmacokinetic model, the program Popdes 4.0 (27) was used to evaluate the design of Study A2121 and Study A2167, and predict the standard errors of the parameter estimates obtained with the “saturated” stochastic model. Once the input functions were deemed adequate, model building was pursued by analysing the pooled data set described in Table I.

Relationship between the demographic variables age and actual bodyweight (BW), and the disposition parameters were tested. Lean bodyweight, fat bodyweight (expected amount of fat for a normal-weight individual) and predicted normal weight (descriptor of weight for overweight and obese individuals) were tested as other surrogates for subjects’ weight (28). Since most subjects were Caucasian (72.0%) males (90.2%), the race and gender variables were not tested as covariates in the model. Potential parameter-covariate relationships were initially identified by graphical assessment of the empirical Bayes estimates of the parameters plotted against individual covariate values, given that the shrinkage magnitude in the structural parameters was not too high ($\leq 30\%$) (29). The selected covariates were then tested in the model by stepwise addition using an OFV drop of $>10.83$ (chi-squared value for $p \leq 0.001$ and a single degree of freedom) as inclusion criteria, followed by stepwise deletion using an OFV increase of $>12.12$ (chi-squared value for $p \leq 0.0005$ and a single degree of freedom) as criteria for retaining the covariate in the model.

Pearl-speaks-NONMEM was used to run a nonparametric bootstrap of 200 samples in order to estimate the standard errors on the model parameters. Since only data from Study A2167 were informative for the estimation of absorption parameters, resampling from the original pooled dataset was stratified by clinical study. Thereby, the proportion of subjects from Study A2121 (73%) and Study A2167 (27%) remained identical in the bootstrap datasets. All runs were included when calculating the bootstrap results.

**Validation of the Pharmacokinetic Model**

The software R was used to perform a visual predictive check that provides an assessment of the final model’s ability to describe the data and its suitability for simulation. The visual predictive check was stratified by study, dose (for Study A2121) and treatment (for Study A2167). For each stratum, 1000 new datasets with identical design to the original data subset were generated using the final model. The concentrations in each original data subset were binned to create concentration intervals corresponding to the nominal observation times. Within each bin, the median concentration, along with the 5th and 95th percentiles, were calculated from the observed subset of data. For each stratum, the 5th, 50th and 95th percentiles were computed for each of the 1000 simulation runs. Thereby, a 95% confidence interval around the median could be calculated for each predicted percentile. To evaluate the predictive performance of the model with respect to both uncensored and left-censored data, the visual predictive check was shown for each stratum in two panels: the top panel compared the median of the predicted concentrations as well as a 90% prediction interval, with the observations; the bottom panels showed the fraction of plasma samples below the quantification limit (BQL) along with a simulation-based 95% confidence interval around the median of the predicted BQL data (30).

An external validation was also performed by comparison of the model with an independent data set. Using Study A2171 data, only the performance of the MR-fasted input function together with the disposition model could be evaluated. Parameter estimates of the final MR-fasted model were used to simulate 1000 new datasets with identical design to Study A2171 (Table II). A visual predictive check was then performed similarly to the internal validation of the model described in the previous paragraph.

**Simulations**

For each formulation and food state, the standard time course of mavoglurant input rate following a single 100-mg dose was simulated in R using Eq. (1) and the final population mean
estimates of the input parameters. Similarly, the typical time course of the bioavailability \( F_A(t) \), was simulated using the following equation:

\[
F_A(t) = F \cdot \sum_{j=1}^{n} f_j \cdot F_{Aj}(t)
\]

with \( F_{A(\infty)} = F \) and where \( F_{Aj}(t) \) is the \( j^\text{th} \) cumulative IG distribution function of time, provided by the R function \( \text{pinvgauss}(\cdot) \) (31). This method allows the analytical solution of the input model (Eq. (1)) to be used for a repeated dose regimen. Three user-defined functions [\( \text{FUNCA} \), \( \text{FUNCC} \), and \( \text{FUNCB} \)], i.e. one for each of the \( n \) weighted IG functions, were defined in a single FORTRAN code (see Appendix 1 in the Supplementary Material online). The user-supplied FORTRAN subroutine was then called in the NM-TRAN code using the “OTHER” option in NONMEM (see Appendix 2 in the Supplementary Material online). When using the first-order or first-order conditional estimation methods, NONMEM requires the first partial derivatives of the functions with respect to the variables associated with random effects to compute the objective function (see NONMEM guide VIII). Although the partial derivatives were not essential for the present simulations, they were provided in the FORTRAN code for further use of the subroutine. Specification of the partial derivatives has been verified using the MR-fasted final model, according to the method described by Shen et al. (31) (details in the Supplementary Material online).

**RESULTS**

Population analysis of the pooled intravenously and orally administered mavoglurant concentration-time data allowed the kinetics of drug input rate and disposition to be described simultaneously. Mavoglurant disposition was best described using a two-compartment model with linear elimination from the central compartment. Using conventional absorption models or the transit compartment model to describe the arbitrarily complex absorption profiles (e.g. double-peak phenomenon), clearly led to model misspecification. In contrast, using a weighted sum of two or three IG functions as an input rate function offered sufficient flexibility to allow a reasonable fitting of the pharmacokinetic model to all data following oral administration of mavoglurant. The use of a single IG function as an input function provided a reasonable description of the concentration-time profiles provided by the IR-fasted treatment, but did not capture the double-peak phenomenon observed in approximately 20% of the individual profiles. The addition of a second IG function substantially increased the flexibility of the input function thereby providing a much better fitting to data from all subjects. Since after the MR-fasted dose, the majority of subjects exhibited a more or less smooth re-increase in their plasma concentrations starting within 8–14 h of administration, a single IG function was not tested to model the drug’s input rate. A sum of two IG functions did not capture the late re-increase in the concentrations. The addition of a third term allowed an acceptable description of the complex concentration-time profiles. The double-peak phenomenon was not observed when the MR tablet was administered within 5 min of completion of a high fat breakfast. However, most of individuals’ concentration-time profile were characterised by a slow initial increase in the plasma concentrations within 2–6 h of administration, followed by a rapid rise toward the maximum. A sum of two IG functions as an input function provided an adequate description of this pattern.

The model was parameterised in terms of plasma clearance (\( CL \)), volume of distribution of the central compartment (\( Vc \)), inter-compartmental clearance (\( Q \)) and volume of distribution of the peripheral compartment (\( Vp \)) for the disposition parameters; in terms of \( F, f_1, f_2, t_{\text{max1}}, t_{\text{max2}}, t_{\text{max3}}, CV_1, CV_2 \) and \( CV_3 \) for the parameters of each input function (Eqs. (1–3)). The final model is illustrated in Fig. 1.

Prediction of the standard errors of the parameter estimates revealed that the study designs did not allow ISV in all parameters to be estimated accurately. Hence, the variances of the random-effects on \( CV_1 \) and \( CV_2 \) of the IR-fasted input function, and on \( f_1 \) of the MR-fed input function, were fixed to a small value (0.0001 to represent a 1% coefficient of variation for a log-normally distributed parameter) rather than being estimated. Since the model included many input parameters that didn’t have any clear biological meanings, correlations were tested only between the disposition parameters. Strong correlations between \( CL \) and \( Vc \) (0.652) and between \( Q \) and \( Vp \) (0.845) were estimated. Nevertheless, for the MR-fasted input model that used a sum of three IG functions, the correlation between \( f_1 \) and \( f_2 \) (0.148) was estimated as they were assigned a multivariate distribution. Since the input function was specific to each period of Study A2167, interoccasion variability was assessed only on the disposition parameters, but the variance estimates were not statistically significantly different from zero. The absorption parameters
were the most variable between individuals, particularly under the MR-fed conditions. An additive error to the logarithmically-transformed plasma concentrations described adequately the unexplained residual variability. The residual error model proposed by Beal, which includes an additional random-effect compared to the additive model (24), did not improve goodness-of-fit plots of the residuals.

The demographic variable age was not tested as a covariate in the model since no marked trend was observed in the plot of the disposition parameters’ empirical Bayes estimates versus individuals’ age. Inclusion of BW as a covariate for Vc and Vp was retained in the model at the 0.0005 significance level. The relationship between BW and Vc was expressed as

\[ V_{ci} = \theta_{Vc} \left( \frac{BW_i}{BW_{med}} \right)^{\eta_{BW,Vc}} \times e(\eta_{Vc,i}) \]  

(9)

where \( V_{ci} \) is the Vc of individual \( i \), \( \theta_{Vc} \) is the standard value of Vc in the population, \( BW_i \) is the BW of individual \( i \), \( BW_{med} \) is the population median BW, \( \eta_{BW,Vc} \) is the exponent of normalised BW on Vc, and \( \eta_{Vc,i} \) is the ISV in Vc for the \( i \)th individual (normally distributed around zero with variance \( \omega^2_{Vc} \)). The effect of BW on Vp was similarly modelled. In the studied population, lean bodyweight, fat bodyweight and predicted normal weight were not better weight descriptors for mavoglurant than BW.

The mean and standard deviation (estimate of standard error) of the bootstrap estimates are presented in Table III for the disposition parameters and in Table IV for the input parameters. Plots of the observations, individual predictions and population predictions versus time for three representative individuals (Fig. 2), confirm that the input functions allowed complex concentration-time profiles following oral administration of mavoglurant to be captured under all conditions (IR-fasted, MR-fasted and MR-fed), even though the absorption pattern was erratic within each sub-population (all individual goodness-of-fit plots are provided

![Diagram of the final structural model for mavoglurant pharmacokinetics.](image)

**Table III** Final Estimates of Mavoglurant Disposition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ( ^a )</th>
<th>% RSE ( ^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (l/h)</td>
<td>29.3</td>
<td>2.48</td>
</tr>
<tr>
<td>Vc (l)</td>
<td>58.7</td>
<td>3.75</td>
</tr>
<tr>
<td>Q (l/h)</td>
<td>24.8</td>
<td>3.72</td>
</tr>
<tr>
<td>Vp (l)</td>
<td>113</td>
<td>4.48</td>
</tr>
<tr>
<td>( \theta_{BW,Vc} )</td>
<td>0.543</td>
<td>26.1</td>
</tr>
<tr>
<td>( \theta_{BW,Vp} )</td>
<td>1.13</td>
<td>12.1</td>
</tr>
<tr>
<td>ISV (% CV)</td>
<td>32.0</td>
<td>11.3</td>
</tr>
<tr>
<td>( \eta_{CL} )</td>
<td>28.1</td>
<td>16.5</td>
</tr>
<tr>
<td>( \eta_{Vc} )</td>
<td>45.6</td>
<td>17.5</td>
</tr>
<tr>
<td>( \eta_{Q} )</td>
<td>43.9</td>
<td>14.3</td>
</tr>
<tr>
<td>Covariance (correlation)</td>
<td>( \eta_{CL} - \eta_{Vc} )</td>
<td>0.652</td>
</tr>
<tr>
<td>Residual variability (% CV)</td>
<td>( \eta_{CL} - \eta_{Vp} )</td>
<td>0.845</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>18.3</td>
<td>5.87</td>
</tr>
</tbody>
</table>

Assessed by nonparametric bootstrapping (\( n = 200 \))

\( ^a \) The bootstrap estimates of the fixed-effects were back-transformed into the log-normal domain prior to calculation of the mean and standard deviation

\( ^b \) RSE: relative standard error of the estimates

\( ^c \) Calculated as \( 100 \cdot \sqrt[3]{\exp(\omega^2) - 1} \)

\( ^d \) Calculated as \( 100 \cdot \sqrt[3]{\exp(\sigma^2) - 1} \)
for each formulation-food condition in the Supplementary Material online). A visual predictive check of the final model, stratified by study and by dose (Study A2121) or treatment (Study A2167), shows that the population pharmacokinetic model developed throughout the analysis adequately describes mavoglurant pooled data (Fig. 3). The lower panels indicate that the fractions of predicted BQL data reasonably accounted for the fractions of BQL data observed across time. Figure 4 demonstrates the performance of the pharmacokinetic model in predicting plasma concentration-time profiles following a single 100-mg dose of the MR tablet of mavoglurant given under fasted conditions.

Figure 5 illustrates the effect of the formulation and food conditions prior to administration, on the time course of drug input rate and fraction of bioavailable-drug provided by a single dose of 100 mg of mavoglurant. The impact of the absorption pattern on both the systemic trend and variability in the concentration-time curves provided by a twice-daily repeated administration (100 mg/dose), is depicted in Fig. 6.

### DISCUSSION

In this article, we present the first model-based analysis of mavoglurant pharmacokinetics in a healthy population. Overall, its population pharmacokinetics in the studied subjects were highly variable. Elimination from the body, which is thought to be primarily mediated by hepatic metabolism (12), was linear within the studied dose range. For a standard 70 kg individual, point estimates of CL, Vc, Q and Vp were 29.3 l/h, 58.7 l, 24.8 l/h and 113 l, respectively. Considering the blood-to-plasma ratio (0.61), the blood clearance for a standard individual would be 48.0 l/h, which corresponds to approximately 55% of the hepatic blood flow ($\approx$ 87.0 l/h). The variability in the disposition process was moderate and partially explained by the effect of BW on Vc and Vp, with the highest impact on Vp. This was expected for a lipophilic compound which is extensively distributed in tissues and organs once it reached the blood stream (12). No effect of BW was identified on CL. Therefore, subjects’ weight is not likely

<table>
<thead>
<tr>
<th>Table IV Final Estimates of Mavoglurant Input Parameters</th>
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<tbody>
<tr>
<td>Parameter</td>
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<tr>
<td></td>
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<tr>
<td>F</td>
</tr>
<tr>
<td>f1</td>
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<td>f2</td>
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<td>tmax1 (h)</td>
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<td>tmax2 (h)</td>
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<td>tmax3 (h)</td>
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<td>CV1</td>
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<td>CV2</td>
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<tr>
<td>CV3</td>
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<tr>
<td>ISV (% CV)</td>
</tr>
<tr>
<td>$\eta_F^c$</td>
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<tr>
<td>$\eta_{f1}^c$</td>
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<td>$\eta_{f2}^c$</td>
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<tr>
<td>$\eta_{tmax1}$</td>
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<tr>
<td>$\eta_{tmax2}$</td>
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<tr>
<td>$\eta_{tmax3}$</td>
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<tr>
<td>$\eta_{CV1}$</td>
</tr>
<tr>
<td>$\eta_{CV2}$</td>
</tr>
<tr>
<td>$\eta_{CV3}$</td>
</tr>
<tr>
<td>Covariance (correlation)</td>
</tr>
</tbody>
</table>

Assessed by nonparametric bootstrapping ($n=200$)

- The bootstrap estimates of the fixed-effects were back-transformed into the log-, logit- or logistic-normal domain prior to calculation of the mean and standard deviation
- RSE: relative standard error of the estimates
- Calculated as follows: 10,000 samples were drawn from a normal distribution using the mean of the bootstrap estimates of both transformed fixed-effects and random-effects; each value was then transformed back into the logit- or logistic-normal domain, and the mean and standard deviation were computed
- Calculated as $100.\sqrt{\exp(\omega^2)-1}$ since these parameters were assumed log-normally distributed
to affect the systemic exposure to mavoglurant. No biological explanation for the correlations between CL and Vc, and between Q and Vp could be proposed since BW didn’t appear to be the source of these correlations. The frequencies of female subjects and of other races than Caucasian were too low to investigate effectively the potential influence of gender and race on mavoglurant disposition. The age range of the studied subjects might also have been too narrow to evaluate the effect of age on the disposition properties.

The absorption and systemic bioavailability of an orally administered compound is governed by a number of factors that includes drug solubility, permeability, in vivo dissolution-or release-rate, and intestinal loss, which in turn depend on physiological, drug-, and formulation-specific factors (32). In particular, the absorption of a poorly soluble and highly permeable compound such as mavoglurant, is likely to be limited by its solubility or dissolution-rate in vivo, depending on the type of formulation used for oral administration. Several formulations have been clinically investigated throughout mavoglurant development. The IR capsule and the MR tablet were the two main formulations assessed in Phase I studies. The MR dosage form was developed subsequently to the IR formulation to improve drug tolerance, and has been selected for Phase II trials. To gain insight into its optimal use in patients, the input characteristics (rate and bioavailability) of the IR and MR formulations were compared in this analysis. Since the slow input process following administration of the MR formulation partly masked mavoglurant disposition, including IV data in the analysis was essential to separate the drug absorption phase from the distribution and elimination phases. Mavoglurant absorption into the systemic circulation was highly variable and difficult to describe accurately.

Fig. 2 Plots of the observations (open circles), individual predictions (solid lines) and population predictions (dashed lines) from the final mavoglurant pharmacokinetic model. Three subjects were selected to illustrate goodness-of-fit for the three formulation-food conditions (IR-fasted, MR-fasted and MR-fed).
regardless of the formulation and food conditions. Although capturing adequately the complex pharmacokinetic profiles is not considered critical for prediction of drug efficacy, it is more important to predict accurately steady-state peak concentrations and anticipate drug tolerance. Using a sum of IG functions as an input rate function was deemed to be the most adequate empirical approach to capture the atypical input profiles observed in all data from Study A2167. The IG density has been previously used as a flexible function to describe the input transit time density for various drugs and extravascular administration routes (26,33–36). Summation of IG densities has been shown to offer even more flexibility to describe input processes of higher complexity such as for sustained-release products (25). Of note, a sum of log-normal density functions has been evaluated as an input rate function in this analysis and provided similar results to the sum of IG densities (results not presented). Nevertheless, the higher flexibility of the IG distribution (37) could be of greater value in other situations such as for more sustained-release formulations. An appealing aspect of such an input function is that the extent of bioavailability could be directly estimated, as it is a parameter of the function. Also, the estimated parameters can be readily used to simulate the time course of the input rate and fraction of bioavailable-drug (Eqs. (1) and (9)), and compare the input properties of different formulations or route of administration. This is of particular value during drug development for bioavailability or bioequivalence studies, and for the development of alternative routes of drug delivery. To do so in the present work, a different input rate function was estimated for each formulation and food status rather than performing a covariate analysis that would have been irrelevant given the complexity of the input model and the lack of biological meaning of the parameters.

Following administration of the IR formulation under fasted conditions, some concentration-time profiles exhibited a double-peak phenomenon, which was adequately captured by a sum of two IG functions (Fig. 1). The fact that this phenomenon was more or less pronounced across the studied formulations. An appealing aspect of such an input function is that the extent of bioavailability could be directly estimated, as it is a parameter of the function. Also, the estimated parameters can be readily used to simulate the time course of the input rate and fraction of bioavailable-drug (Eqs. (1) and (9)), and compare the input properties of different formulations or route of administration. This is of particular value during drug development for bioavailability or bioequivalence studies, and for the development of alternative routes of drug delivery. To do so in the present work, a different input rate function was estimated for each formulation and food status rather than performing a covariate analysis that would have been irrelevant given the complexity of the input model and the lack of biological meaning of the parameters.

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Following administration of the IR formulation under fasted conditions, some concentration-time profiles exhibited a double-peak phenomenon, which was adequately captured by a sum of two IG functions (Fig. 1). The fact that this phenomenon was more or less pronounced across the studied
subjects explains the high ISV estimated in the input parameters. The derived input function indicates that typically, there was an initial rapid entry of mavoglurant into the bloodstream (maximal rate of 29.0 mg/h reached at 0.4 h) followed by a second phase of slower drug input (Fig. 5a). In addition, simulation of the time course of the fraction of bioavailable-dose suggests that the input process was typically complete within 6 h of administration of the IR capsule (Fig. 5b). Since mavoglurant is highly permeable and is a priori not a substrate of any transporters, absorption into the systemic circulation is thought to be limited by drug dissolution which seems to have a more complex and more variable pattern in vivo than in vitro (IR capsule designed to release the drug within approximately an hour of administration). The dissolution of the dosage form is in turn likely limited by the poor solubility of mavoglurant in aqueous media (0.025 mg/ml and pH independent). Any change in the solubility conditions along the gastrointestinal tract would thus explain the multiple-peak phenomenon. For instance, if at a given time and region of the gastrointestinal tract the volume of fluid in which the drug can be dissolved is saturated, part of the drug would remain undissolved or could precipitate until more intestinal fluid is available downstream; hence the dissolution rate would vary and the drug would appear in the circulation in a complex manner. Additional healthy volunteers’ data from a single-ascending dose study of a slightly different IR formulation have shown that both the frequency and magnitude of the double-peak phenomenon was increased with increasing doses, which supports the assumption of a dissolution-limited absorption (internal unpublished data). Since mavoglurant is extensively metabolised in the liver and is substrate of the cytochrome P450 3A4 (abundant in the proximal small intestine’s enterocytes), the absolute bioavailability from the IR capsule (typically 0.436 in the population) is thought to imply a significant first-pass effect due to both gut wall and hepatic metabolism.

Following administration of the MR formulation under fasted conditions, a sum of three IG functions was required to reasonably describe the complex absorption pattern characterised by a second smooth rise in plasma concentration.

Fig. 4 External validation: a visual predictive check of the final population pharmacokinetic model’s ability to predict an independent set of mavoglurant concentration-time data following administration the MR formulation under fasted conditions (Study A2171). In the upper panel, open circles represent the observed data, the solid line is the median of the simulated concentrations, and the grey shaded area represents a 90% prediction interval. The horizontal dotted line is the limit of quantification of the assays. The lower panel shows a simulation-based 95% confidence interval (grey shaded area) around the median (solid line) of the fraction of simulated BQL data (expressed in percentage). The fraction of observed BQL data is represented by the open circles.

Fig. 5 Simulated standard time course of mavoglurant input rate (a) and fraction of bioavailable-dose (b) (using Eqs. (1) and (9), respectively) following a single 100-mg dose under each formulation-food condition of Study A2167, i.e. IR-fasted (solid lines), MR-fasted (dashed lines) and MR-fed (dotted lines). The insert in plot (a) expands the first 6 h.
from approximately 8 h post dose (Fig. 2). Similarly to the IR form, this phenomenon was more or less pronounced within the studied population; hence estimates of ISV in the input parameters were high. The derived input function suggests that, at equal doses, the prolonged release of mavoglurant along the gastrointestinal tract logically resulted in a decreased rate of absorption compared to the IR formulation (maximal input rate of 8.34 mg/h reached around 2 h) which indicates that drug dissolution was the likely rate-limiting step of the input process. As for the IR form, mavoglurant absorption was typically characterised by a second phase of slower drug input (peaking at approximately 12 h) after administration of the MR tablet under fasted conditions (Fig. 5a), which was however maintained at a low rate over approximately 36 h (Fig. 5b). Although no clear physiological explanation for this pattern could be proposed given the empirical nature of the model, similar assumptions to those proposed for the IR formulation can be made for the MR formulation: any change in the dissolution conditions would yield fluctuation in the rate of drug input into the blood stream. The bioavailability from the MR tablet (0.387 for a standard individual) was lower than from the IR capsule. The long duration of the input process (≈36 h) suggests that part of the administered dose was possibly absorbed in the colon during the second phase. The colonic environment is usually less favorable for drug absorption than the small intestine (38,39). Hence, the extent of absorption might have been reduced for the MR formulation relative to the IR formulation.

When the MR tablet was administered shortly after a high fat meal, there was typically an initial delay of approximately 1 h before mavoglurant appeared in the systemic circulation (Fig. 5a). This phenomenon was highly irregular across the studied subjects and was thus reflected by the high ISV estimated for the MR-fed input function (sum of two IG functions). This initial phase of slow input is thought to be due to an erratically delayed gastric emptying of the MR tablet when administered concomitantly with food (40). Subsequently, mavoglurant was more rapidly absorbed into the blood stream than in the fasted state (maximum rate of 37.1 mg/h reached at approximately 2 h) and the late second phase of drug input was not observed. The simulated bioavailability-versus-time curve suggests that the input process can be considered complete 12 h post dose (Fig. 5b). Given the input properties of the MR formulation under fasted conditions, this might be explained by the increased solubility of mavoglurant by the postprandial bile salts secreted in the duodenum, thereby accelerating drug dissolution from the MR tablet. This would in turn explain the higher extent of bioavailability under fed conditions (typically 0.508 in the studied population), as well as the absence of second input phase, since most of the drug would be dissolved and be available for absorption in the small intestine.

Monte Carlo simulation (n = 1000) of the concentration-time profiles produced by the repeated twice-daily administration of mavoglurant IR and MR formulations under fasted and fed (for the MR formulation only) conditions, was performed to gain an understanding of the impact of the formulation- and food-specific input characteristics on the plasma concentration range at steady-state (Fig. 6). Based on visual assessment of the profiles, steady state plasma concentrations should be attained after 48 h of dosing under all formulation-food conditions, although it might be reached earlier under the IR-fasted and MR-fed conditions. Assuming that the pharmacokinetics of mavoglurant are not altered during multiple dosing, the MR formulation is expected to produce a slightly lower concentration range with lower peaks than an equivalent dose of IR capsules. This may be useful information allowing the clinician to adjust dosage of the MR tablet, in order to balance efficacy and safety during dose titration. However, the simulations also suggest a significant

![Fig. 6](https://example.com/fig6.png)
food effect on the pharmacokinetic behaviour of the MR formulation; both the median trend and variability in mavoglurant steady-state plasma concentrations were increased in the fed state compared to the fasted state. As a consequence, the a priori reduced frequency of adverse events in patients chronically treated by mavoglurant MR formulation relative to treatment by the IR formulation, is likely dependent on the food conditions at each drug administration. Nevertheless, the standard Food and Drug Administration high-fat and high-calorie meal used in Study A2167 is not representative of the target patients’ diet, which is more likely to consist of low- and medium-fat meals. Since the food effect is likely explained by the increased solubility of mavoglurant due to elevated bile salts concentrations in the duodenum, it is thought to be correlated to the meals’ proportion of fat. Therefore, a less pronounced impact of the food diet on mavoglurant bioavailability and input kinetics is expected in the patient populations.

The visual predictive check (Fig. 3) indicated that the model performs well and predicts both the central trend and variability in the plasma concentration-time profiles for each route of administration, dose, formulation and food conditions. Also, discarding BQL data during the analysis didn’t lead to obvious model misspecification, as indicated by the adequacy of the observed and predicted fraction of BQL data across time. A significant underprediction of the fraction of BQL data would result in bias in both input and disposition parameters. The cross-validation (Fig. 4) indicates that the model slightly underpredicted the systemic trend in Study A2171 concentration-time data for which the same MR formulation as in Study A2167 was used and healthy volunteers with similar demographics were enrolled. This might be due to the increased frequency and magnitude of the late second phase of drug input in Study A2171 data in comparison with Study A2167 data, which implies that the input function (both fixed and random effects) that would characterise Study A2171 data would be slightly different. This means that Study A2167 subjects were not very representative of the population and emphasises the high specificity of this empirical model to the data included in the analysis. In such situation, even interpolation to a similar population and similar experimental conditions is difficult. Extrapolation beyond the data, such as under different physiological conditions or for other types of formulation, would be even more challenging. Conversely, a physiologically-based model integrating prior information on drug- and formulation-specific characteristics (e.g. in vitro dissolution, in vitro metabolism and in vitro solubility data) would be of greater value for quantitative predictions under different conditions.

CONCLUSIONS

In summary, the disposition of mavoglurant from the body was best described by a two-compartment pharmacokinetic model, and is likely to be influenced by subjects’ BW. Unlike conventional absorption models, using a sum of two or three IG functions as an input rate function described drug absorption into the systemic circulation adequately. Using the IR and MR formulations, mavoglurant input process is thought to be limited by drug dissolution which appeared to be sensitive to the physiological conditions. Even when administered in the IR formulation, mavoglurant exhibited complex and variable input characteristics. The MR formulation showed a prolonged input into the blood stream over more than a day, whereas drug input was typically complete in 6 h for the IR formulation. The bioavailability of the MR formulation was lower than for the IR form, which may be a result of the colonic absorption of part of the administered dose. Hence, dose adjustment might be required to provide a similar efficacy to the IR formulation. A repeated twice-daily administration of the MR tablet is expected to produce smaller peak-to-trough variation than the IR formulation, which might allow a reduction of the frequency of side effects reported in the clinical studies that used the IR form. The input properties of the MR tablet appeared to be strongly governed by the food conditions at drug administration, although the standard high fat meal used to assess food effect on drug input represents an extreme case scenario and is probably not representative of the target patient populations’ diet.

ACKNOWLEDGMENTS AND DISCLOSURES

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Chapter 3: Application of a Bayesian approach to physiological modelling of mavoglurant population pharmacokinetics

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Application of a Bayesian approach to physiological modelling of mavoglurant population pharmacokinetics

Thierry Wendling1,2 · Swati Dumitras2 · Kayode Ogungbenro1 · Leon Aarons1

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Abstract Mavoglurant (MVG) is an antagonist at the metabotropic glutamate receptor-5 currently under clinical development at Novartis Pharma AG for the treatment of central nervous system diseases. The aim of this study was to develop and optimise a population whole-body physiologically-based pharmacokinetic (WBPBPK) model for MVG, to predict the impact of drug–drug interaction (DDI) and age on its pharmacokinetics. In a first step, the model was fitted to intravenous (IV) data from a clinical study in adults using a Bayesian approach. In a second step, the optimised model was used together with a mechanistic absorption model for exploratory Monte Carlo simulations. The ability of the model to predict MVG pharmacokinetics when orally co-administered with ketoconazole in adults or administered alone in 3–11 year-old children was evaluated using data from three other clinical studies. The population model provided a good description of both the median trend and variability in MVG plasma pharmacokinetics following IV administration in adults. The Bayesian approach offered a continuous flow of information from pre-clinical to clinical studies. Prediction of the DDI with ketoconazole was consistent with the results of a non-compartmental analysis of the clinical data (threefold increase in systemic exposure). Scaling of the WBPBPK model allowed reasonable extrapolation of MVG pharmacokinetics from adults to children. The model can be used to predict plasma and brain (target site) concentration–time profiles following oral administration of various immediate-release formulations of MVG alone or when co-administered with other drugs, in adults as well as in children.

Keywords Mavoglurant · Population pharmacokinetics · Physiologically-based pharmacokinetic models · Bayesian analysis · Drug–drug interactions · Paediatrics

Introduction

Mavoglurant (MVG) is an antagonist at the metabotropic glutamate receptor 5 currently under clinical development at Novartis Pharma AG for the treatment of central nervous system diseases. Although MVG can be administered by both the intravenous (IV) and oral route, oral formulations are more practical for chronic treatment of patients. During clinical studies in adults, an immediate-release formulation (hard gelatine capsule) was mainly employed. A population model was recently proposed to describe MVG pharmacokinetics following both IV and oral administration in healthy adult subjects [1]. For a standard individual under fasted conditions, the bioavailability from the capsule formulation was estimated to be 44 % of the administered dose. MVG is a lipophilic neutral drug (logP of 4.7) extensively distributed to organs and tissues [2]. Its steady-state volume of distribution was estimated to be 172 l for a standard 70-kg individual and appeared to be moderately variable in a healthy adult population (coefficient of variation [CV] of 30 %) [3]. This variability was partly
explained by body weight (BW) variations across individuals. Elimination of MVG in humans is believed to be primarily mediated by hepatic oxidative metabolism involving mainly cytochrome P450 (CYP) 3A4, 2C8, 2C9 and 2C19 [2]. Systemic clearance (CL) was reported to be moderately variable in healthy adults (32 % CV) with standard value in the population estimated to be 29.3 l/h [3].

The population pharmacokinetic model previously developed for MVG offered sufficient flexibility to describe the atypical and highly variable concentration–time data resulting from IV and oral administration in healthy adult subjects [3]. However, the empirical nature of the model allowed neither a thorough understanding of MVG pharmacokinetics nor extrapolation outside the studied population (e.g. different age or BW groups) and experimental conditions (e.g. co-administration). Conversely, physiologically-based pharmacokinetic (PBPK) models are mechanistic models that help to gain insight into the absorption, distribution and elimination behaviour of drugs. The main advantages of such models over conventional empirical models are their suitability for prediction of kinetics in various tissues and for extrapolation between species, routes of administration and dosing regimens [4]. The parameters of a PBPK model are of two types: system-specific and drug-specific. While information on system-specific or physiological parameters can be found in the literature, drug-specific parameters are typically derived from the results of pre-clinical in vitro or animal experiments. Both physiological and drug-specific parameters can carry a high degree of uncertainty. One way of introducing uncertainty during parameter estimation is to apply a Bayesian approach which yields statistical distributions of the parameter values (posterior distributions) rather than point estimates. Posterior distributions are consistent with both experimental data and prior beliefs (prior distributions of the parameters) and can be approximated by random draws using Markov Chain Monte Carlo (MCMC) simulations [5]. Also, although it has been shown that a generic whole-body PBPK (WBPBPK) model is globally structurally identifiable under certain assumptions [6], the high number of parameters and the absence of tissue data in human typically result in a numerically unstable analysis. Incorporating prior information on the parameters that are not well informed by the data can help to stabilise the analysis [7]. During drug development, it is desirable to qualify and quantify inter-individual variability (IIV) in the pharmacokinetics of tested compounds. Depending on the quality and amount of clinical data available, this can be done by performing analyses based on hierarchical models to estimate unexplained variability in the drug-specific parameters and improve individual parameter estimates [8]. A Bayesian population analysis outcomes posterior distributions not only for the individual parameters but also for the population parameters [9]. This approach has been applied successfully to physiological pharmacokinetic/toxicokinetic models [10–12] that are especially suited for separating and characterising the physiologic variability from the overall variability in the system, as clear relationships between physiological parameters and individual covariates (i.e. BW, age and gender) have been established [13, 14].

In the present study, we developed a population WBPBPK model to gain mechanistic understanding of MVG pharmacokinetics in adults. Since clinical data following IV administration in healthy adult volunteers were available, we optimised the population model using a Bayesian approach to incorporate prior pre-clinical knowledge on the drug-specific parameters. In this manner, we maintain a continuous flow of information from pre-clinical to clinical studies and possibly help stabilise the estimation of the parameters. The other aim of this study was to illustrate the ability of WBPBPK models to extrapolate pharmacokinetics across experimental conditions and studied populations. As part of MVG clinical development, the drug–drug interaction (DDI) with ketoconazole (strong CYP3A4 inhibitor) was evaluated in adults; the results (unpublished) suggested a threefold increase in the systemic exposure to MVG when orally co-administered with ketoconazole. Also, the efficacy of MVG in treating patients with fragile X syndrome was investigated. Since this mental retardation syndrome is typically diagnosed in young children [3, 15], MVG pharmacokinetics were studied in patients aged from 3 to 11 years (internal unpublished results). A powder for oral suspension (POS) was developed for drug administration in children and was assessed in adult volunteers prior to the paediatric study. Using these clinical data, we evaluated the performance of our model in predicting MVG plasma pharmacokinetics when co-administered with ketoconazole in adults or administered alone in children. To be able to predict concentration–time data after oral administration of the immediate-release formulations used in the DDI (capsule) and paediatric studies (POS), a mechanistic absorption model was also implemented. Scaling the WBPBPK model from adult to children was done by accounting for age-related anatomical/physiological changes in the studied children.

Methods

Work flow for PBPK predictions

The work flow for model development and extrapolation of MVG pharmacokinetics across formulations, dosing
The schematic workflow of the mechanistic modelling and simulation process applied to MVG pharmacokinetics is depicted in Fig. 1. Firstly, a WBPBPK structural model was developed using physiological parameters from the literature and estimates of the drug-specific parameters from in vitro and animal experiments. Secondly, a sensitivity analysis of the WBPBPK model was performed to identify the drug-specific parameters that could be estimated just with plasma data. Thirdly, a hierarchical model accounting for both IIV and uncertainty in the parameters was built and optimised based on clinical IV data. Subsequently, a mechanistic absorption model for oral immediate-release formulations was implemented similarly to the disposition model. Finally, Monte Carlo simulations were performed to predict MVG pharmacokinetics under different experimental conditions (e.g. route of administration, formulation or dosing regimen) and for a different sup-population (e.g. children).

**Clinical data**

Plasma data from four Phase-I clinical studies (Study 1–4) were used to optimise the MVG population WBPBPK model (Study 1) and evaluate its performance in different experimental conditions and sub-populations (Study 2–4). The IV data used to optimise the disposition model (Study 1) were previously described by Wendling et al. [3]. Briefly, 120 healthy volunteers received a single 10-min IV infusion of 25 mg, 37.5 mg or 50 mg of MVG. Most subjects were young (median age of 31 years) Caucasian (62%) male (87%) with a median BW of 83 kg and a median body mass index of 27 kg/m^2.

Study 2 was a two-period single-sequence cross-over study conducted to quantify the impact of the co-administration of ketoconazole on MVG pharmacokinetics. During the first period, each of the 16 healthy subjects enrolled received a single 25-mg dose of MVG alone (immediate-release capsule formulation). Blood samples were collected at pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 24, 36 and 48 h post-dose. Data from this first period were used to check the ability of the model to predict concentration–time data following oral administration of the capsule formulation in adults. Following a washout of a minimum 10 days, subjects received a repeated 400-mg daily oral dose of ketoconazole for 10 days. On Day 5 of this second period, the morning dose of ketoconazole was followed by a single oral administration of 25 mg of MVG. Only pre-dose blood samples were collected for ketoconazole. MVG blood samples were collected at pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 24, 36, 48, 72, 96 and 120 h post-dose. Subjects were mostly young (median age of 34 years) Caucasian (88%) male (100%) with a median BW of 76 kg and a median body mass index of 24 kg/m^2.

Data from Study 3 were used to evaluate the ability of the mechanistic absorption model to predict plasma concentration–time profiles resulting from oral administration of the POS formulation of MVG in adults. The aim of the study was to evaluate the pharmacokinetic properties of two paediatric formulations in healthy adults, prior to studies in children. Only data for the paediatric formulation used in Study 4 were included in the present analysis. 28 healthy young (median age of 32 years) Caucasian (97%) male received a single oral dose of 50 mg of MVG in 5 ml of suspension under fasted conditions. The studied subjects had a median BW of 82 kg and a median body mass index of 26 kg/m^2. Blood samples were collected at pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 10, 14, 24, 36 and 48 h post-dose.

Study 4 was conducted to evaluate MVG pharmacokinetics after single and multiple oral administration in children with fragile X syndrome aged from 3 to 11 years [15]. Only single dose data were used to assess the ability of the WBPBPK model to extrapolate across age groups. 21 subjects received a single oral 15-mg dose of MVG POS formulation (1.5 ml of suspension after reconstitution with water). Blood samples were collected at pre-dose, 0.5, 2, 4, 8, 12 and 24 h post-dose. The demographic characteristics of the children are summarised in Table 1.

All studies were conducted according to the ethical principles of the Declaration of Helsinki and all protocols were approved by the Independent Ethics Committee or Institutional Review Board for each study center. Participants were males and non-pregnant females and all provided full written informed consent prior to inclusion in the studies. Plasma concentrations were determined by a
validated liquid chromatography-tandem mass spectrometry method with a lower limit of quantification of 2 ng/ml for Study 1–3 and 0.2 ng/ml for Study 4 [16]. Concentrations below these limits were labeled as zero.

**Structure of the WBPBPK model for MVG**

**Disposition**

The WBPBPK model for MVG disposition (Fig. 2) comprises 13 tissue compartments, namely lungs (LU), heart (HT), brain (BR), muscle (MU), adipose (AD), skin (SK), spleen (SP), pancreas (PA), liver (LI), stomach (ST), gut (GU), bones (BO) and kidneys (KI). These compartments are connected together by the arterial and venous blood compartments. The LI receives blood both from the splanchnic organs (SP, PA, ST and GU) via the portal vein and directly from the hepatic artery. Since the weight of the selected tissues accounted only for 96 % of total BW, an additional rest-of-body (RB) compartment was included. All tissues were considered as well-stirred compartments, i.e. drug uptake by tissues was assumed perfusion-limited rather than permeability-limited. This assumption was deemed reasonable for a small lipophilic compound like MVG (molecular weight of 313 g/mol). The extent of drug distribution in a tissue is hence characterised by the equilibrium tissue-to-blood partition coefficient ($K_{b,T}$). The dynamics of drug amount in the tissue compartments can be described by the following equation:

\[
\frac{dC_T}{dt} = \frac{C_{VEN/ART} - C_T}{K_{b,T}}
\]

where $C_T$ denotes the concentration ($\mu$g/l), $V_T$ the volume (l) and $Q_T$ the blood flow (l/h) of the different tissues; $C_{VEN/ART}$ is either the venous (for the LU) or arterial (all other tissues) blood concentration ($\mu$g/l). The rate equations for the arterial blood (Eq. 2) and venous blood (Eq. 3) compartments were defined as follows:

\[
\frac{dC_{ART}}{dt} = Q_{LU}C_{LU} - Q_{ART}C_{ART}
\]

\[
\frac{dC_{VEN}}{dt} = \sum Q_T\frac{C_T}{K_{b,T}} - Q_{VEN}C_{VEN}
\]

where $\sum Q_T\frac{C_T}{K_{b,T}}$ includes all tissues except the splanchnic organs; $V_{ART}$ is the volume of arterial blood (l) and $V_{VEN}$ the volume of venous blood (l); $Q_{LU}$ represents the blood flow (l/h), $C_{LU}$ the concentration ($\mu$g/l) and $K_{b,LU}$ the partition coefficient for the LU. MVG plasma concentrations were derived by dividing $C_{VEN}$ by the blood-to-plasma ratio $BP$. Based on the results of a previous pharmacokinetic study in healthy subjects [2], systemic clearance was considered to occur exclusively in the liver. The time dependency of drug amount in the liver was therefore modelled as:

\[
\frac{dC_{LI}}{dt} = Q_{HA}C_{ART} + \sum Q_T\frac{C_T}{K_{b,T}} - Q_{LI}C_{LI} - CL_{int,LI}u_h\frac{C_{LI}}{K_{b,LI}}
\]

**Table 1** Demographic and anthropometric characteristics of Study 4 subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>22.4</td>
<td>18.8</td>
<td>19</td>
<td>23.5</td>
<td>20.5</td>
<td>28.1</td>
<td>33.1</td>
<td>51.8</td>
<td>45</td>
</tr>
<tr>
<td>Body surface area (m$^2$)</td>
<td>0.829</td>
<td>0.742</td>
<td>0.788</td>
<td>0.9</td>
<td>0.82</td>
<td>1.06</td>
<td>1.14</td>
<td>1.41</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Anthropometric variables are given as median
where the sum $\sum Q_i C_i$ includes only the splanchnic organs; $Q_{HA}$ is the blood flow (l/h) from the hepatic artery; $C_LI$ represents the concentration (\(\mu g/l\)); $Q_{LI}$ the blood flow (l/h); $K_{b,LI}$ the partition coefficient and $V_{LI}$ the volume (l) of the liver; $CL_{int,LI}$ denotes MVG intrinsic clearance (l/h) in the liver; $fu_{LI}$ represents the fraction unbound in plasma calculated as the ratio of the fraction unbound in plasma $fub$ to BP. To describe the pharmacokinetics of MVG after IV infusion, the initial conditions of all states of the WBPBPK model were set to zero. The unit of the dose were converted from mg to l and the unit of the infusion time from min to h in order to have an infusion rate in mg/h and concentrations in \(\mu g/l\) (or ng/ml). For an IV bolus of the drug, the initial condition of the venous blood compartment would be set to the administered dose.

**Absorption**

A three-compartment absorption model (Fig. 3) based on the compartmental absorption and transit (CAT) model was applied to the gastro-intestinal (GI) tract [17]. For simplicity, the small intestine lumen (SI) was represented by one compartment instead of seven in the CAT model. Although the simplified model doesn’t describe the small intestinal transit time exactly as the CAT model, the impact on systemic absorption of MVG was deemed negligible. To account for dissolution of the dosage form, two levels distinguishing solid drug from dissolved drug were included for each segment of the GI lumen, i.e. the stomach (ST), SI and colon. The dissolution process was modelled according to Hintz and Johnson [18]. Absorption of dissolved drug was considered only in the small intestine enterocytes (ENT). Pre-systemic clearance was considered from both the ENT and LI compartments. The rate equations for each level of the ST (Eqs. 5, 6), SI (Eqs. 7, 8), colon (Eqs. 9, 10) compartments, as well as for the ENT compartment (Eq. 11) are given below:

$$\frac{dA_{und,ST}}{dt} = -k_{ST} \cdot A_{und,ST} - z \cdot \left( \frac{A_{dis,ST}}{V_{ST}} \right) \cdot A_{und,ST}$$

$$\frac{dA_{dis,ST}}{dt} = z \cdot \left( \frac{S_{ST} - A_{dis,ST}}{V_{ST}} \right) \cdot A_{und,ST} - k_{ST} \cdot A_{dis,ST}$$

$$\frac{dA_{und,SI}}{dt} = k_{SI} \cdot A_{und,SI} - k_{SI} \cdot A_{und,SI} - \frac{A_{dis,SI}}{V_{SI}} \cdot A_{und,SI}$$

$$\frac{dA_{dis,SI}}{dt} = k_{SI} \cdot A_{dis,SI} + z \cdot \left( \frac{S_{SI} - A_{dis,SI}}{V_{SI}} \right) \cdot A_{und,SI} - k_{SI} \cdot A_{dis,SI} - k_{a} \cdot A_{dis,SI}$$

$$\frac{dA_{und,Colon}}{dt} = k_{SI} \cdot A_{und,SI}$$

$$\frac{dA_{dis,Colon}}{dt} = k_{SI} \cdot A_{dis,SI}$$

$$\frac{dC_{ENT}}{dt} = k_{a} \cdot A_{dis,SI} - Q_{ENT} \cdot C_{ENT} - CL_{int,ENT} \cdot fu_{ENT} \cdot C_{ENT}$$

where $A_{und,ST/SI/Colon}$ refers to the amount (\(\mu g\)) of undissolved drug and $A_{dis,ST/SI/Colon}$ to the amount of dissolved drug in either the ST, SI or colon; $k_{ST}$ is the transit rate constant (h\(^{-1}\)) for the ST and $k_{SI}$ the constant for the SI; $z$ is a dissolution constant (l/h/g) independent of the volume of medium and of drug amount and solubility [19]; $S_{ST}$ refers to drug solubility (\(\mu g/l\)) in the ST and $S_{SI}$ to that in the SI; $V_{ST}$ refers to the volume of fluid (l) in the ST and $V_{SI}$ to the volume in the SI; $k_{a}$ is the absorption rate constant calculated as in Eq. 12; $V_{ENT}$ represents the volume (l), $C_{ENT}$ the concentration (\(\mu g/l\)), $Q_{ENT}$ the blood flow (l/h), $CL_{int,ENT}$ the intrinsic clearance (l/h) and $fu_{ENT}$ the fraction unbound in the ENT compartment. The effective permeability of MVG in the jejunum ($P_{eff}$ in cm/h) and the radii of the SI ($r_{SI}$, in cm) were used to estimate $k_{a}$ (h\(^{-1}\)) as follows:

![Fig. 3 Schematic representation of the mechanistic absorption model for MVG immediate-release formulations. See text for definition of symbols](image-url)
\[ k_a = \frac{2P_{off}}{r_{SI}} \]  

(12)

When incorporating the absorption model into the WBPBPK model, the rate equation for the liver (Eq. 4) becomes:

\[
\frac{dC_{LJ}}{dt} \cdot V_{LJ} = Q_{ENT} C_{ENT} + Q_{HA} C_{ART} + \sum Q_T \frac{C_T}{K_{b,T}} \\
- Q_{LJ} \frac{C_{LI}}{K_{b,LJ}} - C_{L_{int,LJ}} \mu_b \frac{C_{LJ}}{K_{e,LJ}}
\]

(13)

All states of the full model had initial conditions equal to zero except for the state representing the undissolved drug in the stomach (Eq. 5) for which the initial condition was set to the administered dose (\(\mu g\)).

Statistical model

To optimise the WBPBPK model using Bayesian statistics, we developed a three-stage hierarchical model to describe both uncertainty and random IIV in the drug-specific parameters, as well as the residual difference between observations and model predictions due to model misspecification, unaccounted intra-individual variability in the parameters and measurement error. Suppose a number \(n_i\) of pharmacokinetic measurements were made for each of the \(K\) individuals, indexed by \(i\). Denote the \(j\)th measurement for the \(i\)th individual by \(y_{ij}\) and the associated time by \(t_{ij}\). Further, denote the \(p\)-dimensional vector of parameters for individual \(i\) by \(\theta_i\), and \(\sigma^2\) the residual variance. At the first stage of the model, log-normality was assumed for the model likelihood (Eq. 14).

\[
p(\log(y_{ij})|\theta_i, \sigma^2) \propto N(\log(f(D_i; t_{ij}; \theta_i)), \sigma^2), \quad i = 1, \ldots, K, \quad j = 1, \ldots, n_i
\]

(14)

In Eq. 14, the structural model \(f(\cdot)\) is a function of the \(i\)th individual-specific dosing regimen \((D_i)\), time \((t_{ij})\) and parameters \((\theta_i)\). At the second stage, distributional assumptions were made for the individual-specific parameters to account for IIV (Eq. 15):

\[
p(\theta_i|\mu, \Omega) = MVLN_p(\mu, \Omega), \quad i = 1, \ldots, K
\]

(15)

where \(MVLN_p(\cdot, \cdot)\) denotes a \(p\)-dimensional multivariate log-normal distribution, \(\mu\) is a vector of \(p\) population parameters and \(\Omega\) is the \(p \times p\) IIV variance–covariance matrix. At the third stage, prior distributions were assigned to both population and individual parameters to account for parameter uncertainty (Eq. 16):

\[
p(\mu) = MVLN_p(\bar{\mu}, \sum), \quad p(\Omega) = IW(\Psi, v)
\]

(16)

where \(\bar{\mu}\) is a vector of \(p\) prior population parameter values; \(\sum\) is the \(p \times p\) variance–covariance matrix that describes the informativeness of the prior distribution of \(\mu\); \(\Psi\) is the scale matrix and \(v\) the degree of freedom of the inverse-Wishart distribution \(IW(\cdot, \cdot)\). \(\Psi\) can be calculated as \(\Psi = v\bar{\Omega}\) where \(\bar{\Omega}\) is the prior expectation of \(\Omega\). No prior information was considered for the variance of the residual error \(\sigma^2\). The hyperparameters of the model \(\bar{\mu}, \sum, \Psi\) and \(v\) must be stated explicitly.

A priori parameter distributions

Physiological parameters

Since WBPBPK models are mechanistic, information on system-related parameters can be extracted from the anatomy/physiology literature. To reduce the number of estimated parameters during the optimisation process and hence reduce the computational burden during the Bayesian analysis, no uncertainty in the physiological parameters was considered. The weight, density and regional blood flow for each organ/tissue of the disposition model are given in Table 2. Note that we didn’t correct the organ weights for residual blood due to lack of data. However, the correction might be important for highly perfused organs like the lungs and kidneys. To account for IIV in blood flows and volumes, these parameters were related to BW of the studied subjects. More specifically, regional blood flows were expressed as fractions of the cardiac output \((f_{CO,T})\) which was in turn defined as a function of BW (Eq. 17) [20]:

\[
CO_i = (187 \cdot BW_i^{0.81}) \cdot 60/1000 \\
Q_{T,j} = f_{CO,T} \cdot CO_i
\]

(17)

where \(CO_i\) is the cardiac output (l/h), \(Q_{T,j}\) the tissue blood flow (l/h) and \(BW_i\) the BW (kg) of individual \(i\). For each tissue of the \(i\)th individual, the volume \(V_{T,j}\) (l) was calculated as a fraction \((f_{BW,T})\) of \(BW_i\) corrected by the density \(d_T\) (kg/l) as in Eq. 18.

\[
V_{T,j} = f_{BW,T} \cdot BW_i/d_T
\]

(18)

To impose physiological constraints, we computed the blood flow and volume of the RB compartment by difference such that for each individual, all blood flows sum to the \(CO_i\) and all organ weights (kg) sum to the \(BW_i\).

Drug-specific parameters

The a priori distributions of the drug-specific parameters of the WBPBPK model are summarized in the first column of Table 3. Prior distributions were constructed based on the results of an in vitro metabolism experiment and a rat distribution study performed at Novartis Pharma AG (internal unpublished data), and using in silico methods to
scale the parameters to human. The prior mean estimate of
MVG hepatic intrinsic clearance \( CL_{\text{int},\text{LI}} \) (l/h) was com-
pared by scaling the in vitro intrinsic clearances determined
in recombinant human CYP enzymes. Details of the
in vitro assay as well as of the extrapolation of the intrinsic
clearance from the in vitro systems to human liver can be
found in the Online Resource (Sect. 1). Briefly, Michaelis–
Menten parameters were estimated from enzyme kinetic
data for CYP 3A4, 2C8, 2C9 and 2C19. The parameter
estimates were then used to calculate in vitro isoenzyme-
specific intrinsic clearances which were in turn scaled to
human liver as described by Howgate and co-workers [21].

The uncertainty in the Michaelis–Menten parameter esti-
mates was propagated to the prior estimate of
\( CL_{\text{int},\text{LI}} \) (26 % CV) using Fieller’s theorem and other basic prop-
erties of variances (Online Resource, Sect. 1) [22].

The a priori \( K_{b,T} \) estimates were computed by extrapolation
of the partition coefficients determined in rat to human.
Prior to clinical development, MVG pharmacoki-
netics was studied in rats (internal unpublished data). In
short, plasma and tissue samples (LU, HT, BR, MU, AD,
SK, LI and KI) were collected from 12 male rats at 0.8, 2, 8
and 24 h (3 rats per sampling time) after an IV bolus of
3 mg/kg of MVG. Concentrations were averaged at each
sample time and the area under the concentration–time
curve (AUC) from 0 to 24 h was calculated for each tissue
using the trapezoidal method. The AUC was extrapolated
to infinity by addition of the term \( C_{\text{last}}/\lambda_T \) where \( C_{\text{last}} \) is the
plasma or tissue concentration at the last sample time and
\( \lambda_T \) is the terminal slope of the curve. The tissue-to-plasma
partition coefficients for the different tissues in rat
\( (K_{p,T}\_\text{rat}) \) were then calculated as follows:

\[
K_{p,T}\_\text{rat} = \frac{AUC_{\text{int},T}\_\text{rat}}{AUC_{\text{int,plasma}}\_\text{rat}}
\tag{19}
\]

where \( AUC_{\text{int},T}\_\text{rat} \) is the AUC to infinity in rat tissues and
\( AUC_{\text{int,plasma}}\_\text{rat} \) that in rat plasma. Extrapolation of
\( K_{p,T}\_\text{rat} \) to human tissue-to-blood partition coefficient
\( (K_{b,T}\_\text{human}) \) was done with the assumption that unbound
tissue-to-plasma partition coefficients are equal between rat
and human (Eq. 20).

\[
K_{\text{pu},T}\_\text{human} = K_{\text{pu},T}\_\text{rat} = K_{p,T}\_\text{rat}/f_{\text{up}\_\text{rat}}
\tag{20}
\]

\[
K_{b,T}\_\text{human} = \frac{K_{\text{pu},T}\_\text{human} \times f_{\text{up}\_\text{human}}}{BP}
\]

In Eq. 20 \( f_{\text{up}\_\text{rat}} \) is MVG fraction unbound in rat plasma
(0.065) and \( f_{\text{up}\_\text{human}} \) that in human plasma (0.028), and
\( K_{\text{pu},T}\_\text{rat} \) and \( K_{\text{pu},T}\_\text{human} \) are unbound tissue-to-plasma
partition coefficients for the rat and human, respectively.
Not all tissues represented in the human WBPBPK model
were sampled in the rat. The equations proposed by
Jansson et al. were used to predict the \( K_{p,T}\_\text{rat} \) for GU and
BO from the MU value [23]. However, no model was
available for the \( K_{p,T}\_\text{rat} \) for SP, PA and ST. Hence,
\( K_{b,T}\_\text{human} \) value for MU was used for these tissues as well
as for the RB compartment of the WBPBPK model. Using
the AUC ratio method to calculate the \( K_{p,T}\_\text{rat} \) values, no
estimates of uncertainty were produced. Consequently, a
log-normal prior distribution with a hypothetical 30 % CV
was assigned to the \( K_{b,T} \) values that were estimated during
the analysis of Study 1 data.

The results of the pre-clinical experiments used to cal-
culate prior estimates of \( CL_{\text{int},\text{LI}} \) and \( K_{b,T}\_\text{human} \) included no
information on the variability in these parameters. How-
ever, based on our previous population pharmacokinetic
analysis, MVG systemic clearance is likely to be variable
in the population [3]. Perhaps just because of variability in
the blood flow and volume of the liver, but the hepatic
intrinsic clearance \( CL_{\text{int},\text{LI}} \) could also be variable in the
population. Hence, we estimated random IIV in \( CL_{\text{int},\text{LI}} \)
from the data using a diffuse inverse-Wishart distribution
as prior for the variance. This was achieved by setting
the degrees of freedom for the inverse-Wishart distribution
equal to the dimension of the variance–covariance matrix,
i.e. equal to one in our hierarchical model, as suggested in
the documentation [24] of the software NONMEM 7.3.0
(ICON Development Solutions, Hanover, Maryland, USA).
We assumed that population variation in the perfusion of
the tissues was sufficiently accounted for by the variability
in tissue volumes and regional blood flows. Also, esti-
mating a random-effect on each of the 14 \( K_{b,T} \) parameters
would have considerably slowed the MCMC simulations.
Therefore, the population variances of all \( K_{b,T} \) values were
fixed to a small value corresponding to a CV of 1 % for a
log-normally distributed variable.

**Sensitivity analysis of the WBPBPK model**

Due to the absence of tissue data, we expected numerical
instabilities to arise during optimisation of the population
disposition model [7]. This is because the model’s response
in the venous blood compartment, and hence in plasma, is
sensitive to only a few drug-specific parameters. To iden-
tify these parameters prior to model fitting, we performed a
sensitivity analysis of the model. The analysis was made
using 1000 sets of parameters randomly drawn from the
multivariate log-normal prior distribution (CV of 26 % for
the \( CL\_\text{int,LI} \) value and of 30 % for the \( K_{b,T} \) values) in order
to account for parameter uncertainty. For each vector of
parameters, the Jacobian matrix for the venous blood
compartment was calculated using the complex-step
derivative approximation [25]. Further, a relative
sensitivity coefficient was calculated for each parameter of each of the 1000 sets as follows [26]:

\[ RS_{VEN,p,k} = \frac{\partial A_{VEN,j,k}}{\partial \theta_{p,k}} \cdot A_{VEN,j,k} \]  

(21)

where \( RS_{VEN,p,k} \) is the venous blood response’s relative sensitivity coefficient for the \( p \)th element of the \( k \)th vector of parameters at time \( j \); \( \frac{\partial A_{VEN}}{\partial \theta_{p,k}} \) is the \( p \times j \) venous blood compartment Jacobian matrix for the \( k \)th set of parameters; \( \theta_{p,k} \) is the \( p \)th element of the \( k \)th vector of parameters; \( A_{VEN,j,k} \) is MVG amount in the venous blood compartment at time \( j \), simulated with the \( k \)th set of parameters. The sensitivity of the venous blood response to the drug-specific parameters was graphically assessed across time (every 0.1 h for 48 h). For a given parameter, a relative sensitivity coefficient equal to an absolute value of 0.1 indicated that 1% variation in the parameter value would yield 0.1% variation in the venous blood response at a given time. Above a value of 0.1, the parameter was deemed to have a significant influence on the venous blood response and thus on the \( j \)th plasma concentration. Theoretically, only the parameters that have a significant influence on the plasma response would have their prior distributions updated by plasma data. Thus, to reduce numerical instabilities during the MCMC simulations, the drug-specific parameters deemed to have negligible impact on the plasma response were not estimated.

**Bayesian computation**

In the present PBPK modelling framework, a Bayesian analysis allowed prior pre-clinical beliefs on the MVG-specific parameters and information from Study 1 data to be combined. The two sources of information are complementary. During drug development, if prior knowledge at the pre-clinical stage was sufficient, clinical studies wouldn’t be needed. On the other hand, due to ethical constraints, clinical data alone are insufficient to provide reasonable and precise estimates of all the parameters of such mechanistic models. Assigning prior distributions to the parameters allowed us to optimise the pharmacokinetic model while considering biological/physiological plausibility. An appealing feature of a Bayesian population approach is that the analysis yields posterior distributional estimates of the parameters of interest for the population as well as for each individual. The posterior distributions of the parameters selected based on the sensitivity analysis were approximated using random draws by Gibbs sampling as implemented in NONMEM. Observations below the lower limit of quantification were discarded from the analysis. Three independent Markov chains were initialized in parallel with different diffuse parameter values (the second and third chains were initialized with values 50% higher and lower, respectively, than the first chain’s values). A Markov chain generates samples from the target distribution only after it has converged to equilibrium. Convergence to approximate equilibrium was monitored

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Definition</th>
<th>Regional blood flow(^a) (%)</th>
<th>Weight(^b) (%)</th>
<th>Density(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART</td>
<td>Arterial blood</td>
<td>100</td>
<td>2.81</td>
<td>1.040</td>
</tr>
<tr>
<td>VEN</td>
<td>Venous blood</td>
<td>100</td>
<td>5.62</td>
<td>1.040</td>
</tr>
<tr>
<td>LU</td>
<td>Lungs</td>
<td>100</td>
<td>0.76</td>
<td>1.051</td>
</tr>
<tr>
<td>HT</td>
<td>Heart</td>
<td>4</td>
<td>0.47</td>
<td>1.030</td>
</tr>
<tr>
<td>BR</td>
<td>Brain</td>
<td>12</td>
<td>2</td>
<td>1.036</td>
</tr>
<tr>
<td>MU</td>
<td>Muscle</td>
<td>17</td>
<td>40</td>
<td>1.041</td>
</tr>
<tr>
<td>AD</td>
<td>Adipose</td>
<td>5</td>
<td>21.42</td>
<td>0.916</td>
</tr>
<tr>
<td>SK</td>
<td>Skin</td>
<td>5</td>
<td>3.71</td>
<td>1.116</td>
</tr>
<tr>
<td>SP</td>
<td>Spleen</td>
<td>3</td>
<td>0.26</td>
<td>1.054</td>
</tr>
<tr>
<td>PA</td>
<td>Pancreas</td>
<td>1</td>
<td>0.14</td>
<td>1.045</td>
</tr>
<tr>
<td>LI</td>
<td>Liver</td>
<td>25.5(^d)</td>
<td>2.57</td>
<td>1.040</td>
</tr>
<tr>
<td>ST</td>
<td>Stomach</td>
<td>1</td>
<td>0.21</td>
<td>1.050</td>
</tr>
<tr>
<td>GU</td>
<td>Gut</td>
<td>14</td>
<td>1.44</td>
<td>1.043</td>
</tr>
<tr>
<td>BO</td>
<td>Bones</td>
<td>5</td>
<td>14.29</td>
<td>1.990</td>
</tr>
<tr>
<td>KI</td>
<td>Kidneys</td>
<td>19</td>
<td>0.44</td>
<td>1.050</td>
</tr>
<tr>
<td>RB</td>
<td>Rest-of-body</td>
<td>7.5</td>
<td>3.86</td>
<td>1.040</td>
</tr>
</tbody>
</table>

\(^a\) Given as a percentage of the cardiac output [34]  
\(^b\) Given as a percentage of total BW [53]  
\(^c\) The value of 1.040 was used when the density was not reported [53]  
\(^d\) Total liver flow
using the potential scale reduction statistic proposed by Gelman and Rubin ($\hat{R}$) [27], as well as by graphical inspection of the Markov chains. Calculation of $\hat{R}$ values was done using the software package CODA [28]. Gelman and Rubin recommend that the chains be sampled until all values for $\hat{R}$ fall below 1.10. To increase the number of samples possibly drawn from the parameter target distribution, $10^6$ iterations were computed for each chain. The WBPBPK model was implemented in NONMEM as a system of 16 ordinary differential equations, which were solved during the analysis using the LSODA solver (ADVAN13 subroutine).

**Monte Carlo simulations**

All simulations, calculations and plots were done in MATLAB R2014a (The MathWorks, Inc., Natick, Massachusetts, USA). For each of the following prediction scenarios, Monte Carlo simulations were performed for 1000 hypothetical individuals by randomly drawing drug-specific parameters from a multivariate log-normal distribution and randomly sampling anthropometric/demographic covariates from the dataset used to evaluate the predictive performance of the model. The model predictive performance was visually assessed by computing the 5th, 50th and 95th percentiles of the simulated concentrations at each sample time, and plotting the median together with a 90 % prediction interval under the observed data.

**Evaluation of the adult population disposition model**

A visual predictive check of the population WBPBPK model’s ability to describe Study 1 data was performed. Since Study 1 data were used to optimise the model, this step of the modelling framework can be considered as an internal validation of the population model. Plasma

### Table 3  Prior and posterior distributions of the drug-specific parameter values for MVG WBPBPK model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Prior distribution</th>
<th>Posterior distribution</th>
<th>$\hat{R}$</th>
<th>$n$ iterations ($\times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$BP$</td>
<td>Blood-to-plasma ratio</td>
<td>0.61</td>
<td>NE</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>$f_{up}$</td>
<td>Fraction unbound in plasma</td>
<td>0.028</td>
<td>NE</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>$CL_{int,LI}$ (l/h)</td>
<td>Hepatic intrinsic clearance</td>
<td>2017 (1.30)</td>
<td>1606 (1.04)</td>
<td>1.04</td>
<td>100</td>
</tr>
<tr>
<td>$K_b$</td>
<td>Blood partition coefficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LU</td>
<td>Lungs</td>
<td>2.3</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td>Heart</td>
<td>3.07</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>Brain</td>
<td>3.04 (1.35)</td>
<td>6.09 (1.16)</td>
<td>2.25</td>
<td>100</td>
</tr>
<tr>
<td>MU</td>
<td>Muscle</td>
<td>1.38 (1.35)</td>
<td>2.01 (1.07)</td>
<td>1.06</td>
<td>100</td>
</tr>
<tr>
<td>AD</td>
<td>Adipose</td>
<td>7.43 (1.35)</td>
<td>10.1 (1.05)</td>
<td>1.08</td>
<td>100</td>
</tr>
<tr>
<td>SK</td>
<td>Skin</td>
<td>0.592</td>
<td>NE</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>SP*</td>
<td>Spleen</td>
<td>1.38</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PA*</td>
<td>Pancreas</td>
<td>1.38</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LI</td>
<td>Liver</td>
<td>5.82</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>ST*</td>
<td>Stomach</td>
<td>1.38</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>GU</td>
<td>Gut</td>
<td>3.33</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>BO</td>
<td>Bones</td>
<td>1 (1.35)</td>
<td>0.784 (1.24)</td>
<td>2.57</td>
<td>100</td>
</tr>
<tr>
<td>KI</td>
<td>Kidneys</td>
<td>3.73</td>
<td>NE</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>RB*</td>
<td>Rest-of-body</td>
<td>1.38 (1.35)</td>
<td>2.04 (1.18)</td>
<td>1.19</td>
<td>100</td>
</tr>
<tr>
<td>$\omega_{CL_{int,LI}}^2$</td>
<td>IIV in $CL_{int,LI}$</td>
<td>0.1 ($\nu = 1$)</td>
<td>0.161 (0.0228)</td>
<td>1.01</td>
<td>100</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>Residual error</td>
<td>–</td>
<td>0.0815 (0.0027)</td>
<td>1.32</td>
<td>100</td>
</tr>
</tbody>
</table>

No prior information was considered for $\sigma^2$

Prior distribution was assumed multivariate log-normal for the fixed-effects and inverse-Wishart for the variance $\omega_{CL_{int,LI}}^2$

All marginal distributions are expressed as geometric mean (geometric standard deviation), except the prior of $\omega_{CL_{int,LI}}^2$ expressed as expected value (degrees of freedom), and the posterior of $\omega_{CL_{int,LI}}^2$ and $\sigma^2$ expressed as arithmetic mean (standard deviation)

NE not estimated

* The $K_b$ value for the MU was used as no prior information was available

\[ J \text{ Pharmacokinet Pharmacodyn (2015) 42:639–657 647} \]
transit rate constants were thus calculated as follows: dosage forms were assumed to be first-order processes. The dataset thereby accounting for IIV in the cardiac output and was randomly sampled from either Study 2 or Study 3 and co-workers [31]. Gastric emptying and SI transit of transformed parameters) as described by Tsamandouras these parameters. The reported means and standard deviations (Study 3) in adults. As for the WBPBPK model, the physiological parameters of the absorption model were extracted from the literature. The transit times of dosage forms in the ST and SI have been reported to be highly variable in human [17, 29, 30]. To integrate random IIV in the transit times while imposing constraints to be consistent with physiology, we assigned a logit-normal distribution to these parameters. The reported means and standard deviations (SD) were translated from the logit-normal domain (untransformed parameters) to the normal domain (logit-transformed parameters) as described by Tsamandouras and co-workers [31]. Gastric emptying and SI transit of dosage forms were assumed to be first-order processes. The transit rate constants were thus calculated as follows:

\[
\begin{align*}
  k_{t,ST,i} &= 1 / TT_{ST,i} \\
  k_{t,SI,i} &= 1 / TT_{SI,i}
\end{align*}
\]

where \(k_{t,ST,i}\) is the gastric emptying rate constant (h\(^{-1}\)) and \(TT_{ST,i}\) the corresponding transit time (h) for the \(i\)th individual; \(k_{t,SI,i}\) is the transfer rate constant in the small intestine lumen (h\(^{-1}\)) and \(TT_{SI,i}\) the small intestine transit time (h) for the \(i\)th individual. The volumes of fluid reported by Schiller et al. (means and SDs) were used to model drug dissolution in the ST and SI [32]. The mean value of 1.75 cm was used for the radius of the SI [33]. BW was randomly sampled from either Study 2 or Study 3 dataset thereby accounting for IIV in the cardiac output and organ volumes. The blood flow to the ENT represents 4.8 % of the cardiac output [34, 35]. The volume of the ENT compartment was extracted from Paine et al. and was independent of individual BW [36]. All drug-specific absorption parameters were derived from the results of in vitro experiments conducted at Novartis Pharma AG (Table 4). The formulation-specific dissolution constant \(z\) was estimated by fitting the model from Hintz and Johnson [18] to the in vitro dissolution profiles of the capsule and POS formulations (more details on the method and fitting results in Sect. 2 of the Online Resource). \(P_{eff}\) was derived from the apparent permeability coefficient determined in an in-house Caco-2 cell monolayer system, and using the in silico method described by Sun et al. to scale from the in vitro system to human jejunum [37] (details in Sect. 3 of the Online Resource). For each of the 1000 hypothetical individual, MVG enterocytic intrinsic clearance \(CL_{int,ENT}\) (Eq. 11) was computed by first back-calculating the recombinant human CYP-specific intrinsic clearances (see Eq. 2 in Sect. 1 of the Online Resource) from \(CL_{int,LI}\), then scaling from the recombinant systems to small intestine enterocytes as described by Howgate et al. [21]. The assumed contributions of CYP3A4 (73 %), CYP2C8 (5 %), CYP2C9 (17 %) and CYP2C19 (5 %) to MVG hepatic metabolism were calculated using our prior in vitro estimates of the CYP-specific intrinsic clearances in human liver microsomes (see Eq. 2 in Sect. 1 of the Online Resource). We assumed that only CYP3A4, CYP2C9 and CYP2C19 enzymes contributed to MVG gut wall metabolism. The abundance of these enzymes in the ENT was extracted from Sjogren et al. [38]. When no information on variability in the absorption parameters was available, a CV of 30 % was included in the present Monte Carlo simulations. The performance of the full model (absorption and disposition) in predicting MVG pharmacokinetics following oral administration of both the capsule and POS formulation was visually assessed as described earlier, using data from Study 2 and Study 3. To gain insight into the effect of first pass metabolism on MVG systemic exposure, the simulations were used to calculate the fraction of dose absorbed into the gut wall (\(F_a\)), the fraction escaping gut wall metabolism (\(F_{g}\)) and the fraction escaping hepatic extraction (\(F_h\)) for both the capsule and POS formulation (details in Sect. 4 of the Online Resource).

**Prediction of the DDI with ketoconazole in adults**

To check the ability of the population PBPK model to extrapolate across dosing regimen, we used the full model to predict MVG pharmacokinetics following oral co-administration with ketoconazole in adults (Study 2). As described in Fig. 1, these simulations were conditional on

\( \text{Eq. 12} \) and \( \text{Eq. 13} \) can be applied to any oral immediate-release formulation for which in vitro dissolution data are available and can be described using the model from Hintz and Johnson (Eq. 5–8) [18]. We used the model to predict plasma concentration–time profiles following oral administration with ketoconazole in adults (Study 2). As for the WBPBPK model, the ability of the population PBPK model to extrapolate from the IV route to the oral administration route. This step of the route to the oral administration route is the gastric emptying rate constant (h\(^{-1}\)) for the \(i\)th individual. The volumes of fluid reported by Schiller et al. (means and SDs) were used to model drug dissolution in the ST and SI [32]. The mean value of 1.75 cm was used for the radius of the SI [33]. BW was randomly sampled from either Study 2 or Study 3 dataset thereby accounting for IIV in the cardiac output and organ volumes. The blood flow to the ENT represents 4.8 % of the cardiac output [34, 35]. The volume of the ENT compartment was extracted from Paine et al. and was independent of individual BW [36]. All drug-specific absorption parameters were derived from the results of in vitro experiments conducted at Novartis Pharma AG (Table 4). The formulation-specific dissolution constant \(z\) was estimated by fitting the model from Hintz and Johnson [18] to the in vitro dissolution profiles of the capsule and POS formulations (more details on the method and fitting results in Sect. 2 of the Online Resource). \(P_{eff}\) was derived from the apparent permeability coefficient determined in an in-house Caco-2 cell monolayer system, and using the in silico method described by Sun et al. to scale from the in vitro system to human jejunum [37] (details in Sect. 3 of the Online Resource). For each of the 1000 hypothetical individual, MVG enterocytic intrinsic clearance \(CL_{int,ENT}\) (Eq. 11) was computed by first back-calculating the recombinant human CYP-specific intrinsic clearances (see Eq. 2 in Sect. 1 of the Online Resource) from \(CL_{int,LI}\), then scaling from the recombinant systems to small intestine enterocytes as described by Howgate et al. [21]. The assumed contributions of CYP3A4 (73 %), CYP2C8 (5 %), CYP2C9 (17 %) and CYP2C19 (5 %) to MVG hepatic metabolism were calculated using our prior in vitro estimates of the CYP-specific intrinsic clearances in human liver microsomes (see Eq. 2 in Sect. 1 of the Online Resource). We assumed that only CYP3A4, CYP2C9 and CYP2C19 enzymes contributed to MVG gut wall metabolism. The abundance of these enzymes in the ENT was extracted from Sjogren et al. [38]. When no information on variability in the absorption parameters was available, a CV of 30 % was included in the present Monte Carlo simulations. The performance of the full model (absorption and disposition) in predicting MVG pharmacokinetics following oral administration of both the capsule and POS formulation was visually assessed as described earlier, using data from Study 2 and Study 3. To gain insight into the effect of first pass metabolism on MVG systemic exposure, the simulations were used to calculate the fraction of dose absorbed into the gut wall (\(F_a\)), the fraction escaping gut wall metabolism (\(F_{g}\)) and the fraction escaping hepatic extraction (\(F_h\)) for both the capsule and POS formulation (details in Sect. 4 of the Online Resource).
the success of MVG pharmacokinetic predictions for the capsule formulation (first period of Study 2). Ketoconazole is a strong competitive inhibitor of CYP3A4 [39] and a moderate one of CYP2C8 [40] and CYP2C9 [41]. In the presence of a competitive inhibitor, the intrinsic clearance of a CYP substrate can be expressed as follows:

\[
CL'_{\text{int,CYP}_i} = \frac{CL_{\text{int,CYP}_i}}{1 + \left[\frac{l_{\text{u,CYP}_i}}{K_{i,u,CYP}_i}\right]}
\]

(23)

where \(CL'_{\text{int,CYP}_i}\) is the isoenzyme-specific intrinsic clearance (l/h) in the presence of inhibitor and \(CL_{\text{int,CYP}_i}\), the clearance in the absence of inhibitor; \([l_{\text{u,CYP}_i}]\) is the inhibitor unbound concentration (\(\mu M\)) in the tissue of interest; \(K_{i,u,CYP}_i\) is the isoenzyme-specific unbound inhibition constant (\(\mu M\)). \(CL_{\text{int,CYP}_i}\) values were derived from \(CL_{\text{int,Li}}\) values using our prior belief of the CYP enzymes’ contribution to MVG hepatic metabolism described earlier. \([l_{\text{u,CYP}_i}]\) was calculated as the product of the inhibitor tissue concentration by the fraction unbound in the tissue. \(K_{i,u,CYP}_i\) was defined as the product of the isoenzyme-specific inhibition constant and the unbound fraction of inhibitor in microsomes. Tissue concentrations of ketoconazole were simulated with Simcyp Version 13 (Simcyp Limited, Sheffield, UK) and the design of Study 2 (400-mg/day for 10 days). Using the minimal PBPK model implemented in Simcyp [42], it is possible to simulate ketoconazole total concentrations in the portal vein and liver (see Fig. 2 in Sect. 5 of the Online Resource). To derive ketoconazole unbound concentrations in the liver, the fraction unbound in the liver was calculated as the ratio of the fraction unbound in plasma to the liver-to-plasma partition coefficient for ketoconazole. To compute ketoconazole unbound concentrations in the ENT, we assumed that total concentrations in the portal vein reflect those in the ENT [42], and used ketoconazole fraction unbound in the ENT to derive unbound concentrations. We resorted to Simcyp default values of the inhibition-related ketoconazole parameters for the present simulation (Table 2 in Sect. 5 of the Online Resource). The simulated ketoconazole concentration–time data were used as forcing functions for the inhibition of the intrinsic clearance in the ENT and LI compartments (Eq. 4, 11 and 23). Interpolation of ketoconazole concentrations from the Simcyp-produced sample times to each time step used by the differential equation solver in MATLAB was done using the function ‘interpl’ (spline interpolation method). To account for uncertainty in the ketoconazole inhibition constant values reported by Simcyp, simulation of MVG concentration–time profiles was performed assuming a CV of 30 % for these parameters. Data from the second period of Study 2 were used to visually evaluate the performance of the model in predicting the impact of the MVG-ketoconazole interaction on MVG pharmacokinetics. To check the consistency of the PBPK modelling approach with the standard non-compartmental analysis approach used to quantify the impact of DDI on drug pharmacokinetics, we calculated for each hypothetical individual the change in AUC to infinity when MVG was co-administered with ketoconazole compared to when administered alone.

**Prediction of oral pharmacokinetics in children**

The suitability of the WBPKPK model for extrapolation of MVG pharmacokinetics from adults to paediatrics was evaluated using data from Study 4. As schematically explained in Fig. 1, this simulation was conditional on the ability of the model to predict pharmacokinetics of the POS formulation in adults (Study 3). Children enrolled in Study 4 were diagnosed with Fragile X syndrome. We assumed that the disease had no impact on MVG pharmacokinetics. Hence, the model was scaled from adult to children by simply integrating the age-related physiological changes in children from 3 to 11 years of age. Information on the age dependencies of the physiological parameters was gathered from the literature for age groups of 1, 5, 10 and 15 years. As for the adult WBPKPK model, regional blood flows were expressed as fractions of the cardiac output, \(f_{\text{CO,T}}\) (Eq. 17) and organ volumes as fractions of BW, \(f_{\text{BW,T}}\) (Eq. 18), thereby accounting for variability in tissue perfusion. The cardiac output was defined as a function of both children’s age and body surface area (BSA) as described by Johnson et al. [43]. \(f_{\text{CO,T}}\) values were computed using the reported age-specific values of the cardiac output [34] and regional blood flows [44]. \(f_{\text{BW,T}}\) values were calculated based on age-related changes in BW and actual organ weights [34]. The same densities as for adults were used for most organs/tissues except for the bones for which information was available in the literature [34]. For this simulation, age and BSA were randomly sampled from Study 4 dataset for each of the 1000 hypothetical individuals. The MATLAB ‘interp1’ function (spline interpolation method) was used to interpolate the physiological parameters from the reference age groups to each individual age. MVG fraction unbound in plasma \(f_{\text{up}}\) was also scaled from adult to children as described by Johnson et al. [43]. For each of the 1000 hypothetical children, the hepatic intrinsic clearance was derived by simply back-calculating the total intrinsic clearance in human liver microsomes from the adult \(CL_{\text{int,Li}}\) (see Eq. 2 in Sect. 1 of the Online Resource), then scaling it again to human liver using the liver weight of the hypothetical child. We thereby assumed that the IIV in \(CL_{\text{int,Li}}\) (estimated from Study 1 data) is the same between adults and children as we had no prior information on the variability in \(CL_{\text{int,Li}}\) in children. Based on the ontogeny of CYP enzymes [45], we assumed that all
enzymes involved in MVG metabolism achieved complete maturity in children aged from 3 to 11 years (same abundances in the ENT and LI as in adults). Finally, to account for variation in drug dissolution between adults and children, the volume of fluid in the SI, $V_{SI}$ (Eq. 7, 8) was defined as a fraction of the volume of the SI cylinder which was calculated using BSA-dependent diameter and length of the SI [43]. The fraction of the cylinder was derived using the adult volume of fluid $V_{SI}$ [32] and volume of the SI cylinder [46] values. The predictive performance of the model for children was graphically evaluated as for the other simulation scenarios.

### Results

Figure 4 shows the results of the sensitivity analysis of the WBPBPK model (disposition only). For each parameter of each of the 1000 parameter vectors drawn from the prior distribution, the absolute value of the venous blood relative sensitivity coefficient $RSVEN_{i,pl,k}$ (Eq. 21) was plotted across time. These results suggest that $CL_{int,LI}$, $K_b,MU$, and $K_b,AD$ are the parameters that have the main influence on the venous blood response, but that the response might be also slightly sensitive to the $K_b,BR$, $K_b,BO$ and $K_b,RR$ parameters. Therefore, only six drug-specific parameters out of 15, namely $CL_{int,LI}$, $K_b,MU$, $K_b,AD$, $K_b,BR$, $K_b,BO$ and $K_b,RR$, were estimated during the Bayesian analysis of Study 1 data.

The posterior distribution of these parameters as well as of the population variance of $CL_{int,LI}$ ($\sigma^2_{CL_{int,LI}}$) and of the residual variance ($\sigma^2$), are summarized in Table 3 and were obtained by pooling the one million parameter vectors from all three Markov chains. On average, one million iterations were completed in approximately 11 days on a medium-sized cluster running Red Hat Enterprise Linux 6.5 on nodes with Intel Xeon E5-2670v2 CPUs with some older nodes running with dual Xeon X5670 CPUs. Each chain was parallelized on 12 different nodes. The nodes are equipped with between 24 and 96 GB of RAM and are interconnected via dedicated Bonded 1 Gbit network cards. As indicated by the $R$ statistic (Table 3), convergence to the target distribution was achieved after 20,000 iterations for $CL_{int,LI}$, $K_b,MU$, $K_b,AD$ and $\sigma^2_{CL_{int,LI}}$ but not for $K_b,BR$, $K_b,BO$, $K_b,RR$ and $\sigma^2$. The distribution of the latter seemed to eventually reach equilibrium after 260,000 iterations. Trace-plots of the three Markov chains run to approximate the posterior distribution are presented in Fig. 5 and show a slower mixing of the chains for the parameters $K_b,BR$, $K_b,BO$ and $K_b,RR$ than for the other parameters. A comparison of the marginal prior and posterior distribution of the parameters for which we had prior knowledge is presented in Fig. 6. All estimated parameter distributions were updated by Study 1 plasma concentration–time data, and appeared to be normal and within plausible biological/physiological limits. The smallest change from the prior mean estimate was for the population estimate of $CL_{int,LI}$ (1.26-fold decrease). The most notable deviation was observed for the estimate of $K_b,BR$ that doubled from the value of 3.04. Overall, the uncertainty in all population parameter values was reduced, especially for $CL_{int,LI}$, $K_b,MU$ and $K_b,AD$ for which the uncertainty CV decreased to a value of 4, 7 and 5 %, respectively. As suggested by the small uncertainty in $\sigma^2_{CL_{int,LI}}$ (CV of 14 %), variability in $CL_{int,LI}$ was well informed by the data and appeared to be high in the studied healthy adult subjects (CV of approximately 108 %). The residual error, which accounts for unexplained intra-individual variability, model misspecification and analytical error for observations in plasma, had a small variance estimate (Table 3) thereby indicating a good fit of the model to Study 1 data.

Figure 7 shows a visual predictive check of the population model’s ability to describe Study 1 data. Observed and simulated concentrations were dose-normalised. Both the median trend and the variability in the data seem to be well captured by the model, confirming the suitability of the population WBPBPK model to describe MVG disposition in adults.

### Table 4 Drug-specific parameters of the absorption model for MVG oral immediate-release formulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Estimate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z_{capsule}$ ($10^{-4}$ l/h/g)</td>
<td>Dissolution constant for the capsule</td>
<td>0.124</td>
<td>Fit to in vitro dissolution profile</td>
</tr>
<tr>
<td>$z_{POS}$ ($10^{-2}$ l/h/g)</td>
<td>Dissolution constant for the POS</td>
<td>2.04</td>
<td>Fit to in vitro dissolution profile</td>
</tr>
<tr>
<td>$S_{ST}$ ($10^6$ ng/ml)</td>
<td>Drug solubility in stomach</td>
<td>0.019</td>
<td>In vitro solubility experiment$^a$</td>
</tr>
<tr>
<td>$S_{SI}$ ($10^6$ ng/ml)</td>
<td>Drug solubility in small intestine</td>
<td>0.037</td>
<td>In vitro solubility experiment$^b$</td>
</tr>
<tr>
<td>$P_{eff}$ (cm/h)</td>
<td>Jejunal effective permeability</td>
<td>5.1</td>
<td>In vitro Caco-2 experiment together with in silico model from Sun et al. [37]</td>
</tr>
</tbody>
</table>

$^a$ Determined in fasted state simulated gastric fluid

$^b$ Determined in fasted state simulated intestinal fluid

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The results of the Monte Carlo simulations for extrapolation of MVG pharmacokinetics beyond Study 1 population and experimental conditions are presented in Fig. 8. For each simulation scenario, the model could predict reasonably well both the median trend and the variability in MVG plasma concentration–time data, which shows the adequacy of the population PBPK model for extrapolation across routes of administration and formulations (Fig. 8a, b), dosing regimen (Fig. 8c) and age groups (Fig. 8d). Nevertheless, the variability in the early concentration–time data is overall slightly under-predicted (see Fig. 8b, c). Moreover, the model slightly over-predicts the median trend in the paediatric data, especially during the first 5 h post-dose during which drug absorption occurs. In adults, the geometric mean value of the simulated $F_a$ was slightly higher for the POS formulation (0.65) than for the capsule formulation (0.52). However, taking into account the variability, there was no statistically significant difference between the $F_a$ values of the two formulations (the 90% confidence interval was [0.34; 0.73] for the capsule and [0.39; 0.87] for the POS formulation). The geometric mean values of $F_g$ and $F_h$ were 0.93 (CV of 3%) and 0.64 (CV of 17%), respectively, regardless of the formulations. The DDI simulations suggest that on average, the systemic
exposure to MVG is increased by 3.8 fold (geometric mean of the AUC ratio) when co-administered with 400 mg of ketoconazole compared to administration of MVG alone. However, this increase in systemic exposure might be highly variable in a healthy adult population (90% confidence interval of [1.4; 9.8]).

Discussion

We have reported the development, optimisation and applications of a population WBPBPK model for MVG to gain understanding of its pharmacokinetics during its clinical development and to evaluate the ability of the model to
extrapolate beyond the data analysed. Since the generic WBPBPK model proposed is mechanistic in nature, its parameters could be defined a priori using knowledge from the literature (system-specific parameters) and pre-clinical experiments (drug-specific parameters). Bayesian statistics were applied to leverage our prior knowledge of the drug-specific parameters while avoiding biologically unrealistic estimates as well as numerical instabilities due to the absence of tissue data. Using a Bayesian population approach, the current pharmacokinetic model could be progressively updated by new clinical data in order to propagate the information throughout the drug development process.

To decrease the computational burden during the MCMC simulations, we estimated only the parameters that were deemed to have a significant influence on the venous blood response. Although this approach can be considered subjective, we believed that accounting for uncertainty in all parameters of the WBPBPK model, including physiological parameters (i.e. blood flows and organ volumes), would have yielded significant numerical instabilities given

Fig. 7 A visual predictive check of the population WBPBPK model’s ability to describe Study 1 data. Open circles are observed concentrations plotted across time, the solid red line is the median of the simulated concentrations and the grey area represents a 90% prediction interval. Both observed and predicted concentrations were dose-normalised. The insert expands the first 2 h of the concentration–time data plotted on linear scales (Color figure online)

Fig. 8 A visual evaluation of the model’s ability to predict MVG pharmacokinetics when orally administered alone in adults using either the capsule (a) or the POS formulation (c), when co-administered with ketoconazole in adults using the capsule formulation (b), and when administered alone in children using the POS formulation (d). Open circles are observed concentrations plotted across time, the solid red lines are the medians of the simulated concentrations and the grey areas represent 90% prediction intervals. The horizontal dotted black lines represent the lower limit of quantification of the assays (Color figure online)
the available data. In fact, a preliminary analysis of Study 1 data in which we estimated all drug-specific parameters (but not the physiological parameters) showed that the posterior distribution of the parameters couldn’t reach approximate equilibrium even after one million iterations (Fig. 3 in Sect. 6 of the Online Resource) possibly because of high correlations between parameters and/or because the priors were too vague given the complexity of the model. Hence, we decided to estimate only the six drug-specific parameters selected based on the results of the sensitivity analysis. An alternative to this approach could be to reduce the WBPBPK model using proper lumping techniques [47] to possibly shorten the data analysis and stabilise the MCMC simulations. Using proper lumping, the parameters of the lumped compartments can be directly related to the parameters of the original model. Hence, prior knowledge on the parameters of the WBPBPK model can be used to construct prior distribution of the reduced model parameters. Nevertheless, an optimal lumping scheme for a model is specific to its structure and distribution of the parameter values. Therefore, the use of reduced PBPK models for extrapolation of pharmacokinetics across experimental conditions and sub-populations is challenging as it often requires the incorporation of additional mechanisms in the model (e.g. DDI mechanism) and/or to scale the parameter distributions (e.g. from adult to children).

The Bayesian population analysis of Study 1 data provided better and more precise estimates of the population parameters $CL_{int,Lt}$, $K_{h,MU}$, $K_{h,AD}$, $K_{h,BR}$, $K_{h,BO}$ and $K_{h,BO}$ while maintaining biologically plausible values (Table 3). The data contained information mostly for $CL_{int,Lt}$, $K_{h,MU}$ and $K_{h,AD}$ as indicated by the higher reduction in the uncertainty in these parameters compared to the other parameters (Fig. 6). This is consistent with the results of the sensitivity analysis (Fig. 4) and with the fact that target distributions were reached much faster for $CL_{int,Lt}$, $K_{h,MU}$ and $K_{h,AD}$ (20,000 iterations) than for $K_{h,BR}$, $K_{h,BO}$ and $K_{h,BO}$ (260,000 iterations) during the MCMC simulations. The estimates of the tissue-to-blood partition coefficients indicate that MVG is extensively distributed into the adipose tissue and the brain (target site), which was expected for a small neutral lipophilic drug that is a priori not substrate to any efflux transporter [2]. Our simulations suggest that on average, the brain exposure might be four times higher than the plasma exposure to the drug. Although we have no data to check whether these predictions are reasonable or not, exploratory simulations can be performed to evaluate the impact of the brain exposure on drug response under different scenarios as well as the sensitivity of the system to parameter uncertainty and variability. Such exposure–response simulation study can be of clinical value to predict a dosing regimen that best fits efficacy or safety requirements.

Using a population approach, we could quantify IIV in $CL_{int,Lt}$, even though we didn’t have prior knowledge of the variability in this parameter. This variability is likely explained by the heterogeneity of the CYP enzymes’ functionality within the human population. For instance there is evidence for genetic polymorphism of CYP2C9 [48]. Information on individual genotypes was however not available and could therefore not be tested in the population model as a covariate for $CL_{int,Lt}$. Note that the high variance estimate for $CL_{int,Lt}$ could be inflated if variability in the physiological parameters was underestimated. In this study, we chose BW as explanatory variable for the physiologic variability in the organ volumes and blood flows. However, there is evidence that for a given age and gender, the variability in the organ volumes is better correlated with body height (e.g. heart, lungs and liver) or independent of BW and body height (e.g. brain), and that the variability in the blood flows is better explained by body height [14]. This should be taken into consideration if the model were to be used for exploratory simulations, especially for prediction of the target site exposure to the drug as discussed in the previous paragraph.

Before discussing the results of our Monte Carlo simulations, it should be stressed that the simulations were performed without taking into account the uncertainty around the population and individual parameter estimates, meaning that our predictions represent the mean of the predictions derived using the full posterior parameter density. Making full use of the posterior parameter distribution can be of value during drug development to account for parameter uncertainty when predicting clinical endpoints. However, the objective of the present simulations was to evaluate the ability of the model to extrapolate MVG pharmacokinetics rather than to address specific efficacy or safety concerns.

Incorporating a mechanistic absorption model into the WBPBPK model allowed extrapolation of MVG pharmacokinetics from the IV administration route to the oral route. The prior knowledge that we had on the system-, drug- and formulation-specific parameters appeared good enough for prediction of MVG oral pharmacokinetics without having to optimise any absorption parameters (Fig. 8a, b), although the slight under-prediction of the variability at the early time points suggests that the absorption parameters might be more variable than what we accounted for. An advantage of this so-called “bottom-up” approach is that, using available formulation-specific dissolution data, the model can be applied to predict pharmacokinetics of other oral immediate-release
formulations of interest. This can be of value during clinical development to anticipate the dose that would yield a desirable steady-state exposure for a new formulation. In our work, this was rather a pre-requisite to be able to predict the impact of the DDI with ketoconazole on the pharmacokinetics of the capsule formulation in adults, and predict the pharmacokinetics of the POS formulation in children. Of note, more complex models could be implemented to describe more mechanistically the dissolution process [19, 49, 50]. Another advantage of mechanistic absorption models is that a better understanding of the systemic bioavailability and the first-pass effect can be gained. Our simulations suggest that for both the capsule and POS formulation, on average more than 50 % of an orally administered dose of MVG is absorbed into the gut wall. This is consistent with the reported results of a clinical study of the pharmacokinetics of 14C-radiolabeled MVG orally administered to four healthy adult subjects using the capsule formulation [2]. The high value of $F_g$ (0.93) indicates a low extent of gut wall metabolism of MVG, which however strongly relies on our assumption that the drug is bound to proteins in the enterocytes to the same extent as in the plasma. The systemic bioavailability of MVG was predicted for a standard individual in the population to be 31 % for the capsule and 39 % for the POS formulation. It should be noted that the capsule formulation used in Study 2 is different from the capsule formulation assessed in our previous population analysis of MVG pharmacokinetics in healthy adults (different dissolution characteristics) [3]. Therefore, it wasn’t relevant to compare the present estimate of the systemic bioavailability with the one from our previous analysis.

One of the main applications of PBPK models is to predict the impact of DDIs on pharmacokinetics of therapeutic drugs. Since our population WPBPK model could adequately predict the pharmacokinetics of the capsule formulation in the adult subjects of Study 2, we subsequently used it to predict the effect of ketoconazole on MVG pharmacokinetics, without using the data to optimise the inhibition-related parameters. The model predictions were in good agreement with the available clinical data (Fig. 8c). In addition, the predicted increase in AUC (3.8-fold) was consistent with the results (unpublished) of a former in-house non-compartmental analysis of Study 2 data (threefold). Nevertheless, other types of DDIs (e.g. mechanism-based inhibition and induction) should be investigated with the proposed PBPK model to gain confidence in the metabolic pathways involved in MVG elimination. Moreover, since MVG can be associated to serious neurological adverse events [51, 52] and since its efficacy is believed to be closely related to the brain exposure, it is important to identify DDIs that have significant effect on brain concentrations in order to suggest possible dose adjustments.

It has been recognised that the PBPK modelling approach is ideal for extrapolation of pharmacokinetics of therapeutic drugs from an adult to a paediatric population because the differences in the concentration–time profiles are mainly due to age-related differences in anatomy and physiology [44]. Including the age-related changes in the physiological parameters of our WPBPK model as well as in some drug-specific parameters (i.e. $f_{up}$ and $CL_{int}$) allowed reasonable prediction of MVG pharmacokinetics in children from 3 to 11 years of age, without having to fit the model to the clinical data (Fig. 8d). The over-prediction of the median pharmacokinetic trend in the first 5 h post-dose could be explained by unaccounted age-related changes in the absorption parameters. No strong evidence of differences in the absorption-related physiological parameters (e.g. gastric and small intestine transit times) between adults and children could be found in the literature. However it is likely that dissolution of the POS formulation (rate-limiting step of MVG absorption) has a different pattern in children compared to adults possibly because the dynamic of fluid in the small intestine is different and/or the change in the fluid volume is more important than what we accounted for. Nevertheless, the 21 children enrolled in Study 4 might not be representative of a population aged from 3 to 11 years (see Table 1) which could also explain the discrepancies between the model predictions and the observations. It should also be noted that the maturation of CYP enzymes involved in MVG metabolism was considered to be achieved in children aged from 3 years. To predict pharmacokinetics in younger subjects, age-related changes in the enzyme abundances (both in the liver and gut wall) should be incorporated in the model using ontogeny equations [45].

Conclusions

In conclusion, population physiological modelling of MVG pharmacokinetics provided further insight into its absorption, distribution and elimination mechanisms in human, including the source and magnitude of variability. The Bayesian approach offered a continuous flow of information from pre-clinical to clinical studies and helped to reduce the uncertainty in some drug-specific parameter values. This approach could be applied to new clinical data to update our current knowledge of MVG population pharmacokinetics and maintain the information flow during drug development. The model can be used to predict plasma and brain (target site) concentration–time profiles following administration of various oral immediate-release formulations of MVG alone or when co-administered with other drugs, in adults as well as in children. While predicting the pharmacokinetic
properties of new formulations is not of particular interest for the current clinical development of MVG, being able to predict the DDI risk of compounds likely to be co-administered with MVG, across different age groups, could be useful for the design of better clinical studies. For that purpose, the data used to evaluate the predictive performance of the model (Study 2–4) could now be analysed to improve our current estimates of the parameters.

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Compliance with ethical standards

Conflict of interest

Thierry Wending is an employee of Novartis Pharma AG and a Ph.D. student at the University of Manchester.

References

Chapter 4: Reduction of a whole-body physiologically-based pharmacokinetic model to stabilise the Bayesian analysis of clinical data

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Reduction of a Whole-Body Physiologically Based Pharmacokinetic Model to Stabilise the Bayesian Analysis of Clinical Data

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ABSTRACT. Whole-body physiologically based pharmacokinetic (PBPK) models are increasingly used in drug development for their ability to predict drug concentrations in clinically relevant tissues and to extrapolate across species, experimental conditions and sub-populations. A whole-body PBPK model can be fitted to clinical data using a Bayesian population approach. However, the analysis might be time consuming and numerically unstable if prior information on the model parameters is too vague given the complexity of the system. We suggest an approach where (i) a whole-body PBPK model is formally reduced using a Bayesian proper lumping method to retain the mechanistic interpretation of the system and account for parameter uncertainty, (ii) the simplified model is fitted to clinical data using Markov Chain Monte Carlo techniques and (iii) the optimised reduced PBPK model is used for extrapolation. A previously developed 16-compartment whole-body PBPK model for mavoglurant was reduced to 7 compartments while preserving plasma concentration-time profiles (median and variance) and giving emphasis to the brain (target site) and the liver (elimination site). The reduced model was numerically more stable than the whole-body model for the Bayesian analysis of mavoglurant pharmacokinetic data in healthy adult volunteers. Finally, the reduced yet mechanistic model could easily be scaled from adults to children and predict mavoglurant pharmacokinetics in children aged from 3 to 11 years with similar performance compared with the whole-body model. This study is a first example of the practicality of formal reduction of complex mechanistic models for Bayesian inference in drug development.

KEY WORDS: Bayesian population approach; mavoglurant; PBPK extrapolation; physiologically based pharmacokinetic models; proper lumping.

INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models are increasingly used in drug development for their ability to predict drug concentrations in clinically relevant tissues (e.g. target site of the drug) and to extrapolate across species, experimental conditions (e.g. co-administration with a metabolic inductor/inhibitor) and special populations (e.g. children). Since PBPK models are mechanistic in nature, parameters can be defined a priori using knowledge from the anatomy/physiology literature (system-specific parameters) and preclinical experiments (drug-specific parameters), and can thus be used for exploratory simulations. Nevertheless, it is often desirable to fit the model to animal or clinical data to improve the estimates of the drug-specific parameters. A whole-body PBPK (WBPBPK) model is typically expressed as a system of ordinary differential equations (ODEs), in which each state represents a tissue or an organ space. During clinical studies, tissue concentrations are usually not measured due to ethical constraints. As a consequence, fitting a WBPBPK model to clinical data commonly yields a numerically unstable analysis as many parameters (e.g. tissue-to-blood partition coefficients) cannot be informed just by plasma data. To stabilise the analysis, prior knowledge on the parameter values can be incorporated in the model using a Bayesian approach. Such an approach also allows biological/physiological constraints to be maintained when estimating the parameters. A Bayesian population analysis produces posterior distributions of both the population and individual parameters, which can be approximated using Markov Chain Monte Carlo (MCMC) simulation (1). Optimising complex systems, such as WBPBPK models, using MCMC techniques can be time consuming, especially when analysing a large amount of sparse and noisy data typically collected during clinical trials (2). In addition, depending on the informativeness of the priors assigned to the model parameters, the statistical model might not be identifiable for a high-dimensional system (3, 4).
The dimension of a dynamical system can be reduced using model order reduction methods (5). Lumping is one of these methods that allows all states of the original system to be transformed to fewer pseudo-states, thus reducing the number of ODEs and parameters accordingly (6). Proper lumping is a specific case of lumping methods where each state of the original model contributes to only one of the states of the reduced model. Therefore, the parameters of the reduced model can be directly related to those of the original model. This is of particular value in a Bayesian framework since prior knowledge on the original model can still be used to construct a prior distribution for the parameters of the reduced model. WBPBPK models are usually reduced in a way that the compartments of clinical interest remain unlumped. The reduced model should be able to describe the main features of the original model (e.g. response in blood and target site) while perhaps neglecting some less important aspects (e.g. response in the splanchnic organs). An automatic algorithm for proper lumping that can handle constraints (to avoid all possible combination of states) has been proposed by Dokoumetzidis and Aarons (7). In general, a model is reduced specifically for a particular set of parameter values. However, parameters always carry uncertainty and should hence be considered as random variables following statistical distributions rather than single values. Dokoumetzidis and Aarons proposed a robust Bayesian method to account for parameter uncertainty during the lumping process (8).

We have recently reported the MCMC analysis of mavoglurant (MVG) Phase-I clinical data using a generic WBPBPK model (9). We have found that in spite of assigning informative prior distributions to all population median parameters, the posterior distribution of the parameters could not converge to equilibrium, which suggested that the model was probably numerically unidentifiable. We therefore proposed to estimate only the population median parameters that have a significant influence on the plasma response (6 out of 15 parameters) and fix the others based on a sensitivity analysis of the model. However, although this approach permitted convergence to be achieved, it can be deemed too subjective as we assumed a priori that the available plasma data would not inform the majority of the parameters. In addition, fixing parameters while optimising some others can distort the covariance structure of the parameters and produce biased estimates (10).

The aim of the current study was thus to propose an alternative approach to stabilise the Bayesian analysis of clinical data with a PBPK model, which is the use of a reduced model obtained by proper lumping of a WBPBPK model in order to retain the mechanistic nature of the system. The WBPBPK model for MVG was used to illustrate this approach. The second goal of this study was to investigate whether the reduced yet mechanistic model could still be used to extrapolate MVG pharmacokinetics from adults to children as it was successfully done in our previous study of the WBPBPK model (9).

**METHODS**

The work flow for reducing the WBPBPK model for MVG, fitting the reduced model to clinical data and using the optimised reduced model for extrapolation from adults to children, includes the following steps:

1. Development of the original model
2. Model order reduction using proper lumping
3. Sensitivity analysis of the reduced model
4. Bayesian data analysis
5. Extrapolation

Thereby, prior information on the model parameters is propagated all along the modelling work flow and is combined with the information in the data to then perform possibly better predictions.

**WBPBPK Model for MVG**

The WBPBPK model used to describe the time course of MVG plasma concentrations after intravenous (IV) administration in healthy adult subjects has been described in detail by Wendling et al. (9) and is depicted in Fig. 1. Briefly, the model comprises 14 tissue compartments (lungs (LU), heart (HT), brain (BR), muscle (MU), adipose (AD), skin (SK), spleen (SP), pancreas (PA), liver (LI), stomach (ST), gut (GU), bones (BO), kidneys (KI) and rest-of-body (RB)) and 2 blood compartments (arterial and mixed venous), that is 16 states in total. Each tissue compartment is assumed well-stirred, with the extent of distribution being characterised by the equilibrium tissue-to-blood partition coefficient ($K_{b,T}$).

![Fig. 1. Schematic representation of the WBPBPK model for MVG. See text for definition of symbols. The numbers refer to the state numbers of the system](image-url)
The rate equation for the tissue compartments can be expressed as follows:

$$\frac{dA_T}{dt} = \frac{Q_T}{V_{VEN/ART}} A_{VEN/ART} + \frac{Q_T}{V_T K_b, T} A_T$$

(1)

where $A_T$ denotes the amount (µg), $V_T$ the volume (l) and $Q_T$ the blood flow (l/h) for the different tissues, and $A_{VEN/ART}$ and $V_{VEN/ART}$ are the amount (µg) and volume (l), respectively, of either mixed venous blood (for the LU) or arterial blood (all other tissues). Drug elimination is assumed to occur entirely in the liver compartment via oxidative metabolism, as extrahepatic metabolism and renal excretion are thought to be minor for MVG (11). The rate equation for the liver is thus defined as:

$$\frac{dA_{LI}}{dt} = \frac{Q_{HA}}{V_{ART}} A_{ART} + \frac{Q_{SP}}{V_{SP} K_{SP}} A_{SP} + \frac{Q_{PA}}{V_{PA} K_{PA}} A_{PA} + \frac{Q_{ST}}{V_{ST} K_{ST}} A_{ST}$$

+ $\frac{Q_{GU}}{V_{GU} K_{GU}} A_{GU}$

+ $\frac{Q_{CL}}{V_{CL} K_{CL}} A_{CL}$

+ $\frac{Q_{LJ}}{V_{LJ} K_{LJ}} A_{LJ}$

+ $\frac{Q_{LI}}{V_{LI} K_{LI}} A_{LI}$

(2)

where $Q_{HA}$ is the blood flow (l/h) from the hepatic artery, $C_{L\text{int.LI}}$ is the intrinsic clearance in the liver (l/h) and $f_u$ is the fraction unbound in blood.

Since the model is highly mechanistic, all parameters have a biological/physiological interpretation and can be defined a priori based on former experiments. These parameters are of two types: system-specific and drug-specific. The organ/tissue volumes and regional blood flows are the system-specific parameters and are typically gathered from the anatomy/physiology literature. The drug-specific parameters are the $C_{L\text{int.LI}}$ and the $K_{b, T}$ and were extrapolated from an in vitro metabolism experiment and a distribution study in rats, respectively, as described in (9).

Both system- and drug-specific parameter values can carry uncertainty due to errors in assumptions, hypotheses, observations, experiments and handling of the system studied (12). However, no uncertainty in the system-specific parameters was considered in our previous Bayesian analysis with the WBPBPK model in order to decrease the computational burden and potential numerical instabilities (9). On the other hand, a multivariate log-normal analysis with the WBPBPK model in order to decrease the system-specific parameters with uncertainty coefficients (CV) of 26% for the $C_{L\text{int.LI}}$ value and of 30% for the $K_{b, T}$ values. The values of the organ/tissue blood flows and volumes as well as the marginal prior distributions of $C_{L\text{int.LI}}$ and $K_{b, T}$ can be found in (9).

**Reduction of the WBPBPK Model for MVG**

The lumping methodology applied in this study has been described in detail in (7). Nevertheless, to understand how the ODEs for the reduced system were extracted, the basics of this technique are briefly described below.

The WBPBPK model for MVG can be expressed as

$$\frac{dy}{dt} = f(y) = K \cdot y$$

(3)

where $y$ is a vector of $n$ states representing the amount of drug in the organ spaces and tissues, and $K$ is the corresponding matrix of transfer rate constants. $y$ can be reduced to a vector of $\hat{n} < n$ pseudo-states $\hat{y}$ such that:

$$\hat{y} = M \cdot y$$

(4)

where $M$ is the lumping matrix of dimension $\hat{n} \times n$ that determines the transformation from $y$ to $\hat{y}$. The inverse transformation is given by $y = M^\top \cdot \hat{y}$. The inverse transformation is expressed as follows:

$$\frac{d\hat{y}}{dt} = \hat{K} \cdot \hat{y}$$

(5)

or

$$\frac{d\hat{y}}{dt} = \hat{K} \cdot \hat{y}$$

(6)

where $\hat{K}$ is the matrix of transfer rate constants for the reduced system and can be calculated for a linear system as in Eq. 7.

$$\hat{K} = M \cdot K \cdot M^\top$$

(7)

The lumping matrix $M$ contains only 1 and 0 s, which describe the lumping scheme. The objective of model order reduction is to determine an appropriate matrix $M$ such that the reduced system satisfies a specific property or criterion. We used the algorithm proposed by Dokoumetzidis and Aarons (7) to determine the optimal lumping matrix $M$ such that an objective function accounting for the statistical distributions of the parameters (BOF) is minimised (Eq. 8).

$$\text{BOF} = \int_s (P(\theta) \cdot OF) d\theta$$

(8)

In Eq. 8, $P(\theta)$ is the probability density function of the prior distribution of the parameters $\theta$, $S$ is the parameter space and OF is the local objective function that takes the sum of squares of the differences of the responses between the reduced and original systems for all states (8).

Some advantages of this algorithm are: it is automated and was developed to combat combinatorial explosion; it can handle constraints that have a physiological meaning or force some organs/tissues of clinical interest to remain unlumped; it accounts for the prior distribution of the parameters as in a Bayesian modelling framework. Using this algorithm, the lumping matrix $M$ is calculated for a desired number of pseudo-states $\hat{n}$ of the reduced system. Thus, determining the optimal lumping matrix $M$ also involves determining manually the minimal value of $\hat{n}$ so that the reduced model still satisfies the property/criterion of interest. This was done in two steps. Firstly the predictions in the lumped states for the reduced system and original system were visually compared. To do so, the states of the original system were lumped in the same way as the reduced system but after the simulation using Eq. 4. Note that at this stage, all simulations and calculations done to determine the optimal lumping scheme were based on amounts as the ODEs for the WBPBPK model were defined to describe the temporal change of drug amount in

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**References:**


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the organ/tissue compartments. Secondly, the plasma concentration-time profiles predicted with the reduced and original models were visually compared. All calculations/simulations were performed using MATLAB R2014a (The MathWorks, Inc., Natick, Massachusetts, USA) and were speeded up using the Parallel Toolbox on a 4 core Intel® Core™ i5-3570 processor (3.40 GHz, 8 GB of RAM). Once the optimal lumping matrix $M$ was obtained, the ODEs for the reduced model were determined using Eqs. 6 and 7 and the Symbolic Math Toolbox in MATLAB.

The way a PBPK model is reduced is specific to the aim of the subsequent modelling exercise. For MVG, the objective was to have a simplified model that would be able to predict the response in plasma and the brain (target site of the drug) similarly to the WBPBPK model. If no constraints are imposed, any state could be grouped with any other as the lumping method groups states purely based on kinetics (8). Hence, the brain was forced to remain unlumped, and the blood compartments were allowed to be lumped only together as well as with the lungs since these compartments are in series (Fig. 1). Similarly, the liver compartment (elimination site) was forced to remain unlumped and the splanchnic organs (SP, PA, ST and GU) were allowed to be grouped only together to maintain a clear anatomical interpretation. This also allows the incorporation of a mechanistic absorption model to predict pharmacokinetics after oral administration of MVG as it will be done later in this study.

**Bayesian Analysis of MVG Clinical Data Using the Reduced Model**

**Data**

The simplified PBPK model for MVG was optimised based on data from a Phase-I clinical study in healthy adult subjects. The design of the study and characteristics of the subjects enrolled have been described in (13). Briefly, plasma concentration-time data were collected from 120 healthy volunteers after a single 10-min IV infusion of 25, 37.5 and 50 mg of MVG. The subjects were young (median age of 31 years) Caucasian (62%) males (87%) with median body weight (BW) of 83 kg and median body mass index of 27 kg/m². The study was conducted according to the ethical principles of the Declaration of Helsinki, and the protocol was approved by the Independent Ethics Committee for the study centre. Participants were males and nonpregnant females, and all provided full written informed consent prior to inclusion in the study.

**Structural Identifiability of the Reduced Model**

It has been shown that a generic WBPBPK model, with well-stirred compartments and one elimination site, is globally structurally identifiable even when only blood/plasma concentrations are measured (14). This is because for each tissue compartment, the influx rate $k_{in,T}$ is known a priori as it is defined as $k_{in,T} = Q_T/V_{VEN/ART}$ (see Eq. 1) and $Q_T$ and $V_{VEN/ART}$ are assumed known. This is true whether each tissue sub-system is parameterised in terms of efflux rate $k_{out,T}$ (Eq. 9) or in terms of blood flow $Q_T$, volume $V_T$ and partition coefficient $K_{b,T}$ as it is assumed that $K_{b,T}$ is the only unknown variable.

$$k_{out,T} = Q_T/V_T K_{b,T}$$

In general, any PBPK model based on the well-perfused mammillary model should be globally identifiable if the influx rates are known and only the efflux rates are estimated. Using proper lumping, the transfer rate constants for the reduced model, obtained by extracting the matrix $\hat{K}$ (Eq. 7), can be directly related to the blood flows, volumes and partition coefficients for the organ/tissue compartments of the original WBPBPK model. Thus, for simulation, the model can still be parameterised in terms of the original model parameters. However, the efflux rates for the lumped compartments are functions of the blood flows, volumes and partition coefficients of all the tissue compartments that were merged together (shown in the ‘RESULTS’) and are therefore functions of more than one unknown variables (partition coefficients). Hence, for the reduced model to be globally structurally identifiable during its optimisation, the lumped compartments have to be parameterised in terms of efflux rates. The unlumped compartments however, can still be parameterised in terms of the original model parameters.

**Statistical Model**

MVG plasma data were analysed using a three-stage hierarchical model to account for both population variability and uncertainty in the reduced PBPK model parameters. The same statistical model was used and described in detail in our previous analysis with the WBPBPK model (9). Briefly, we assumed at the first stage a log-normally distributed residual error (mean zero, variance $\sigma^2$) to account for model misspecification, unaccounted intra-individual variability in the parameters and measurement error. At the second stage, the model parameters were assumed to follow a multivariate log-normal distribution in the population. At the third stage, assumptions on the prior distribution of both the population and individual parameters were made, i.e. a multivariate log-normal distribution for the population median parameters and an inverse-Wishart distribution for the variability variance-covariance matrix (diagonal elements denoted $\sigma^2$).

**Definition of Priors**

The prior distributions of the WBPBPK model parameters (9) were used to construct priors for the reduced model parameters. When fitting a complex mechanistic model to data, providing prior information on the parameters is important to reduce numerical instabilities and impose biological/physiological constraints on the parameter estimates. For the compartments that remained unlumped, the marginal prior distributions of the $K_{b,T}$ (and of $CL_{int,L1}$ for the liver) could be readily used. However, additional calculations were required to construct the marginal prior distributions of the efflux rates for the lumped compartments. While the means of the distributions could be directly calculated using the relationship between the efflux rates
and the original model parameters, calculation of the variances required applying the delta method, which is a technique for approximating the moments of functions of random variables (15), as it will be shown in the RESULTS.

In our previous study, although we had no prior preclinical information on the variability in the WBPBPK drug-specific parameters, we could accurately estimate from the data the variability in $CL_{\text{int, L1}}$ using an uninformative prior (9). However, we did not attempt to quantify the variability in the other 14 parameters (partition coefficients) in order to decrease the computational burden. In the present study, we performed a first analysis run under similar conditions, i.e. with a random effect only on $CL_{\text{int, L1}}$. Depending on how much the model and thus the number of parameters could be reduced, we then ran the analysis again with random effects on all parameters. This was to check the impact of the statistical model on the stability of the Bayesian analysis. In each case, an uninformative prior distribution was assigned to the variance-covariance matrix by setting the degrees of freedom for the inverse-Wishart distribution equal to the size of the matrix (16).

**Sensitivity Analysis of the Model**

A sensitivity analysis of the reduced PBPK model was performed to identify the parameters that have a significant influence on the plasma response and are thus expected to be updated by the clinical data. To account for uncertainty in the parameter values, 1000 sets of parameters were randomly drawn from the multivariate log-normal prior distribution. The sensitivity of the model to each parameter was then assessed by calculating and plotting over time a relative sensitivity coefficient as explained in (9).

**MCMC Simulation**

The posterior distribution of the parameters was summarised using random draws by Gibbs sampling as implemented in NONMEM 7.3.0 (ICON Development Solutions, Hanover, Maryland, USA). Three independent Markov chains were run in parallel for one million iterations, starting at different diffuse parameter values. Computations were performed on a medium-sized cluster running Red Hat Enterprise Linux 6.5 on nodes with Intel Xeon E5-2670v2 CPUs with some older nodes running with dual Xeon X5670 CPUs. Each chain was parallelized on 12 different nodes. The nodes are equipped with between 24 and 96 GB of RAM and are interconnected via dedicated Bonded 1 Gbit network cards. Convergence of the posterior distribution to approximate equilibrium was monitored using the potential scale reduction statistic proposed by Gelman and Rubin ($\hat{R}$) (17), as well as by visual inspection of the Markov chains. $\hat{R}$ values were calculated using the software package CODA (18). The reduced ODE system was solved using the LSODA solver as implemented in the ADVAN13 NONMEM subroutine.

**Scaling the Reduced PBPK Model from Adults to Children**

In our previous study, the WBPBPK model could extrapolate reasonably well MVG oral pharmacokinetics from adults to children (9). This was done in three steps. Firstly, the WBPBPK model was fitted to IV adult data by estimating only the most influential parameters, as explained in the introduction. Secondly, to be able to predict pharmacokinetics after oral administration of the drug in adults, a mechanistic absorption model incorporating system-specific (e.g. gastric and small intestine transit times), drug-specific (e.g. solubility) and formulation-specific (e.g. dissolution constant) parameters was implemented. The reader is referred to (9) for details on the structure of the model as well as on the distribution of the absorption parameters. Thirdly, the full model was scaled from adult to children and used for Monte Carlo simulation. The predictive performance of the model for children was visually evaluated using data from a Phase-I clinical study of a single 15-mg dose of MVG administered to 21 subjects aged from 3 to 11 years (9).

In the current study, the same three steps were followed to evaluate the ability of the reduced model to predict MVG oral pharmacokinetics in children. Although at the third step, the reduced model predictions were checked not only against the paediatric data but also against the WBPBPK model predictions. For both the reduced and whole-body model, the Monte Carlo simulation were performed exactly as in (9) using MATLAB. Since the liver compartment was forced to remain unlumped during the reduction process, the absorption model could be integrated in the reduced PBPK model as for the WBPBPK model.

A generic WBPBPK model can be easily scaled from adults to children by incorporating in the model the age-related changes in the physiological parameters (i.e. organ/tissue volumes and blood flows) as well as in some drug-specific parameters such as the intrinsic clearance and the fraction unbound in plasma, as it was done for MVG in our previous study (9). The same can be done for a formally reduced model as the parameters retain mechanistic meanings. Yet, the efflux rates for the lumped compartments are a mix of physiological and drug-specific parameters and are therefore also expected to vary with age. It is thus not sufficient to scale the model from adult to children by only accounting for the age-related changes in the physiological parameters, but the efflux rates should also be scaled, especially if most compartments are pseudo-states resulting from lumping. One approach to scale efflux rates across age groups is to use the following allometric scaling relationship (19):

$$k_i = k_{\text{std}} \left( \frac{BW_i}{70} \right)^{-0.25}$$

(10)

where $k_i$ is the efflux rate for the $i$th individual with body weight $BW_i$ and $k_{\text{std}}$ is the efflux rate for a standard individual in the population. Hence, the approach that we propose to scale a formally reduced PBPK model across age groups involves both what we call ‘physiological scaling’ and allometric scaling. The method used for physiological scaling is described in detail in (9).

**RESULTS**

**Reduction of the WBPBPK Model for MVG**

To account for parameter uncertainty during the order reduction of the WBPBPK model for MVG, the Bayesian objective function BOF (Eq. 8) implemented in the lumping
algorithm that we used was minimised based on 1000 parameter sets drawn by Latin hypercube sampling from the prior distribution of the parameters. Calculation of the objective function value for each of the 1000 samples is computationally intensive as it requires solving the original ODE system 1000 times. Nevertheless, parallelising the optimisation on 4 cores helped to reduce the run time to less than an hour.

The lumping schemes determined for reduction of the 16 original states to 7 and 6 states are shown in Table 1, columns 2 and 3, respectively. For both schemes, the blood compartments were lumped together with the lungs to form a central compartment from which plasma concentrations are derived. Figure 2 shows plots of the reduced model predictions versus the original model predictions in most lumped states are reasonably consistent especially in the terminal phase of the profile (Fig. 2b). As a consequence, the system reduced to seven states was selected as a simplified PBPK model for MVG. The structure of the model is depicted in Fig. 4. The matrix of transfer rates for this model was calculated using Eq. 7 and can be expressed as follows:

\[
\mathbf{K} = \begin{pmatrix}
-k_{\text{out, LUM}} & Q_{\text{BR}}/(K_{b, \text{BR}} \cdot V_{\text{BR}}) & Q_{\text{M}}/(K_{b, \text{M}} \cdot V_{\text{M}}) & Q_{\text{AD}}/(K_{b, \text{AD}} \cdot V_{\text{AD}}) & 0 & Q_{\text{LI}}/(K_{b, \text{LI}} \cdot V_{\text{LI}}) \\
Q_{\text{LUM}}/V_{\text{CEN}} & -k_{\text{out, LUM}} & 0 & 0 & 0 & 0 \\
Q_{\text{BR}}/V_{\text{CEN}} & 0 & -Q_{\text{BR}}/(K_{b, \text{BR}} \cdot V_{\text{BR}}) & 0 & 0 & 0 \\
Q_{\text{M}}/V_{\text{CEN}} & 0 & 0 & -Q_{\text{M}}/(K_{b, \text{M}} \cdot V_{\text{M}}) & 0 & 0 \\
Q_{\text{AD}}/V_{\text{CEN}} & 0 & 0 & 0 & -Q_{\text{AD}}/(K_{b, \text{AD}} \cdot V_{\text{AD}}) & 0 \\
Q_{\text{SPL}}/V_{\text{CEN}} & 0 & 0 & 0 & 0 & -k_{\text{out, SPL}} \\
Q_{\text{HA}}/V_{\text{CEN}} & 0 & 0 & 0 & 0 & 0 \\
\end{pmatrix}
\]

with

\[V_{\text{CEN}} = 3 \cdot V_{\text{ART}}\]

\[Q_{\text{LUM}} = Q_{\text{HT}} + Q_{\text{SK}} + Q_{\text{KI}} + Q_{\text{BO}} + Q_{\text{RB}}\]

\[Q_{\text{SPL}} = Q_{\text{SP}} + Q_{\text{PA}} + Q_{\text{ST}} + Q_{\text{GU}}\]

\[Q_{\text{HA}} = Q_{\text{LI}} \cdot Q_{\text{SPL}}\]

\[k_{\text{out, LUM}} = Q_{\text{HT}}/(5 \cdot K_{b, \text{HT}} \cdot V_{\text{HT}}) + Q_{\text{SK}}/(5 \cdot K_{b, \text{SK}} \cdot V_{\text{SK}}) + Q_{\text{KI}}/(5 \cdot K_{b, \text{KI}} \cdot V_{\text{KI}}) + Q_{\text{BO}}/(5 \cdot K_{b, \text{BO}} \cdot V_{\text{BO}}) + Q_{\text{RB}}/(5 \cdot K_{b, \text{RB}} \cdot V_{\text{RB}})\]

\[k_{\text{out, SPL}} = Q_{\text{SP}}/(4 \cdot K_{b, \text{SP}} \cdot V_{\text{SP}}) + Q_{\text{PA}}/(4 \cdot K_{b, \text{PA}} \cdot V_{\text{PA}}) + Q_{\text{ST}}/(4 \cdot K_{b, \text{ST}} \cdot V_{\text{ST}}) + Q_{\text{GU}}/(4 \cdot K_{b, \text{GU}} \cdot V_{\text{GU}})\]

where \(f_{ub}\) is the fraction unbound in blood calculated as the ratio of the fraction unbound in plasma to the blood-to-plasma ratio, \(V_{\text{CEN}}\) is the volume (l) for the CEN compartment (from which plasma concentrations are derived), \(k_{\text{out, LUM}}\) and \(Q_{\text{LUM}}\) are the efflux rate (h\(^{-1}\)) and blood flow (l/h), respectively, for the LUM compartment, \(Q_{\text{HA}}\) is the blood flow (l/h) from the hepatic artery, and \(k_{\text{out, SPL}}\) and \(Q_{\text{SPL}}\) are the efflux rate (h\(^{-1}\)) and blood flow (l/h), respectively, for the SPL compartment (see Fig. 4 and Table 1 for definition of the lumped compartments). It should be stressed that the relationships in Eqs. 11 and 12 are direct results from the matrix operation in Eq. 7.

**Bayesian Analysis of MVG Clinical Data Using the Reduced Model**

**Structural Identifiability of the Reduced Model**

In the WBPBPK model for MVG, the unknown variables are the CL\(_{\text{out, LI}}\) and \(K_{b, \text{LI}}\). In the reduced model, the efflux rates for the LUM and SPL compartments are thus functions of more than one unknown variable (Eq. 12) meaning that for the model to be structurally identifiable, it cannot be fully parameterised in
terms of the WBPBPK model parameters but has to be expressed as in Eq. 11. Hence, by reducing the system from 16 to 7 states, the number of parameters is reduced from 15 to 7 and the parameters are $C_{int, L1}$, $k_{out, LUM}$, $K_{bh}$, $BR$, $K_{bh, MO}$, $K_{bh, AD}$, $k_{out, SPL}$, and $K_{bh, L1}$. BW was included as a covariate for the efflux rates $k_{out, LUM}$ and $k_{out, SPL}$ as in Eq. 10, in order to explain part of the population variability in these parameters and to further scale them from adults to children using allometry as explained in the ‘METHODS’.

### Priors

The prior distribution of the reduced PBPK model parameters is presented in the first column of Table II. For $k_{out, LUM}$ and $k_{out, SPL}$, the means of the marginal distributions were simply calculated by using the relationships in Eq. 12, and the blood flow and volume values as well as the prior means of the partition coefficients reported in (9). To calculate the variances, the following relationships were determined by applying the delta method:

\[
\begin{align*}
\text{var}(k_{out, LUM}) &= CV^2 \left( \frac{Q_{HT}}{5 \cdot K_{bh, HT} \cdot V_{HT}} \right)^2 + \left( \frac{Q_{SK}}{5 \cdot K_{bh, SK} \cdot V_{SK}} \right)^2 + \left( \frac{Q_{KI}}{5 \cdot K_{bh, KI} \cdot V_{KI}} \right)^2 + \left( \frac{Q_{BO}}{5 \cdot K_{bh, BO} \cdot V_{BO}} \right)^2 + \left( \frac{Q_{RB}}{5 \cdot K_{bh, RB} \cdot V_{RB}} \right)^2 \\
\text{var}(k_{out, SPL}) &= CV^2 \left( \frac{Q_{SP}}{4 \cdot K_{bh, SP} \cdot V_{SP}} \right)^2 + \left( \frac{Q_{PA}}{4 \cdot K_{bh, PA} \cdot V_{PA}} \right)^2 + \left( \frac{Q_{ST}}{4 \cdot K_{bh, ST} \cdot V_{ST}} \right)^2 + \left( \frac{Q_{GU}}{4 \cdot K_{bh, GU} \cdot V_{GU}} \right)^2 
\end{align*}
\]

(13)

It should be noted that the relationships in Eq. 13 could be simplified because we assumed in our previous Bayesian analysis the same coefficient of variation CV for all $K_{bh}$. *$	extbf{Sensitivity Analysis of the Model}$*

The results of the sensitivity analysis for the reduced model shown in Fig. 5 suggest that the model is sensitive to all parameters except to $k_{out, SPL}$ and $K_{bh, LI}$ that seem to have a negligible influence on the response in the central compartment and thus on the plasma response. Uncertainties in these

---

Fig. 2. Drug amount in the lumped states predicted with a reduced system of size 7 (a) and size 6 (b) plotted against the original system predictions. The simulation was performed every 0.1 h for 48 h using a 50-mg IV dose (used in the clinical study) for 1000 parameter sets sampled from the prior distribution. The subtitles refer to the state numbers of the original system (see Fig. 1; Table I)

Fig. 3. Plots of the confidence intervals (90%) around MVG median plasma concentration-time profiles after an IV dose of 50 mg. Confidence intervals produced with the original WBPBPK model and with the seven-state reduced model are represented by the solid black lines and solid red lines (superimposed), respectively, whereas the intervals are shown by the dashed blue lines for the six-state reduced model. The simulation was performed every 0.1 h for 48 h, for 1000 parameter sets sampled from the prior distribution. Plasma concentrations were derived from the amount of drug in the venous blood compartment for the original system and in the central compartment for the reduced systems.
parameters are therefore not expected to be reduced by the clinical data. On the other hand, as for the WBPBPK model, $\text{CL}_{\text{int}}, \text{LJ}, \text{Kb}_{\text{MU}}$ and $\text{Kb}_{\text{AD}}$ are the parameters that have the main influence on the plasma response and that might have their marginal distributions updated by the data.

**MCMC Simulation**

The Bayesian analysis of MVG clinical data with the reduced PBPK model was run with two different statistical models: in the first, referred to as ‘STATMOD 1’, the effect of inter-individual variability was estimated only on $\text{CL}_{\text{int}}, \text{LI}$ and was fixed to a small value (corresponding to a variability CV of 1% for a log-normally distributed variable) for the other parameters; in the second, referred to as ‘STATMOD 2’, we estimated random effects on all seven parameters. For both statistical models, it took on average less than 3 days to run one million iterations on the cluster described in the ‘METHODS’ and by parallelising each Markov chain on 12 different nodes. The total run time was only few hours longer for STATMOD 2 than for STATMOD 1. The posterior distribution of the parameters is summarised in the supplemental online (Table I) for STATMOD 1 and in Table II for STATMOD 2 and was generated by pooling the one million samples from the three Markov chains. Using both models, the posterior distribution of the population and individual parameters converged to approximate equilibrium. However, as indicated by the $\hat{R}$ statistic, convergence was achieved after 360,000 iterations for STATMOD 1 versus 8000 iterations for STATMOD 2. In addition, the trace plots of the Markov chains suggest a much slower mixing of the chains for STATMOD 1 (Fig. 1 in the Supplemental online material) than for STATMOD 2 (Fig. 6). Slow mixing of Markov chains suggests that the MCMC samples are highly autocorrelated.

### Table II. Prior and Posterior Distributions of the Drug-Specific Parameter Values for MVG-Reduced PBPK Model (STATMOD 2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prior distribution</th>
<th>Posterior distribution</th>
<th>$\hat{R}$</th>
<th>Iterations ($\times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CL}_{\text{int}}, \text{LJ}$ (l/h)</td>
<td>2017 (1.30)</td>
<td>1650 (1.04)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$k_{\text{out}, \text{LUM}}$ (h$^{-1}$)</td>
<td>19.72 (1.22)</td>
<td>7.38 (1.10)</td>
<td>1.01</td>
<td>1.00</td>
</tr>
<tr>
<td>$\text{Kb}_{\text{BR}}$</td>
<td>3.04 (1.35)</td>
<td>4.79 (1.22)</td>
<td>1.19</td>
<td>1.06</td>
</tr>
<tr>
<td>$\text{Kb}_{\text{MU}}$</td>
<td>1.38 (1.35)</td>
<td>2.04 (1.09)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\text{Kb}_{\text{AD}}$</td>
<td>7.43 (1.35)</td>
<td>9.49 (1.06)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$k_{\text{out}, \text{SPL}}$ (h$^{-1}$)</td>
<td>26.13 (1.17)</td>
<td>17.5 (1.44)</td>
<td>1.33</td>
<td>1.06</td>
</tr>
<tr>
<td>$\text{Kb}_{\text{LI}}$</td>
<td>5.82 (1.35)</td>
<td>9.75 (1.28)</td>
<td>1.18</td>
<td>1.09</td>
</tr>
<tr>
<td>$\alpha^2_{\text{CL}_{\text{int}}, \text{LJ}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.186 (0.0260)</td>
<td>1.03</td>
<td>1.00</td>
</tr>
<tr>
<td>$\alpha^2_{\text{kout}, \text{LUM}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.214 (0.0566)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\alpha^2_{\text{Kb}, \text{BR}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.0102 (0.0133)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\alpha^2_{\text{Kb}, \text{MU}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.513 (0.109)</td>
<td>1.01</td>
<td>1.00</td>
</tr>
<tr>
<td>$\alpha^2_{\text{Kb}, \text{AD}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.119 (0.0327)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\alpha^2_{\text{kout}, \text{SPL}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.0109 (0.0215)</td>
<td>1.10</td>
<td>1.02</td>
</tr>
<tr>
<td>$\alpha^2_{\text{Kb}, \text{LI}}$</td>
<td>0.1 ($v=1$)</td>
<td>0.0114 (0.0178)</td>
<td>1.06</td>
<td>1.03</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>–</td>
<td>0.0448 (0.0017)</td>
<td>1.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

$\hat{R}$ is the potential scale reduction statistic. No prior information was considered for the residual variance $\sigma^2$. Prior distribution was assumed multivariate log-normal for the population median parameters and inverse-Wishart for the variances. For the population median parameters, both prior and posterior marginal distributions are expressed as geometric mean (geometric standard deviation). For the variances ($\alpha^2$), prior marginal distributions are expressed as expected value (degrees of freedom of the inverse-Wishart distribution $v$) and posterior marginal distributions as arithmetic mean (standard deviation).
within each chain, which increases the uncertainty of the estimation of posterior quantities of interest (e.g., means, variances or quantiles). The degree of autocorrelation can be measured by the effective sample size of the sequence of interest. Using the CODA software package, we estimated for each model parameter the effective sample size of the posterior sequences obtained with STATMOD 1 and STATMOD 2 (Table II in the Supplemental online material). The smaller the effective sample size estimate, the higher autocorrelation between the MCMC samples and the higher uncertainty in the posterior quantities. The estimates suggest that the MCMC samples for $K_{b, BR}$, $k_{out, SPL}$ and $K_{b, LI}$ are highly autocorrelated for STATMOD 1 whereas there are not so much for STATMOD 2 which was thus considered as numerically more stable. Finally, the estimates of the residual variance suggest that STATMOD 2 provides a better description of individual profiles than STATMOD 1. We therefore focus on STATMOD 2 in the following paragraph.

Monitoring the $\hat{R}$ statistic during the MCMC simulation (Table II) suggested that most marginal distributions seemed to have reached equilibrium after 2000 iterations except for $K_{b, BR}$, $k_{out, SPL}$, $K_{b, LI}$ and $\omega^2_{K_{b, BR}}$ for which additional sampling was required. The marginal posterior and prior distributions of the population median parameters are compared in Table II (see also Fig. 2 of the Supplemental online material). All distributions were updated by the plasma data. The smallest deviation from the prior mean was for $CL_{int, LI}$ (18%) whereas the most notable change was for $K_{b, LI}$ (68%). However, uncertainty in the parameter value was only slightly decreased for $K_{b, LI}$ (from 30% to 25% CV) and was even increased for $k_{out, SPL}$ (from 17% to 36% CV). The estimates of the inter-individual variances suggest that $K_{b, MU}$ is the most variable parameter in the population (CV of 72%) and that the data had no valuable information for the variability in $K_{b, BR}$, $k_{out, SPL}$ and $K_{b, LI}$ (uncertainty CV of 130%, 197% and 156% for $\omega^2_{K_{b, BR}}$, $\omega^2_{k_{out, SPL}}$ and $\omega^2_{K_{b, LI}}$, respectively). As seen in Fig. 7, the population model seems to provide a good description of both the median trend and the variability in the analysed plasma concentration-time data.

**Scaling the Reduced PBPK Model from Adults to Children**

Prior to scaling the reduced PBPK model from adults to children, we checked that the reduced model together with the mechanistic absorption model could predict adequately MVG oral pharmacokinetics in adults (results not presented) as it was done for the WBPBPK model in (9). We then applied physiological scaling to the organ/tissue volumes and...
blood flows, the $CL_{int,LI}$, the fraction unbound in plasma and the volume of fluid in the small intestine (absorption parameter), and allometric scaling to the efflux rates $k_{out,LUM}$ and $k_{out,SPL}$ (Eq. 9). For the purpose of comparing the performance of the reduced model with that of the whole-body model in extrapolating MVG pharmacokinetics from adults to children, STATMOD 1 was used for the Monte Carlo simulation with the reduced model as it was also used in our previous simulation with the WBPBPK model (9). The results of the simulation are shown in Fig. 8. The scaled reduced model appears to be consistent with the scaled WBPBPK model. Both models slightly over-predict the median trend in the data in the first five hours post-dose. As explained in our previous study (9), this is likely due to unaccounted age-related changes in the absorption parameters which are the same between the reduced and the original model. Note that the predictions made with the reduced and the whole-body model are not expected to be identical simply because the reduced model is an approximation of the whole-body model. Hence, it is not surprising that analysing the same data yields slightly different posterior estimates of the population median and variance parameters and thus provides different prediction intervals. It should also be noted that the simulation was performed by simply using the posterior means of the population and individual parameters and of the residual error. Although simulating with uncertainty, by sampling the parameters from the posterior distribution, can be of value in drug development, e.g. to select a safe dosing regimen prior to a clinical study, it was deemed not critical in evaluating the predictive performance of the reduced model.

**DISCUSSION**

Bayesian population physiological modelling represents a useful translational approach to bridge preclinical and clinical pharmacokinetic data during drug development. Among PBPK models, whole-body models are especially suited for between- or within-species extrapolation as all the model parameters (system- and drug-specific) have clear mechanistic meanings. However, fitting a WBPBPK model to sparse and noisy clinical data is challenging due to the high number of parameters and due to the difficulty of defining priors that are informative enough given the complexity of the model. For instance, the WBPBPK model previously developed for MVG appeared to be numerically unidentifiable when analysing dense clinical data, possibly because of severe correlations between parameters and of too diffuse priors given the dimension of the system (9). One approach to reduce the number of parameters and make the model numerically identifiable is to fix the parameters that have a negligible influence on the plasma response to values a priori.
known from preclinical experiments (9). This approach has the problem of possibly producing biased estimates and underestimating the uncertainty in the system. Using MVG as an example, we therefore evaluated in the present study an alternative approach that is to formally reduce a WPBPK model and its number of parameters, and use the reduced model for the Bayesian data analysis. Finally, we proposed an approach to scale reduced PBPK models across age groups so that the model can still be used for extrapolation.

The WBPBPK model for MVG could be reduced from 16 to 7 states while maintaining the main features of the model, i.e. kinetics in the plasma (Fig. 3) and the brain (Fig. 2a). The fact that lumping the adipose tissue with the muscle tissue (six-state model) significantly affects the plasma kinetics (Fig. 3) is likely due to the extensive distribution of MVG into the adipose tissue which equilibrates much slower than the muscle tissue (see Fig. 3 in the Supplemental online material). Using the proper lumping algorithm proposed by Dokoumetzidis and Aarons (8), we could easily give emphasis to specific tissues (e.g. brain and liver) and organs spaces (e.g. mixed blood) and avoid combinations that have no anatomical meaning (e.g. splanchnic organs lumped with the brain). Thereby, we retained the mammillary structure of the model (Fig. 4) which offered the advantage of facilitating the determination of the structural identifiability of the model as explained in the ‘METHODS’. If the mammillary structure of the model is perturbed, a formal structural identifiability analysis should be performed. Another advantage was that a mechanistic absorption model could be directly integrated and can be used to investigate the bioavailability of the drug (first-pass effect) or even the impact of drug-drug interactions (e.g. inhibition of the hepatic metabolic clearance). Nevertheless, incorporating a drug-drug interaction mechanism in the model often means incorporating a nonlinear mechanism. Depending

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**Fig. 7.** A visual predictive check of the ability of the population reduced PBPK model (STATMOD 2) to describe MVG plasma data after IV administration in adult subjects. *Open circles* are observed concentrations plotted across time, the *solid red line* is the median of the simulated concentrations and the *grey area* represents a 90% prediction interval. Both observed and predicted concentrations were dose-normalised. The insert expands the first 2 h of the concentration-time data plotted on linear scales.

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**Fig. 8.** A visual evaluation of the ability of the scaled PBPK model to predict MVG pharmacokinetics after oral administration of a 15-mg dose in children aged from 3 to 11 years. *Open circles* are observed concentrations plotted across time, the *dashed red lines* are the 5th, 50th and 95th percentiles of the concentrations simulated with the WBPBPK model from Wendling et al. (9), the *solid blue lines* are the 5th, 50th and 95th percentiles of the concentrations simulated with the reduced PBPK model (STATMOD 1), and the *horizontal dotted line* represents the lower limit of quantification of the assay.
on the experimental conditions and on the characteristics of the drug, this can significantly affect kinetics in the tissues and hence the validity of the lumping scheme. If the model is meant to be used to predict the impact of drug-drug interactions as was done in our previous analysis with the WBPBPK model (9), we strongly recommend that the inhibition/induction mechanism is firstly incorporated in the model before it is reduced. A difficulty may arise in extracting the ODEs as the system would be nonlinear and Eq. 7 would not be valid anymore, but for simulation purpose, the ODEs of the reduced system are not needed as the system can be derived using Eq. 5.

The lumping algorithm appeared to be suitable for a Bayesian PBPK modelling framework as it takes into account the prior distribution of the parameters. Thereby, the lumping scheme is robust to variation in parameter values due to uncertainty that arises from the preclinical experiments and extrapolation methods. In addition, the parameters of the reduced model retain a clear physiological interpretation (Eqs. 11 and 12). Hence, we could easily propagate the prior information on the system- and drug-specific parameters of the WBPBPK model to all the parameters of the reduced model including the more empirical efflux rates for the lumped compartments. This was deemed important to stabilise the MCMC analysis and produce biologically/physiologically plausible posterior values.

As opposed to the MCMC analysis with the WBPBPK model (when estimating all drug-specific parameters), convergence of the posterior parameter distribution could be achieved with the reduced PBPK model (with both STATMOD 1 and STATMOD 2) without having to fix population median parameters. However, due to highly autocorrelated MCMC samples, intensive Gibbs sampling (360,000 samples) was required to reach approximate equilibrium when the statistical model was limited to a random effect on CLint, LI (STATMOD 1) as in our previous WBPBPK analysis (9). In population modelling, it is desirable to assign random effects to all model parameters to learn from the data about variability in an objective manner. Since the number of parameters was significantly reduced from 15 to 7, we could also assess a full statistical model (STATMOD 2) without having to fit a covariate model if the variance estimates are thought to be physiologically unrealistic.

Using proper lumping, the biological/physiological interpretation of the parameters is retained in the reduced model. In other words, the model can be expressed using the parameters of the original model (see Eqs. 11 and 12). Hence, scaling across age groups can easily be done by incorporation of the age-related anatomical and physiological changes in the subjects. A slight difficulty arises when the model is fitted to data prior to being used for extrapolation, as the rate equations for the lumped states have to be expressed using the more empirical efflux rate parameters (Eq. 11).

Nevertheless, it is common to scale a parameter describing a transfer rate using the allometric relationship expressed in Eq. 10. However, there is evidence that body height is a better explanatory variable for the population variability in the blood flows and volumes than BW (24). Particular attention to physiologic variability, as well as to parameter uncertainty, should be paid if the model were to be used for exploratory simulations to determine for instance a dosing regimen that best fits efficacy or safety requirements. In that regard, it is informative to quantify the variability in all drug-specific parameters (using a ‘full’ statistical model) in order to potentially refine the covariate model if the variance estimates are thought to be physiologically unrealistic.

parameters $CL_{int}$, $L_{I1}$, $k_{out}$, $LUM$, $K_b$, $BR$, $K_b$, $MU$, $K_b$, $AD$ and $K_b$, $L_{I1}$ than our prior preclinical estimates. The uncertainty in the parameter values was especially reduced for $CL_{int}$, $L_{I1}$, $k_{out}$, $LUM$, $K_b$, $MU$ and $K_b$, $AD$ for which convergence of the marginal distributions was achieved after only 2000 iterations (Table I). In addition, population variability in these four parameters could be accurately estimated in contrast to the variability in $K_b$, $BR$, $k_{out}$, $SPL$ and $K_b$, $L_{I1}$. This suggests that the data had information mainly for $CL_{int}$, $L_{I1}$, $k_{out}$, $LUM$, $K_b$, $MU$ and $K_b$, $AD$ which is consistent with the results of the sensitivity analysis (see Fig. 5). No clear explanation could be given for the increase in uncertainty around the population median of $k_{out}$, $LUM$ although it might be due to numerical issues specific to the MCMC algorithm implemented in NONMEM. The variability estimate for $k_{out}$, $LUM$ (CV of 46%) suggests that the allometric relationship in Eq. 10 helps to explain only partially the overall variability in this parameter which is probably due to variation in the volumes and blood flows of the HT, SK, KI, BO and RB compartments. Also, the high population variability estimate for $K_b$, $MU$ is likely explained by unaccounted variability in the organ/tissue volumes and blood flows (21) perhaps because BW is not the most appropriate covariate for the volumes and blood flows. We used BW for simplicity, based on (22, 23).

Using proper lumping, the biological/physiological interpretation of the parameters is retained in the reduced model. In other words, the model can be expressed using the parameters of the original model (see Eqs. 11 and 12). Hence, scaling across age groups can easily be done by incorporation of the age-related anatomical and physiological changes in the subjects. A slight difficulty arises when the model is fitted to data prior to being used for extrapolation, as the rate equations for the lumped states have to be expressed using the more empirical efflux rate parameters (Eq. 11). Nevertheless, it is common to scale a parameter describing a transfer rate using the allometric relationship expressed in Eq. 10. Hence, a formally reduced PBPK model can be scaled across age groups as a WBPBPK model (changes in blood flows, volumes, intrinsic clearance, fraction unbound in plasma etc.) with additional allometric scaling of the efflux rates for the lumped states. For MVG, the extrapolation workflows with the WBPBPK model (9) and with the reduced PBPK model performed similarly (Fig. 8). Yet, the more the model is reduced, the less mechanistic it is and the more allometric scaling predominates. A major limit of allometric scaling is that it fails to account for age-related maturity changes in active processes such as enzymatic elimination or transporter-mediated efflux. Incorporating maturity differences in a PBPK model is critical to predict pharmacokinetics in young children (25). If appropriate constraints are imposed
during the lumping process, emphasis can be given to active processes so that maturity and physiological differences can still be incorporated when scaling the model. The same method could be applied to between-species scaling as anatomical and physiological changes over organisms can be found in the literature. Although, a more robust method would be to account for the distribution of the parameter over different animal species during the lumping process (8). This also applies to within-species scaling like between age groups as it was done in the present study. However, defining the prior distribution of the parameters over different species or age groups can be difficult as the parameter values follow multimodal distributions rather than log-normal distributions.

The Bayesian workflow proposed in this study (see ‘METHODS’) could be applied to any complex mechanistic pharmacokinetic and pharmacodynamic model. The choice of reducing the system depends on its complexity and on the purpose of the subsequent simulation as the ability of a reduced model to extrapolate under different scenarios is limited compared to a fully mechanistic model (e.g. prediction of drug-drug interactions with a PBPK model). The difficulty with pharmacodynamic models is that the systems are often nonlinear due to feedback mechanisms. Hence, extraction of the ODEs to be able to fit the reduced model to data is not trivial. Also, determining the structural identifiability of the simplified model requires a formal identifiability analysis. Yet, such an approach has been recently successfully applied to the coagulation network in human, although a frequentist analysis was performed to further model fibrinogen concentration-time data following brown snake envenomation (26). The choice of reducing a model also depends on the amount of prior information available on the structural parameters as well as on the variability terms. Jonsson et al. have shown an example of the impact of the informativeness of the priors for population variances on the stability of the Bayesian analysis of a mechanistic pharmacokinetic/pharmacodynamic model (3). Recently, Garcia et al. have also shown that increasing the informativeness of the priors for variability terms can help a statistical PBPK model to be identifiable (4). However, in both analyses, the informativeness was increased by naively fixing the variability terms for which no priors were available to zero. If prior information is restricted to the population median parameters, it might be desirable to reduce the model to be able to quantify the variability in all parameters and thus learn more objectively about the overall variability in the system.

CONCLUSIONS

We conclude that the present study represents a first example of the practicality of formally reduced PBPK models for Bayesian inference in clinical pharmacokinetics. The Bayesian proper lumping method allowed propagation of MVG preclinical pharmacokinetic information from the original to the reduced system. Using MCMC techniques, the information could then be combined with clinical data to improve the population model without having to fit neither variability terms nor structural drug-specific parameters. Finally, the reduced yet mechanistic model could still be used for PBPK extrapolation across age groups. Nevertheless, more examples are needed to prove the advantages of such Bayesian mechanistic modelling approaches in drug development.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST Thierry Wending is an employee of Novartis Pharma AG and a Ph.D. student at the University of Manchester.

REFERENCES

Chapter 5: Predicting survival of pancreatic cancer patients treated with gemcitabine using longitudinal tumour size data

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In preparation
5.1. Abstract

5.1.1. Background

Measures derived from longitudinal tumour size data have been utilized to predict survival of patients with solid tumours. In this study, the predictive value of such measures was examined for patients with metastatic pancreatic cancer undergoing gemcitabine therapy.

5.1.2. Methods

The control data from two Phase III studies were retrospectively used to develop (271 patients) and validate (398 patients) survival models. Firstly, 31 baseline variables were screened from the training set using penalised Cox regression. Secondly, tumour shrinkage metrics were interpolated for each patient by hierarchical modelling of the tumour size time-series. Subsequently, survival models were built by applying two approaches: the first aimed at incorporating model-derived tumour size metrics in a parametric model, the second simply aimed at identifying empirical factors using Cox regression. Finally, the performance of the models in predicting patient survival was evaluated on the validation set.

5.1.3. Results

Depending on the modelling approach applied, albumin, body surface area, neutrophil, baseline tumour size and tumour shrinkage measures were identified as potential prognostic factors. The distributional assumption on survival times appeared to affect the identification of risk factors but not the ability to describe the training data. The two survival modelling approaches performed similarly in predicting the validation data.

5.1.4. Conclusions

A parametric model that incorporates model-derived tumour size metrics in addition to other baseline variables could predict reasonably well survival of patients with metastatic pancreatic cancer. However, the predictive performance was not significantly better than a simple Cox model that incorporates only baseline characteristics.
5.2. Introduction

Pancreatic cancer is a common cause of cancer-related death and is difficult to treat as diagnosis is often made late and patients present with metastatic disease [1]. Despite recent improvements in diagnostic techniques, the prognosis of patients with pancreatic cancer is poor, with a 5-year survival rate of 0.4-4% [2]. The only potential curative treatment is surgical resection although only 15-20% of patients are eligible for surgery [1]. Gemcitabine chemotherapy is the standard palliative care [3], with a median survival of 5.7 months and 20% 1-year survival rate [4]. However, the prognosis of patients receiving palliative chemotherapies varies depending on their clinical characteristics [5]. It is therefore important to identify subgroups of patients that would benefit from chemotherapies. Several prognostic factors for patients with metastatic pancreatic cancer (MPC) have been previously identified: pancreatic cancer location [6], albumin level (ALB), carbohydrate antigen 19-9 level (CA19-9), alkaline phosphatase level (ALP), lactate dehydrogenase level, white blood cell count, aspartate aminotransferase level, blood urea nitrogen level [7], long-standing diabetes [8], Eastern Cooperative Oncology Group (ECOG) performance status, C-reactive protein level [9], the status of unresectable disease, carcinoembryonic antigen level and neutrophil-lymphocyte ratio [10].

Identifying prognostic factors with predictive value for patient risk stratification is an important task in cancer research to monitor and assess clinical trials, and individualise patient care. Multivariate statistical modelling methods are commonly used to investigate survival in relation to a factor of interest (e.g. treatment exposure) while adjusting for others (e.g. genotype). The Cox proportional hazards (CPH) model [11] is the most frequently used multivariate regression method, mainly because no assumption on the distribution of survival times is required (‘semi-parametric’ method) although it relies on the key assumption that the group-specific hazards are proportional over time. A useful alternative when the proportional hazards assumption does not hold is to use the accelerated failure time (AFT) model [12] which offers the advantage of an easier interpretation of the covariate effects (directly on the survival time) as compared to the CPH model (effects on the hazard rate). Since the AFT model is fully parametric, it is also more suitable for simulations that can be of value to extrapolate patient survival data and to optimise the design of oncology clinical trials. Nevertheless, the assumption on the survival
time distribution might be deemed too strict as most of the time the distribution (or even a close approximation) is unknown.

In oncology drug development, decision making and trial design are traditionally based on the Response Evaluation Criteria In Solid Tumours (RECIST), an empirical categorical measure of antitumor activity [13,14]. More recently, several studies have shown that model-derived tumour size (TS) metrics can also be used as predictors for survival of patients with solid tumours [15-18]. In drug development, a parametric survival model that includes early change in TS as predictor variables might be beneficial for trial design and early assessment of drug efficacy. To our knowledge, this approach has not been evaluated in MPC.

The objective of this study is to apply a fully parametric drug development approach (referred to as the ‘PAR approach’) that utilises longitudinal TS data, to predict the mortality risk of patients with MPC. We used the control arm data from two Phase III MPC studies to build and validate the models as it would be done in the clinic. Early changes in TS were interpolated by hierarchical nonlinear modelling of the TS time-series and were tested as predictors for survival in an AFT model. We also compared the predictive performance between the PAR approach and a more conventional clinical approach that uses a CPH regression of empirical risk factors.

5.3. Materials and methods

5.3.1. Data

The models were built on the data of a Phase III study in which 546 patients with MPC were randomly assigned to receive either gemcitabine (n = 275) or aflibercept (n = 271) [19]. The predictive performance of the models was then evaluated using an independent (validation) data set from a Phase III study of 861 patients with MPC randomly assigned to nab-paclitaxel plus gemcitabine (n = 431) or gemcitabine (n = 430) [20]. Only gemcitabine data (control arms) were available for the present analysis. Although our work was done retrospectively, the choice of the training and validation sets was based on the chronology of the clinical studies in order to mimic the time constraints of statistical analyses in clinical research. Details of patient characteristics and the study designs can be found in the original study reports.
The primary endpoint in both studies was overall survival, defined as the time from randomisation to the time of death from any cause. We instead used as model outcome the survival time defined as the time from randomisation to the time of death from the disease. Event times and baseline clinical characteristics were available for 271 patients in the training set and for 398 patients in the validation set. Censored times were extracted from the date of the last visit (for assessment of tumour response or laboratory variables). Among the patients who died in the training set (n = 147) and in the validation set (n = 370), only 13% and 8%, respectively, died from other causes than cancer progression. In both studies, antitumor activity was evaluated every 8 weeks according to RECIST version 1.0 criteria.

5.3.2. Statistical analysis

Firstly, potential risk factors were screened among previously reported prognostic factors and patient clinical characteristics from the training set. Secondly, TS reduction metrics were interpolated at relevant times for each patient of both the training and validation set, by modelling separately the TS time-series from the two data sets. Subsequently, we developed a multivariate survival model using two different approaches: the PAR approach that aims at relating model-derived TS reduction metrics to survival time using the AFT model, and a more classic clinical approach that aims at identifying empirical risk factors using a Cox regression model (referred to as the ‘COX approach’). Finally, the performance of these two approaches in predicting patient mortality risk was compared using the validation set.

5.3.2.1. Screening risk factors

31 prognostic factors or baseline clinical characteristics for all 271 patients of the training set (128 death from disease) were screened to identify potential survival predictors: gender, race, age, body surface area (BSA), body weight, body height, ECOG status, cancer location (CLOC, defined categorically as entire pancreas or pancreas head versus pancreas tail or pancreas body), stage of cancer (from I to IV), diabetes status (yes or no), smoking status (yes or no), baseline TS, ALB, ALP, alanine transaminase, aspartate aminotransferase, bilirubin level (BILI), calcium, CA19-9, creatinine, glucose, haemoglobin, potassium, magnesium, neutrophil count
(NEUT), phosphorus, platelets, total protein, white blood cell count and sodium levels. Details on the distribution of these variables can be found in the original study report. The ALP, CA19-9 and NEUT variables were log-transformed as their distributions in the studied patients are highly skewed. 21 variables had less than 30% of missing values which were multiply imputed using complete cases of all variables including the response variable (death status) [21].

Variable selection was done using a CPH regression with the least absolute shrinkage and selection operator (LASSO) penalty [22]. Using LASSO regularization, the coefficients of the variables that are apparently not important shrink to zero, thereby producing a sparse model. Since the number of death events was small (n = 128), the algorithm was forced to retain no more than 10 variables in the sparse model. All continuous variables were standardised prior to the regression as they were not in the same units. The optimal value of the penalty tuning parameter was determined by k-fold cross-validation using the model partial-likelihood deviance as loss function. Regardless of the results, factors deemed clinically relevant (e.g. biomarker or established risk factors) were evaluated in the subsequent survival analysis aiming at quantifying the predictive value of the selected factors and of early change in TS metrics.

5.3.2.2. Modelling tumour size time-series

TS measures were derived as the sum of the longest diameters of the target lesions based on the RECIST criteria. In order to further compare empirical and model-derived early changes in TS metrics as survival predictors, only patients who had a pre-treatment and at least one on-treatment TS assessment were included in the time-series analysis (152 and 385 patients for the training and validation set, respectively). To account for random interpatient and residual variability in the data, a three-stage hierarchical model was developed. At the first stage, the model likelihood was assumed lognormally distributed. A mixed model with exponential-decay and linear-growth components was used to describe the time course of TS change, using the same nomenclature as Wang et al. [15] (Eq. 5.1).

\[
TS_i(t) = BASE_i \cdot e^{-SR_i \cdot t} + PR_i \cdot t
\]  
(5.1)
In Eq. 5.1, $TS_i(t)$ is the TS at time $t$ for the $i^{th}$ patient, $BASE_i$ is the baseline TS, $SR_i$ is the decay or tumour shrinkage rate constant and $PR_i$ is the re-growth or tumour progression rate constant. At the second stage, we assumed that each parameter follows a lognormal distribution in the patient population. For example, the distribution of the baseline TS was defined as follows:

$$BASE_i = e^{\theta_{BASE} + \eta_{BASE,i}} \quad (5.2)$$

where $\theta_{BASE}$ is the population mean of the log-transformed parameter and $\eta_{BASE,i}$ is the difference between the individual and the population mean log-transformed values that is assumed normally distributed with mean 0 and standard deviation $\omega_{BASE}$. Finally at the third stage, we assigned uninformative prior distributions to the model parameters as well as to the residual standard deviation $\sigma$ (see Stan code in Appendix A4.1).

Sampling from the posterior distribution of the parameters was done using the No-U-Turn variant of the Hamiltonian Monte Carlo algorithm [23], as implemented in the software Stan [24]. Convergence to approximate equilibrium was monitored using the potential scale reduction statistics [25]. Since the purpose of the modelling was to interpolate TS for each patient, the main diagnostic criterion for the model was its ability to capture observed individual profiles. We then used the posterior mean of the individual parameters to predict for each patient the percentage TS change from baseline (PTR) at early time points, i.e. 2, 4, 6, 8 and 10 weeks, in order to further evaluate the predictive value of these model-derived TS metrics for survival. Since the COX approach aims at investigating the predictive value of empirical factors only, we also calculated from the observed individual TS data the best percentage change from baseline within 12 weeks (PTR$_{\max}$), as a surrogate of antitumor response to gemcitabine.

5.3.2.3. Survival analysis

To be able to compare the PAR approach with the more empirical COX approach, only patients included in the TS model-based analysis of the training set and with complete cases for the screened risk factors were retained for the multivariate survival analysis. For clarity, the resulting data set is referred to as the ‘reduced-
training’ set, which includes baseline risk factors, the model-derived PTR at week 2 to 10, and the empirical PTR_{max} metric for all patients.

Since the PAR approach aims at developing a quantitative tool to further guide trial design, a parametric AFT model was used to relate baseline risk factors and model-derived PTR metrics to survival times. The lognormal and Weibull distributions were evaluated for the distribution of survival times \( T \). The model can be expressed as follows:

\[
T \sim \text{lognormal}(\mu, \sigma) \\
\text{or} \\
T \sim \text{Weibull}(\alpha, \lambda)
\]

where \( \mu \) (\(-\infty \) to \(+\infty \)) and \( \sigma \) (0 to \(+\infty \)) are the mean (location) and scale parameters, respectively, for the lognormal distribution, and \( \alpha \) (0 to \(+\infty \)) and \( \lambda \) (0 to \(+\infty \)) are the shape and scale parameters, respectively, for the Weibull distribution.

In order to parameterise the models as AFT models, the covariates effects were tested only on \( \mu \) for the lognormal model, and on \( \lambda \) for the Weibull model. For instance \( \lambda \) was defined as follows:

\[
\lambda = e^{\theta_0 + \theta_x x}
\]

where \( \theta_0 \) is the intercept (typical value) of the natural logarithm of \( \lambda \) and \( \theta_x \) the coefficient (effect) of the variable \( x \). This parameterisation ensures that the parameter do not take negative values for any patient or group. We chose to implement the model in the software Stan [24] as it provides an easy way to model censored data and offers flexibility on the input data format and model parameterisation. Diffuse prior distributions were assigned to the model parameters (see example of Stan code in Appendix A4.2). Posterior distributions were approximated using the No-U-Turn sampling algorithm. Selection of an appropriate survival distribution was based on approximate leave-one-out cross-validation (LOO) as computed in the R package \texttt{loo} [26]. Once a base model was selected, covariates were included in a forward way based on LOO estimates and the 95\% credible intervals of the regression coefficients. The ability of the proposed model to describe the data in the reduced-training set (calibration ability) was evaluated by visual comparison of the observed and predicted survival curves. One thousand
parameter sets were randomly sampled from the posterior distributions. For each parameter set, individual survival curves were simulated on the basis of patient covariates and were then averaged to derive a population survival curve. The median population survival curve and a 95% credible interval were then calculated based on the 1,000 samples. The observed survival curves (median and 95% confidence interval (CI)) were derived using Kaplan-Meier estimates [27].

A more frequently used approach to model survival data and identify risk factors is to perform a multivariate CPH regression, as was done for the original training set to screen for potential risk factors. Note that to be able to do a head-to-head comparison of the PAR and COX approaches, we repeated the multivariate CPH regression on the reduced-training set to possibly relate baseline risk factors and the empirical \( \text{PTR}_{\text{max}} \) metric to the survival time. In addition, we also built a CPH model based only on the baseline clinical characteristics of the patients, i.e. excluding TS-related variables. Using a penalized regression like the LASSO method, appropriate estimation of parameter uncertainties is not trivial. Therefore, variable reduction was instead done by backward deletion using a significance level of 0.05. The proportional hazards assumption was evaluated for the remaining variables by incorporation of a time interaction and by visual inspection of the scaled Schoenfeld residuals [28]. The discriminative ability of the model was assessed by computing the concordance probability corrected for the bias due to model optimism [29]. Empirical 95% CIs around the regression coefficient estimates were calculated from 1,000 bootstrap resamples. The analysis was carried out using the R package \texttt{rms} [30].

### 5.3.2.4. Validation

The performance of the PAR and COX approaches in predicting the survival time of new patients was evaluated by applying the models to the validation set and computing the area under a receiver operating characteristic (ROC) curve at different relevant time points. Only patients with complete cases for the proposed risk factors were retained in the validation set. Each ROC curve was derived using patient survival probabilities at a given time \( t \) as predictor, and patient death status at time \( t \) as binary outcome. Survival probabilities were predicted on the basis of patient covariates in the validation set. For the AFT model, posterior mean parameters were
used to simulate survival probabilities. The 95% CI for the time-dependent area under a ROC curve (AUROC) was computed on the basis of bootstrapping resampling method. In addition, the integrated AUROC values across all time points was calculated as a global predictive accuracy or discrimination measure, using the R package risksetROC [31]. Finally, since the follow-up period in the validation set was longer than in the training set, we also check the ability of the PAR approach to extrapolate survival data beyond the training follow-up period, by visual comparison of the observed and predicted survival curves.

5.4. Results

5.4.1. Baseline risk factors

Among the 31 variables screened for the 271 patients of the training set, ECOG score (0 versus 1 or 2), ALB, ALP (log-transformed), BSA, BILI, CA19-9 (log-transformed), NEUT (log-transformed) and baseline TS (TS0) were retained in the sparse model produced by the LASSO penalised CPH regression, that is 8 variables in total. While an increase in most variables is associated with worse prognosis, there is an inverse relationship between ALB and BSA, and patient survival times. There is evidence in the literature that cancer of the body and tail of the pancreas are associated with poorer survival than head lesions [6]. In addition, it has been shown that diabetes might affect survival of patient with MPC [8]. Although CLOC and diabetes status were not found to be important risk factors when analysing the training set, these variables were further re-assessed on the reduced-training set.

5.4.2. Tumour size time-series model

The hierarchical nonlinear model provided a good description of the median trend and the variability in the TS time-series of both the training and validation set (Figure A4.1 in Appendix A4.3). Of note, the TS model was fitted to the validation data which were used to validate only the survival models. The predicted median trends in the TS time-series suggest that under gemcitabine treatment, tumours (target lesions) typically undergo slight shrinkage followed by smooth re-growth. Although this trend varies across the 152 patients of the training set and across the
385 patients of the validation set (e.g. TS only increasing for some patients), the model was flexible enough to reasonably capture individual profiles (Figure 5.1).

Figure 5.1 Observed (black open circles) and predicted (solid black lines) tumour size time course for six representative patients of the training set. The grey areas represent 95% credible intervals for the individual predictions.

The posterior distributions of the model parameters are summarised in Table 5.1. Convergence was considered achieved for all marginal parameter distributions based on the potential scale reduction statistics (all less than 1.1). The uncertainty in all parameter values was reasonably small as indicated by the standard deviations.
Table 5.1 Posterior quantities for the parameters of the tumour size time-series models for the training and validation sets

| Parameter | Training | | | Validation | | |
|-----------|----------|---------|-------------|----------|---------|
|           | Mean     | SD      | Mean        | SD       |
| θ_{BASE}  | 4.58     | 0.0414  | 4.52        | 0.0367   |
| θ_{SR}    | -6.78    | 0.166   | -6.92       | 0.0702   |
| θ_{PR}    | -3.17    | 0.301   | -5.79       | 0.643    |
| ω_{BASE}  | 0.648    | 0.0307  | 0.682       | 0.0267   |
| ω_{SR}    | 1.32     | 0.135   | 1.27        | 0.0976   |
| ω_{PR}    | 0.835    | 0.226   | 2.45        | 0.368    |
| σ         | 0.150    | 0.0065  | 0.235       | 0.0071   |

Abbreviation: SD, standard deviation
Symbols: θ, log-transformed population means; ω, interpatient standard deviations; σ, residual standard deviation
Posterior means and SDs calculated on 2,000 samples obtained by pooling 4 chains of 500 samples after burn-in for each

5.4.3. Survival analysis

Among the 152 patients for whom PTR at week 2 to 10 could be interpolated and PTR_{max} could be calculated, 120 patients had complete cases for ECOG score, ALB, ALP, BSA, BILI, CA19-9, CLOC, diabetes status, NEUT and TS_{0} (observed), and were therefore retained in the reduced-training set. This data set was used to build a multivariate survival model by application of either the PAR or the COX approach. The distributions of the variables in the reduced-training set are presented in the first column of Table 5.2. Fifty-eight patients (48%) had died by the end of the study, with a median follow-up time of 19 weeks for the 62 patients still alive. The median survival time estimate for the studied group is 33 weeks (95% CI from 29 to 42 weeks).
Table 5.2 Baseline characteristics and tumour size reduction metrics for patients by the reduced-training set (complete cases from the original training set) and the validation set

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reduced-training set (120 patients, 58 deaths)</th>
<th>Validation set (235 patients, 204 deaths)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Categorical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLOC</td>
<td>Entire pancreas or head (68) versus body or tail (32)</td>
<td>NE</td>
</tr>
<tr>
<td>DIAB</td>
<td>yes (32) versus no (68)</td>
<td>NE</td>
</tr>
<tr>
<td>ECOG</td>
<td>0 (37) versus 1 or 2 (63)</td>
<td>NE</td>
</tr>
<tr>
<td><strong>Continuous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALB (G/l)</td>
<td>41 (26 to 62)</td>
<td>43 (28 to 53)</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>161 (5.04 to 1686)</td>
<td>NE</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.78 (1.31 to 2.29)</td>
<td>1.84 (1.34 to 2.49)</td>
</tr>
<tr>
<td>BILI (µmol/l)</td>
<td>10.3 (1.71 to 34.4)</td>
<td>NE</td>
</tr>
<tr>
<td>CA19-9 (IU/ml)</td>
<td>1426 (0.6 to 294800)</td>
<td>NE</td>
</tr>
<tr>
<td>NEUT (G/l)</td>
<td>5.75 (1.55 to 34.7)</td>
<td>5.40 (1.50 to 17.3)</td>
</tr>
<tr>
<td>TS₀ (mm)</td>
<td>107 (19 to 378)</td>
<td>95 (10 to 358)</td>
</tr>
<tr>
<td>PTR (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk2</td>
<td>1.00 (-5.20 to 25.8)</td>
<td>1.45 (-17.7 to 51.32)</td>
</tr>
<tr>
<td>wk4</td>
<td>1.97 (-10.4 to 44.3)</td>
<td>2.87 (-35.4 to 75.7)</td>
</tr>
<tr>
<td>wk6</td>
<td>2.89 (-15.7 to 57.4)</td>
<td>4.25 (-53.1 to 87.1)</td>
</tr>
<tr>
<td>wk8</td>
<td>3.78 (-21.0 to 66.7)</td>
<td>5.61 (-70.9 to 92.2)</td>
</tr>
<tr>
<td>wk10</td>
<td>4.66 (-26.3 to 73.1)</td>
<td>6.94 (-88.7 to 94.4)</td>
</tr>
<tr>
<td>PTRₘₐₓ (%)</td>
<td>9.59 (-71.4 to 78.9)</td>
<td>7.14 (-582 to 100)</td>
</tr>
</tbody>
</table>

Abbreviations: NE, not extracted; CLOC, pancreatic cancer location; DIAB, diabetes status; ECOG, Eastern Cooperative Oncology Group status score; ALB, albumin; ALP, alkaline phosphatase; BSA, body surface area; BILI, bilirubin; CA19-9, carbohydrate antigen 19-9; NEUT, neutrophil count; TS₀, baseline tumour size; PTRₘₐₓ, percentage tumour size change from baseline at week x; PTRₘₐₓ, best percentage tumour size change from baseline within 12 weeks

5.4.3.1. The PAR approach

The AFT models using a lognormal distribution (AFTₐₙ) or a Weibull distribution (AFT₇₉) for the distribution of survival times provided similar performance in describing the reduced-training set as indicated by the LOO estimates (-265.9 and -265.8 for the AFTₐₙ and AFT₇₉ models, respectively) and their standard errors (21.52 and 21.23 for the AFTₐₙ and AFT₇₉ models, respectively). A visual evaluation of the descriptive performance of the models is depicted in Figure A4.2 (Appendix A4.3). Hence, both models were used for the multivariate analysis and were further validated. Using the AFTₐₙ, TS₀ (centred at the median), ALB (centred at the median) and PTR at week 2 (PTRₘₐₓ) were found to be the best predictor of
patient time to death. When analysing the survival data with the AFT\_WB model, only \( TS_0 \) and BSA (centred at the median) were identified as predictors. It should be noted that the predictive values of PTR at week 2, 4, 6, 8 and 10 were the same when using the AFT\_LN model. Nevertheless, we chose to carry on the analysis with the earliest TS reduction metric, i.e. PTR at week 2. In the AFT\_WB model, the coefficients of all PTR variables have credible intervals that include zero. An example of Stan code for the AFT\_WB model is presented in Appendix A4.2. Potential scale reduction statistics (all \(< 1.1\)) indicated that all parameter distributions seemed to have converged to approximate equilibrium. The posterior marginal distributions are summarised in Table 5.3.

Table 5.3 Posterior quantities for the parameters of the lognormal and Weibull accelerated failure time models for the survival data of the reduced-training set

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>95% credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lognormal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \log(\mu) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_0 )</td>
<td>1.25</td>
<td>[1.21; 1.29]</td>
</tr>
<tr>
<td>( \theta_{TS0} )</td>
<td>-0.001</td>
<td>[-0.0015; -0.0005]</td>
</tr>
<tr>
<td>( \theta_{PTRwk2} )</td>
<td>1.07</td>
<td>[0.189; 2.01]</td>
</tr>
<tr>
<td>( \theta_{ALB} )</td>
<td>0.0079</td>
<td>[0.0006; 0.0151]</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>0.544</td>
<td>[0.453; 0.661]</td>
</tr>
<tr>
<td>Weibull</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \log(\lambda) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \theta_0 )</td>
<td>3.80</td>
<td>[3.67; 3.93]</td>
</tr>
<tr>
<td>( \theta_{TS0} )</td>
<td>-0.0037</td>
<td>[-0.0052; -0.0022]</td>
</tr>
<tr>
<td>( \theta_{BSA} )</td>
<td>0.762</td>
<td>[0.103; 1.44]</td>
</tr>
</tbody>
</table>

Lognormal distribution: \( \mu \) is the mean and \( \sigma \) the scale parameter
Weibull distribution: \( \alpha \) is the shape and \( \lambda \) the scale parameter
Intercepts \( \theta_0 \) and coefficients \( \theta_x \) are for the log-transformed parameters that include covariates (see METHODS, and example of Stan code in Appendix A4.2)
Posterior means and percentiles were calculated on 2,000 samples obtained by pooling 4 chains of 500 samples after burn-in for each

5.4.3.2. The COX approach
After backward deletion of the 11 variables (including the TS\(_0 \) and PTR\(_{\text{max}} \) variables) evaluated in the multivariate CPH model, only TS\(_0 \) (centred at the median), BSA (centred at the median) and PTR\(_{\text{max}} \) were found to be significant risk factors (\( P \) values < 0.0001, of 0. 0087 and of 0.0008, respectively). However, the bootstrap
95% CI for the BSA coefficient included zero (-3.97 to 0.485). Hence, only TS₀ (in mm) and PTRₘₐₓ were retained in the CPH model (referred to as the ‘COX₁’ model) with coefficient estimates of 0.0064 (95% CI from 0.0032 to 0.0134) and -1.29 (95% CI from -4.40 to -0.184), respectively. Interaction of these two variables with time did not significantly improve the model (all P values > 0.2) which suggests that the assumption of proportional hazards is reasonable (see also Figure A4.3 in Appendix A4.3). When excluding the TS related variables TS₀ and PTRₘₐₓ from the multivariate regression, only NEUT (log-transformed) was identified as significant survival predictor (P = 0.0006) with coefficient estimate of 0.759 (95% CI from 0.336 to 1.72). The interaction with time also did not significantly improve the model (P > 0.1). This model is referred to as the ‘COX₂’ model in the rest of the report. The bias-corrected concordance probability was estimated to be 0.71 and 0.66 for the COX₁ and COX₂ models, respectively, which suggests that the COX₁ model distinguish high-risk patients from low-risk patients better than the COX₂ model.

5.4.4. **Predictive performance**

Only patients with complete cases for ALB, BSA, NEUT, PTRₘₐₓ, PTRₚₖ₂ and TS₀ were retained in the validation set (235 patients, 204 deaths) to evaluate the performance of the PAR and the COX approaches in predicting MPC survival data. The distribution of these variables are summarised in the second column of Table 5.2. The median follow-up time for patients still alive was 57 weeks and the median survival time estimate for the group is 35 weeks (95% CI from 30 to 40 weeks).

The predictive performance of the two approaches was compared by calculating the AUROC at 3, 6, 9 and 12 months. This time points were chosen because the median survival times reported in recent randomized trials [19,20] fall in this range. The time-dependency of AUROC for the AFTₜₙ, AFTₜₚ, COX₁ and COX₂ models is depicted in Figure 5.2. The AFTₜₙ model typically performs better than the other models. Also, the performance of the COX models seems to decrease with time. However, for clarity, the AUROC-time curves are plotted without their confidence bands which all overlap at any time.
The integrated AUROC values across all time points were 0.68 (95% CI, 0.60 to 0.75), 0.60 (95% CI, 0.51 to 0.68), 0.65 (95% CI, 0.57 to 0.73) and 0.64 (95% CI, 0.55 to 0.72) for the AFT\textsubscript{LN}, AFT\textsubscript{WB}, COX\textsubscript{1} and COX\textsubscript{2} models, respectively. The 95% CIs of the integrated AUROC values overlap, which suggests that all four models perform globally, similarly on the validation set. The extrapolation ability of the AFT\textsubscript{LN} and AFT\textsubscript{WB} models is illustrated in Figure 5.3 and seems to be higher for the AFT\textsubscript{LN}.
Figure 5.3 A visual evaluation of the ability of the lognormal and Weibull accelerated failure time models to predict the survival data in the validation set. The observed median survival curves (solid black lines) are plotted along with their 95% confidence intervals (dashed black lines) as well as with the simulated median survival curves (solid grey lines) and their 95% credible intervals (grey areas). The vertical dashed lines represent the last follow-up time in the reduced-training set, i.e. 69 weeks.

5.5. Discussion

Prognostic models for survival of patients with MPC are essential to identify stratification variables and control for known important variability in the data when conducting large, prospective, Phase III randomised controlled trials. It is also essential to individualise care and treat patients more effectively. In this study, two different survival modelling approaches have been evaluated retrospectively using the control arm data of two independent Phase III studies of patients under gemcitabine treatment. The first approach, referred to as the PAR approach, aims at incorporating model-predicted TS reduction metrics into a parametric survival model that can be used for clinical trial simulations. This approach utilises time-series of imaging data to interpolate early change in TS for all patients. The second approach, referred to as the COX approach, simply aims at identifying empirical prognostic factors with the commonly used multivariate CPH regression model. Our results suggest that the two approaches perform similarly in predicting survival probability of new MPC patients, as indicated by the 95% CIs of the integrated AUROC values.

Regardless of the modelling approach applied, the baseline variable TS$_0$ appears to be a significant prognostic factor for patients with MPC, although the effect is weak overall: in the COX$_1$ model for instance, the hazard ratio (HR) for 1 cm increase in
TS_0 is 1.07 (95% CI, 1.03 to 1.14); in the AFT_{LN} model, the ratio of median survival time (AF) for 1 cm increase in TS_0 is 0.990 (95% credible interval, 0.985 to 0.995). The other risk factors identified were different depending on which approach was applied. With the PAR approach, the metric PTR at week 2 was identified as strong survival predictors in the AFT_{LN} model (AF ratio of 2.92 for 1% increase, with 95% credible interval from 1.21 to 7.46) although it was not in the AFT_{WB} model. The fact that the predictive value of the PTR metric was similar at each time point assessed (week 2 to 10) is consistent with the predicted median TS-dynamic trend in the studied patients treated with gemcitabine (Figure A4.1 in Appendix A4.3). Also, patients with higher albumin levels were found to survive slightly longer (AF ratio of 1.08 with 95% credible interval from 1.01 to 1.16), which is consistent with biology (inflammation marker) and with a previously reported MPC prognostic model [7]. Finally, increase in BSA was related to increase in median survival time (AF ratio of 2.14 with 95% credible interval from 1.11 to 4.22) in the AFT_{WB} model. Since patients undergoing gemcitabine chemotherapy are dosed by BSA, this means that patients with higher doses might survive longer, which suggests that the standard dose for gemcitabine (1000 mg/m^2) might not be optimal for all studied MPC patients. Using the COX approach, PTR_{max} was identified as significant risk factor with HR estimate of 0.275 (95% CI, 0.0123 to 0.832). However, the CPH model that includes only NEUT as covariate (COX_2 model) performed as well as the model with both TS_0 and PTR_{max} (COX_1 model) in predicting patient survival, thereby suggesting that variables derived from TS imaging data might not be better prognostic factors for patients with MPC than some of the toxicology markers like baseline neutrophils. Patients with high pre-treatment neutrophil counts are probably more at risk than patients with lower counts (HR ratio of 2.14 with 95% CI from 1.40 to 5.58), as it was suggested in a previous study [10]. It should be noted that only NEUT was retained in the CPH model when analysing the reduced-training set (excluding TS-related variables) compared to 8 variables when analysing the original training set using the LASSO method. This can be explained by the decreased power of the analysis when reducing the training set and probably also by the difference of variable selection method.

The choice of the modelling approach clearly depends on the purpose of the survival analysis. The PAR approach has been recommended to aid oncology drug
development decisions such as compound screening, dose selection and trial design [15,16,18,17]. In the present example for MPC, this approach could reasonably predict the mortality risk of patients from an independent study, with similar performance compared to the more conventional COX approach. However, our work suggests caveats against the PAR approach. Firstly, a unique AFT model could not be selected on the basis of the training set (see Figure A4.2 in Appendix A4.3), possibly due to the small study sample size (120 patients, 58 deaths). This emphasises the difficulty of defining the distribution of survival times when using parametric models with limited survival data. Although, the AFT_{LN} and AFT_{WB} models could predict the validation data with similar global predictive accuracy, the distributional assumption on time to death seems to affect the identification of prognostic factors (Table 5.3) as well as the extrapolation ability of the model (Figure 5.3). It should be stressed that using the Weibull distribution, longitudinal TS metrics were not identified as better survival predictors than other baseline characteristics. Nevertheless, we acknowledge that other commonly used AFT models, such as the log-logistic and generalised gamma distributions, were not assessed in this analysis and might provide a better description of the training data than the lognormal and Weibull distributions. Secondly, the PAR approach is more time consuming than the COX approach as it involves modelling the TS dynamics rather than simply evaluating empirical metrics. Also, the individual model parameters, used to interpolate early change in TS for each patient, always carry uncertainty. For simplicity, we ignored parameter uncertainty in the present analysis by using the posterior means of the individual parameters. However, this can introduce bias in the subsequent evaluation of these variables as survival predictors. Ideally, several sets of TS reduction metrics should be produced by sampling from the posterior distribution of the individual parameters, if a Bayesian approach is applied. This issue has also been addressed in a frequentist modelling approach where shrinkage of individual parameter estimates can affect the type I error of falsely detecting or failing to detect TS metrics as predictors of survival [32].

We developed the parametric AFT models using a Bayesian approach mainly because we found a probabilistic programming language convenient for the analysis of censored data and because credible intervals for the covariate effects are obtained. The identification of prognostic factors was simply done by forward selection based
on LOO estimates and the 95% credible intervals of the coefficients. Alternatively, methods that use shrinkage priors could be employed for variable selection [33,34]. These methods are similar to frequentist penalised regressions, in the sense that the coefficients of (apparently) irrelevant covariates would have credible intervals that include zero, although it offers the advantage of readily producing the uncertainty distribution of the parameters.

In conclusion, the PAR modelling approach that utilises model-derived TS metrics in addition to baseline patient characteristics could predict reasonably well survival of patients with MPC undergoing gemcitabine chemotherapy. However, determining the distribution of survival times appeared challenging with data from only one small study, and seems to affect the identification of risk factors. Moreover, the predictive performance was not significantly better than a simple CPH model that incorporates only baseline neutrophil count as covariate. Nevertheless, our findings should be confirmed by analysing data sets that have higher power for multivariable survival regression. In particular the predictive value of the new potential prognostic factors BSA (gemcitabine dose) and TS-related metrics should be re-assessed together with established risk factors on a larger MPC study.

5.6. Acknowledgements

This study is based on research using information obtained from www.projectdatasphere.org, which is maintained by Project Data Sphere, LLC. Neither Project Data Sphere, LLC nor the owner(s) of any information from the web site have contributed to, approved or are in any way responsible for the contents of this work. We wish to thank Emma Martin (Manchester Pharmacy School, The University of Manchester, Manchester, UK) for fruitful discussions.

5.7. Conflict of interest

The authors declare that they have no conflict of interest.
5.8. References


Chapter 6: General discussion

Pharmacokinetic and pharmacodynamic models are mainly employed to guide clinical and drug development decision-making. Using mavoglurant as an example of a compound under clinical development, the first part of this thesis illustrates how simulations based on a population pharmacokinetic model can help to select an appropriate pharmaceutical formulation and dosing regimen during the development process. In neuroscience, promising chemical entities, i.e. those that can cross the blood-brain barrier, are often associated with complex absorption profiles due to their physicochemical properties (lipophilic drugs, see Figure 1.4). Although simple compartmental absorption models can easily be linked to compartmental disposition models and are therefore convenient for ‘semi-mechanistic’ data modelling, our work shows that highly empirical input models are more adequate to accurately characterise complex concentration-time relationships often due to complex absorption patterns. In particular, we found that input functions defined as weighted sums of Gaussian basis functions are very useful to describe pharmacokinetic profiles with multiple peaks and are easier to implement in a population model than other flexible nonlinear functions such as splines. Characterising input profiles precisely is essential in drug development to adequately evaluate the impact of absorption factors, such as food status at drug administration, and determine the best conditions of use of an experimental formulation. On the other hand, physiologically-based absorption models can be employed to generate hypotheses and possibly gain insight into the input process. For mavoglurant for instance, physiologically-based simulations could be performed to provide possible explanations for the complex multiple-peak concentration-time pattern typically observed after oral administration of the modified-release formulation. Although, exploring ‘what-if’ scenarios can be challenging and time consuming as the absorption process is a result of the interplay between a number of complex pre-systemic mechanisms. Nevertheless, mechanistic models enable extrapolation of the pharmacokinetic knowledge beyond the experimental conditions under investigation.

The use of PBPK models for population analysis of clinical data has received increased interest as it allows prediction of various clinical scenarios (e.g. pharmacokinetics in children) to possibly guide trial design (e.g. selection of a safe
dose) and conduct better studies. To adequately perform such population PBPK analyses, applying a Bayesian approach is critical, although not always sufficient for complex PBPK models. The combination of preclinical and clinical information on mechanistic parameters not only provides better and plausible estimates of the parameters for a standard individual in the population, but also allows quantification of the inter-individual variation while accounting for known biologically-meaningful covariate effects (e.g. polymorphisms of metabolic enzymes). The work in this thesis provides a general Bayesian modelling workflow to adequately perform population data analysis with a complex PBPK model (Figure 6.1). We encountered numerical issues when conducting the Bayesian population analysis of mavoglurant data with a whole-body PBPK model, possibly because we had prior information only for the population mean parameters and not for the population variances, and because of the high dimension of the system and perhaps high correlations between parameters. These numerical issues could also be attributed to the random walk behaviour of the Markov chain Monte Carlo algorithm used for the analysis. It would be very interesting to instead use the Hamiltonian Monte Carlo sampling algorithm implemented in the software Stan to see if suppression of this behaviour allows reduction of numerical instabilities. However, there is currently no efficient differential equation solver implemented in Stan, which makes population analyses with high dimensional systems almost impossible. We thus proposed two options to stabilise a Markov chain Monte Carlo analysis of a whole-body PBPK model: naively increasing the informativeness of the priors by fixing parameters, or formally simplifying the model using Bayesian proper lumping. We have shown that a formally reduced PBPK model can still be used for extrapolation. However, to fully preserve the predictive ability of the original model, the model should be reduced by accounting for the distribution of the physiological parameter values across all sub-populations (children, obese, elderly etc.) or species for which pharmacokinetics are intended to be predicted.

The proposed Bayesian mechanistic modelling workflow can also be applied to highly mechanistic pharmacodynamic models, also known as system pharmacology models, although additional difficulties arise compared to PBPK models. For instance, as our knowledge of the mammillary anatomy and physiology is high, we are quite certain in the overall structure of PBPK models, at least for chemicals that
perfuse organs well. Conversely, the structure of system pharmacology models often carries uncertainty due to our lack of knowledge on the biological networks that the models are meant to describe. Although not trivial, taking into account this uncertainty is important to provide credible model-based predictions. Moreover, the dimension of system pharmacology models is often higher compared to PBPK models. Hence the need for formal reduction of the models to facilitate data analysis is even more important than in pharmacokinetics. However, since pharmacodynamic systems are often nonlinear, adequate extraction of the differential equations of the system appears very challenging when proper lumping is applied. Perhaps other model reduction methods, such as elimination of states, are more appropriate for simplification of complex nonlinear dynamical systems. Nevertheless, when integrated into a Bayesian work flow, population mechanistic modelling is currently considered as a very promising quantitative translational tool for trial design in drug development. Although we acknowledge that more examples for different types of drugs and diseases are needed to show the benefit of such a mechanistic approach over more empirical analyses for more complex pharmacokinetic and pharmacodynamic scenarios, especially when prior information are lacking.

**Figure 6.1** Schematic work flow for Bayesian mechanistic modelling of population pharmacokinetic and/or pharmacodynamic data

Using pancreatic cancer as an example, the second part of this thesis illustrates how pharmacodynamic modelling can help guide clinical or drug development decisions in oncology. The main aim of anticancer drug development is to compare survival
between a control group of patients (e.g. patients receiving the standard of care) and a group receiving the experimental treatment. The incorporation of longitudinal tumour size measures into a parametric survival model has been increasingly used and suggested for early assessment of the benefit of an investigational treatment and for trial design optimisation. Our work shows that such a survival modelling approach has some limitations that seem to be mostly due to clinical constraints: small sample size of studies, slow patient recruitment rate, time to obtain sufficient amount of tumour imaging data, delay between ‘learning’ and ‘confirming’ studies, etc.. In the clinic, the aim of survival analysis is to predict individual risk rather than group risk, i.e. the risk of mortality or disease progression of a patient given its clinical and antitumor response characteristics. The work in this thesis shows that for this purpose, a more empirical and simpler survival modelling approach (i.e. Cox regression of empirical factors) might be sufficient. However, more comparative studies need to be conducted to evaluate the predictive performance of different survival approaches and antitumor response markers. For example, it would be informative to perform head-to-head comparisons of the prognostic value of RECIST variables and model-derived tumour size metrics for various types of cancer, to see whether it is worth modelling longitudinal tumour size data to summarise the information as a continuous variable rather than as a categorical variable. In particular, the evaluation of RECIST over time rather than just at patients’ first visit seems to be of increasing interest. It should be noted that although RECIST variables are categorical, they include more information than continuous variables derived from tumour size defined as the sum of longest diameters of the target lesions, as they also take into account the non-target lesions and the appearance of new lesions.

To conclude, depending on the objectives of pharmacokinetic and pharmacodynamic model-based analyses, a number of valuable modelling tools and methods can be employed to make useful inference from clinical data. However, to provide credible and effective clinical guidance, scientists should make an effort to understand and acknowledge the limitations of these tools. Finally, it is important to bear in mind that poorly designed studies produce data from which constructive learning is difficult, even when sophisticated analysis methods are employed.
Appendix A1: Supplementary material for Chapter 2
A1.1. Supplementary methods

A1.1.1. Implementation of dose superimposition in NONMEM using a sum of inverse Gaussian functions as an input rate function

Shen et al. recently proposed two approaches to implement dose superposition in NONMEM [1]. One approach is to perform dose superposition in a user-defined FORTRAN subroutine; the other is to simply perform it in a NM-TRAN abbreviated code without using any additional subroutine. Since the implementation of the latter method is easier and subject to fewer possible sources of error, this method was considered as a reference. The two approaches were adapted for the function used to model mavoglurant input following oral administration of the modified-release formulation under fasted conditions (MR-fasted model) (see Appendices A1.3 and A1.4 for the user-supplied subroutine itself and the corresponding NONMEM code, respectively, and Appendix A1.5 for the reference method). Using the reference method, the maximum number of doses that can be administered among the subject population has to be pre-defined and hence, the dosing events have to be indexed for each individual in the dataset (DOSENO data item in Appendix A1.5).

A1.1.1.1. Comparison of the FORTRAN subroutine approach with the reference method, using simulations

Two datasets of 30 standard subjects (body weight of 70 kg) were generated using the MR-fasted final model (see Chapter 2 for details on the model) and either the user-supplied FORTRAN subroutine or the reference method to perform dose superposition. Each subject initially received a 25-mg intravenous bolus followed by seven 100-mg oral doses administered every 12 h, with the first oral dose given 48 h after the intravenous dose. The predicted concentration-time profiles obtained with both methods were then compared, and for each individual the difference between the concentrations was calculated at each time point.
A1.1.1.2. Verification of the partial derivatives defined in the FORTRAN subroutine

As mentioned in the manuscript, parameter estimation using the first-order method (FO) or the first-order conditional method with interaction (FOCE-I) requires the first partial derivatives of the user-defined functions (FUNCA, FUNCB and FUNCC) with respect to the variables defined with random-effects to compute the objective function (see NONMEM guide VIII) [2]. To verify that these partial derivatives were correctly specified, the objective function value (OFV) of the MR-fasted model was computed for each dose superposition method. To do so, an estimation step was run in NONMEM with the option MAXEVAL=0, which allows calculation of the OFV based on the parameter initial values (final estimates of the MR-fasted model, also used for simulation). The dataset simulated with the reference method was analysed. For each approach, the FO and FOCE-I methods were both used to compute the OFV.
A1.2. Supplementary results

A1.2.1. Individual goodness-of-fit plots for each formulation-food conditions

Figure A1.1 Plots of the observations (open blue circles) and individual predictions (solid red lines) versus time for all individuals under IR-fasted conditions (43 subjects)
Figure A1.2 Plots of the observations (open blue circles) and individual predictions (solid red lines) versus time for all individuals under MR-fasted conditions (29 subjects)
Figure A1.3 Plots of the observations (open blue circles) and individual predictions (solid red lines) versus time for all individuals under MR-fed conditions (28 subjects)

A1.2.2. Implementation of dose superimposition in NONMEM using a sum of inverse Gaussian functions as an input rate function

A1.2.2.1. Comparison of the FORTRAN subroutine approach with a reference method using simulations

The predicted concentration-time profiles from the 30 hypothetical individuals are depicted in Figure A1.4. The profiles generated with the user-supplied FORTRAN subroutine approach and with the reference method were identical. For all individuals, the difference in concentrations was at any time equal to zero.
Figure A1.4 Plots of mavoglurant individual predicted concentrations versus time, using the MR-fasted final model (see Chapter 2 for details on the model). Although the Monte Carlo simulation ($n=30$) was performed with both inter-subject variability and unexplained residual variability, the individual concentrations are plotted without residual errors (IPRED output in NONMEM) to reduce the noise in the simulated data.

A1.2.2.2. Verification of the partial derivatives

The OFVs computed for the model using the user-supplied subroutine and the model defined as per the reference method were identical (-5037.714 with FO and -5623.275 with FOCE-I), which confirms that the first-order partial derivatives of FUNCA, FUNCB and FUNCC (Appendix A1.3) were correctly specified.
A1.3. User-defined FORTRAN functions FUNCA, FUNCB and FUNCC (subroutine referred as sumdose\_3IG.f90 in Appendix A1.4)

<table>
<thead>
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<tr>
<td>-----------------------------------------------</td>
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<tr>
<td>1st weighted IG function</td>
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<td>-----------------------------------------------</td>
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</tbody>
</table>

FUNCTION FUNCA(X,X1,X2)
USE SIZES, ONLY: DPSIZE, ISIZE
REAL(KIND=DPSIZE) :: EVTREC
INTEGER(KIND=ISIZE) :: FIRSTEM
REAL(KIND=DPSIZE) :: X,X1,X2,FUNCA
DIMENSION X(9),X1(9),X2(9,9)

! 9 is a fixed dimension in NMTRAN and PREDPP. It may not be changed.
! Input: X
! X(1) = Flag (0 - initialize ; 1 - dose from PK ; 2 - call from DES)
! X(2) = DOSE TIME (from PK) or T (from DES)
! X(3) = DOSE AMOUNT
! X(4) = MIT1
! X(5) = CV1
! X(6) = FR1

! Output: X1(9), X1(i) = 1st partial derivative of FUNCA w.r.t X(i)
! Does not compute derivatives of FUNCA w.r.t X(3) (AMT) because BIO is used outside
! this function and F1=0, so AMT can't have a partial derivative.
! Output: X2(9,9), X2(i,j) = 2nd partial derivative of FUNCA w.r.t. X(i), X(j)
! X2 ARE NOT COMPUTED BY THIS FUNCTION.
! DO NOT USE FOR LAPLACIAN METHOD

INTEGER ndos,i,maxndos
PARAMETER (MAXNDOS=10000)
REAL(KIND=DPSIZE) :: amt(maxndos),dosetime(maxndos)
REAL(KIND=DPSIZE) :: IPT,MIT1,CV1,FR1,t,deltatime
REAL(KIND=DPSIZE) :: pi
SAVE
pi = 3.141592
FUNCA=0

SELECT CASE (int(X(1)))
  CASE(0)  ! From PK at ICALL=0 or ICALL=1
    ! Initialization
    ndos=0
    CASE(1)  ! From PK at ICALL=2 with a dose
      ! Save dose information if amt>0
      if (X(3) > 0) then
        ndos=ndos+1
        if (ndos > maxndos) then
          ! The following is too drastic, but is more informative than an error caused by
          ! exceeding an array bound.
          WRITE (6,*) 'ERROR in FUNCA: No. of doses > maxndos:',maxndos
          STOP
        endif
        
        dosetime(ndos)=X(2)
        amt(ndos)=X(3)
      endif
    endif
  CASE(2)  ! From DES
    ! Compute superimposed (total) input at time T
    if (ndos > 0) then
      t = X(2)
      mit1 = X(4)
      cv1 = X(5)
      fr1 = X(6)
      do i=1,ndos
        deltatime = t-dosetime(i)
        if (deltatime > 0) then
          r1 = MIT1/(2*pi*CV1**2*deltatime**3)
          sqrt1 = sqrt(r1)
          exp1 = exp(-(deltatime-MIT1)**2/(2*CV1**2*MIT1*deltatime))
          r1, sqrt1 and exp1 are intermediate variables
          ipt = amt(i)*fr1*sqrt1*exp1
        endif
      enddo
    endif
FUNCA = FUNCA + IPT
! X(2) = partial derivative of FUNCA w.r.t. T
X(2)=X(2) + IPT * ((MIT1**2-deltatime**2)/(2*CV1**2*MIT1*deltatime**2) - 3*MIT1/(4*pi*CV1**2*deltatime**4*R1))
! X(4) = partial derivative of FUNCA w.r.t. MIT1
X(4)=X(4) + IPT * (1/(2*MIT1) + (deltatime**2-MIT1**2)/(2*CV1**2*MIT1**2*deltatime))
! X(5) = partial derivative of FUNCA w.r.t. CV1
X(5)=X(5) + IPT * ((deltatime-MIT1)**2/(CV1**3*MIT1*deltatime) - 1/CV1)
! X(6) = partial derivative of FUNCA w.r.t. FR1
X(6)=X(6) + IPT/FR1
endif
enddo
endif
END SELECT
return
end FUNCTION FUNCA

! 2nd weighted IG function
!----------------------------------------------------------------------------------
FUNCTION Funcb(X,X1,X2)
USE SIZES, ONLY: DPSIZE, ISIZE
REAL(KIND=DPSIZE) :: EVTREC
INTEGER(KIND=ISIZE) :: FIRSTEM
REAL(KIND=DPSIZE) :: X,X1,X2,FUNCB
DIMENSION X(9),X1(9),X2(9,9)
INTEGER ndos,i,maxndos
PARAMETER (MAXNDOS=10000)
REAL(KIND=DPSIZE) :: amt(maxndos),dosetime(maxndos)
REAL(KIND=DPSIZE) :: IPT,MIT2,CV2,FR2,t,deltatime
REAL(KIND=DPSIZE) :: pi
SAVE
pi = 3.141592
FUNCB=0
X1=0
SELECT CASE (int(X(1)))
CASE(0)
! From PK at ICALL=0 or ICALL=1
! Initialization
ndos=0
CASE(1)
! From PK at ICALL=2 with a dose
! Save dose information if amt>0
if (x(3) > 0) then
  ndos=ndos+1
  if (ndos > maxndos) then
    ! The following is too drastic, but is more informative than an error caused by exceeding an array bound.
    WRITE (6,*) 'ERROR in FUNCA: No. of doses > maxndos:',maxndos
    STOP
  endif
  dosetime(ndos)=X(2)
  amt(ndos)=X(3)
endif
CASE(2)
! From DES
! Compute superimposed (total) input at time T
if (ndos > 0) then
  t = X(2)
  mit2 = X(4)
  cv2 = X(5)
  fr2 = X(6)
  do i=1,ndos
    deltatime = t-dosetime(i)
    if (deltatime > 0) then
      R2 = MIT2/(2*pi*CV2**2*deltatime**3)
      SQRT2 = sqrt(R2)
      EXP2 = exp(-deltatime-MIT2)**2/(2*CV2**2*MIT2**2*deltatime))
      IPT = amt(i)*FR2*SQRT2*EXP2
      FUNCB = FUNCB + IPT
    endif
  enddo
endif
END FUNCTION Funcb
! X1(5) = partial derivative of FUNCB w.r.t. CV2
X1(5)=X1(5) + IPT * ((deltatime-MIT2)**2/(CV2**3*MIT2*deltatime) - 1/CV2)
! X1(6) = partial derivative of FUNCA w.r.t. FR2
X1(6)=X1(6) + IPT/FR2
endif
enddo
ENDDO
RETURN
END FUNCTION FUNCB

! 3rd weighted IG function
FUNCTION FUNCC(X,X1,X2)
USE SIZES, ONLY: DPSIZE, ISIZE
REAL(KIND=DPSIZE) :: EVTREC
INTEGER(KIND=ISIZE) :: FIRSTEM
REAL(KIND=DPSIZE) :: X,X1,X2,FUNCC
DIMENSION X(9),X1(9),X2(9,9)
INTEGER ndos,i,maxndos
PARAMETER (MAXNDOS=10000)
REAL(KIND=DPSIZE) :: amt(maxndos),dosetime(maxndos)
REAL(KIND=DPSIZE) :: IPT,MIT3,CV3,FR3,t,deltatime
REAL(KIND=DPSIZE) :: pi
SAVE
pi = 3.141592
FUNCC=0
X1=0
SELECT CASE (int(X(1)))
CASE(0) ! From PK at ICALL=0 or ICALL=1
!! Initialization
ndos=0
CASE(1) ! From PK at ICALL=2 with a dose
!! Save dose information if amt>0
if (x(3) > 0) then
  ndos=ndos+1
  if (ndos > maxndos) then
    ! The following is too drastic, but is more informative than an error caused by exceeding an array bound.
    WRITE (6,*) 'ERROR in FUNCA: No. of doses > maxndos: ',maxndos
    STOP
  endif
  dosetime(ndos)=X(2)
  amt(ndos)=X(3)
endif
CASE(2) ! From DES
!! Compute superimposed (total) input at time T
if (ndos > 0) then
  t = X(2)
  mit3 = X(4)
  cv3 = X(5)
  fr3 = X(6)
  do i=1,ndos
    deltatime = t-dosetime(i)
    if (deltatime > 0) then
      R3 = MIT3/(2*pi*CV3**2*deltatime**3)
      SQRT3 = sqrt(R3)
      EXP3 = exp(-((deltatime-MIT3)**2)/(2*CV3**2*MIT3*deltatime))
      IPT = amt(i)*FR3*SQRT3*EXP3
      FUNCC = FUNCC + IPT
    endif
  enddo
! X1(2) = partial derivative of FUNCC w.r.t. T
X1(2)=X1(2) + IPT * ((MIT3**2-deltatime**2)/(2*CV3**2*MIT3*deltatime**2) - 3/MIT3/(4*pi*CV3**2*deltatime**4*R3))
! X1(4) = partial derivative of FUNCC w.r.t. MIT3
X1(4)=X1(4) + IPT * ((1/(2*MIT3)) + (deltatime**2-MIT3**2)/(2*CV3**2*MIT3*deltatime**2))
! X1(5) = partial derivative of FUNCC w.r.t. CV3
X1(5)=X1(5) + IPT * ((deltatime-MIT3)**2/(CV3**3*MIT3*deltatime) - 1/CV3)
! X1(6) = partial derivative of FUNCA w.r.t. FR3
X1(6)=X1(6) + IPT/FR3
endif
enddo
A1.4. Dose superposition in NONMEM using the usersupplied FORTRAN subroutine (example of the MR-fasted model using a sum of three IG functions as input rate function)

$PROBLEM Dose superposition
$INPUT ID TIME DV AMT
$DATA data.csv IGNORE=@
$SUBROUTINE ADVAN13 TOL=9 OTHER=sumdose_3IG.f90
$MODEL
COMP = (CENTRAL, DEFDOS, DEFOBS)
COMP = (PERIPH)
$PK
;-----------------------------------------------
; Dose superposition
;-----------------------------------------------
CALLFL = -2
IF(NEWIND < 2) THEN
    VECTRA(1)=0.
    VECTRB(1)=0.
    VECTRC(1)=0.
    X=FUNCA(VECTRA)
    X=FUNCB(VECTRB)
    X=FUNCC(VECTRC)
ENDIF
;-----------------------------------------------
; Model parameters
; The distributions assigned to the parameters are not relevant in this example
;-----------------------------------------------
F1  = 0
; F1=0 for oral route -> dose explicitly used in input function
; Disposition
CL  = ...
V1  = ...
Q   = ...
V2  = ...
S1  = V1
K10  = CL/V1
K12  = Q/V1
K21  = Q/V2
; Input
BIO  = ...
; absolute bioavailability
tm1  = ...
; 1st-input mode (time-to-peak)
tm2  = ...
; 2nd-input mode (>= tm1)
tm3  = ...
; 3rd-input mode (>= tm2)
CV1  = ...
; CV of MIT1
CV2  = ...
; CV of MIT2
CV3  = ...
; CV of MIT3
FR1  = ...
; weight for the 1st IG pdf
FR2  = ...
; weight for the 2nd IG pdf
FR3  = ...
; weight for the 3rd IG pdf
a1  = sqrt(1+9/4*CV1**4) - 3/2*CV1**2
a2  = sqrt(1+9/4*CV2**4) - 3/2*CV2**2
a3  = sqrt(1+9/4*CV3**4) - 3/2*CV3**2
MIT1 = tm1/a1
; Mean Input Time
MIT2 = tm2/a2
MIT3 = tm3/a3
;-----------------------------------------------
; Dose superposition
;-----------------------------------------------
IF(DOSTIM==0)THEN
VECTRA(2) = TIME ; assign event dose time
VECTRB(2) = TIME
VECTR(2) = TIME
VECTRA(3) = AMT ; assign event dose amount
VECTRB(3) = AMT
VECTR(3) = AMT
ELSE
VECTRA(2) = DOSTIM ; assign non-event (ADDL) dose time
VECTRB(2) = DOSTIM
VECTR(2) = DOSTIM
VECTRA(3) = AMT
VECTRB(3) = AMT
VECTR(3) = AMT
ENDIF

IF (AMT > 0) THEN
VECTRA(1) = 1. ; set FLAG for PK dose recording
VECTRB(1) = 1.
VECTR(1) = 1.
X = FUNCA (VECTRA) ; call subroutine for dose recording
; X is a dummy variable because nothing is returned by FUNCA
X = FUNCB (VECTR)
X = FUNCC (VECTR)
ENDIF

; ODE system
------------------------------------------------------------------
$DES
VECTRA(1) = 2 ; set FLAG for computation of drug input at time T
VECTRA(2) = T
VECTRA(3) = 0 ; unused argument
VECTRA(4) = MIT1
VECTRA(5) = CV1
VECTRA(6) = FR1
VECTRB(1) = 2
VECTRB(2) = T
VECTRB(3) = 0
VECTRB(4) = MIT2
VECTRB(5) = CV2
VECTRB(6) = FR2
VECTR(1) = 2
VECTR(2) = T
VECTR(3) = 0
VECTR(4) = MIT3
VECTR(5) = CV3
VECTR(6) = FR3

INPT = FUNCA (VECTRA) + FUNCB (VECTR) + FUNCC (VECTR) ; call subroutine to compute
drug input

DADT(1) = BIO * INPT - A(1) * (K10 + K12) + A(2) * K21
DADT(2) = A(1) * K12 - A(2) * K21

; Residual error model
------------------------------------------------------------------
$ERROR
IPRED = F
IF (F > 0) LNIPRED = LOG (IPRED)
Y = LNIPRED + EPS(1)

; Initial values of THETAs, OMEGAs and SIGMAs
------------------------------------------------------------------
$THETA
$OMEGA
$SIGMA

; Simulation method and outputs
------------------------------------------------------------------
$SIM (123456) ONLYSIM
$TABLE ID TIME IPRED INPT AMT ONEHEADER NOPRINT FILE=sdtab
A1.5. Dose superposition performed solely in a NM-TRAN abbreviated code for mavoglurant MR-fasted model (reference method)

$SIZES DIMNEW=2000 ; To increase the total number of intermediate variables
$INPUT ID TIME DV AMT DOSENO ROUTE MDV EVID
$DATA fit.csv IGNORE = @
$SUBROUTINE ADVAN13 TOL=9
$MODEL
COMP = (CENTRAL, DEFDOS, DEFOBS)
COMP = (PERIPH)
$ABBREVIATED DERIV2=NO ; To avoid to print 2nd-order partial derivatives
$PK
; Definition of dosing regimen
CALLFL = -2
IF(NEWIND < 2) THEN
T1=0
T2=0
T3=0
T4=0
T5=0
T6=0
T7=0
T8=0
DOSE1=0
DOSE2=0
DOSE3=0
DOSE4=0
DOSE5=0
DOSE6=0
DOSE7=0
DOSE8=0
ENDIF
IF(DOSENO == 1) THEN
T1=TIME
DOSE1=AMT
ENDIF
IF(DOSENO == 2) THEN
T2=TIME
DOSE2=AMT
ENDIF
IF(DOSENO == 3) THEN
T3=TIME
DOSE3=AMT
ENDIF
IF(DOSENO == 4) THEN
T4=TIME
DOSE4=AMT
ENDIF
IF(DOSENO == 5) THEN
T5=TIME
DOSE5=AMT
ENDIF
IF(DOSENO == 6) THEN
T6=TIME
DOSE6=AMT
ENDIF
IF(DOSENO == 7) THEN
T7=TIME
DOSE7=AMT
ENDIF
IF(DOSENO == 8) THEN
T8=TIME
DOSE8=AMT
ENDIF
; Definition of the routes of administration
IV=0 ; Oral administration
IF (ROUTE==1) IV=1 ; IV bolus

; Model parameters
; The distributions assigned to the parameters are not relevant in this example
; F1 = 0 ; F1=0 for oral route -> dose explicitly used in input function

; Disposition
CL = ...
V1 = ...
Q = ...
V2 = ...
S1 = V1 ; amount in central compartment into concentration
K10 = CL/V1
K12 = Q/V1
K21 = Q/V2

; Input
BIO = ... ; absolute bioavailability
tm1 = ... ; 1st-input mode (time-to-peak)
tm2 = ... + tm1 ; 2nd-input mode (>= tm1)
tm3 = ... + tm2 ; 3rd-input mode (>= tm2)
CV1 = ... ; CV of MIT1
CV2 = ... ; CV of MIT2
CV3 = ... ; CV of MIT3
FR1 = ... ; weight for the 1st IG pdf
FR2 = ... ; weight for the 2nd IG pdf
FR3 = ... ; weight for the 3rd IG pdf
a1 = sqrt(1+9/4*CV1**4) - 3/2*CV1**2 ; mode = mean * a
a2 = sqrt(1+9/4*CV2**4) - 3/2*CV2**2
a3 = sqrt(1+9/4*CV3**4) - 3/2*CV3**2
MIT1 = tm1/a1 ; Mean Input Time
MIT2 = tm2/a2
MIT3 = tm3/a3

; ODE system

$DES
IG1=0
IG2=0
IG3=0

IPT1=0 ; IV bolus
IPT2=0
IPT3=0
IPT4=0
IPT5=0
IPT6=0
IPT7=0
IPT8=0

IF (T>T2.AND.DOSE2>0) THEN
IG1 = sqrt((MIT1/(2*pi*CV1**2*(T-T2)**3)))*exp(-(((T-T2)-MIT1)**2)/((2*CV1**2*MIT1*(T-T2))))
IG2 = sqrt((MIT2/(2*pi*CV2**2*(T-T2)**3)))*exp(-(((T-T2)-MIT2)**2)/((2*CV2**2*MIT2*(T-T2))))
IG3 = sqrt((MIT3/(2*pi*CV3**2*(T-T2)**3)))*exp(-(((T-T2)-MIT3)**2)/((2*CV3**2*MIT3*(T-T2))))
IPT2 = DOSE2 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF (T>T3.AND.DOSE3>0) THEN
IG1 = sqrt((MIT1/(2*pi*CV1**2*(T-T3)**3)))*exp(-(((T-T3)-MIT1)**2)/((2*CV1**2*MIT1*(T-T3))))
IG2 = sqrt((MIT2/(2*pi*CV2**2*(T-T3)**3)))*exp(-(((T-T3)-MIT2)**2)/((2*CV2**2*MIT2*(T-T3))))
IG3 = sqrt((MIT3/(2*pi*CV3**2*(T-T3)**3)))*exp(-(((T-T3)-MIT3)**2)/((2*CV3**2*MIT3*(T-T3))))
IPT3 = DOSE3 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF (T>T4.AND.DOSE4>0) THEN
IG1 = sqrt((MIT1/(2*pi*CV1**2*(T-T4)**3)))*exp(-(((T-T4)-MIT1)**2)/((2*CV1**2*MIT1*(T-T4))))
IG2 = sqrt((MIT2/(2*pi*CV2**2*(T-T4)**3)))*exp(-(((T-T4)-MIT2)**2)/((2*CV2**2*MIT2*(T-T4))))
IG3 = sqrt(MIT3/(2*pi*CV3**2*(T-T4)**3))*exp(-(((T-T4)-MIT3)**2)/(2*CV3**2*MIT3*(T-T4)))
   IPT4 = DOSE4 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF(T>T5.AND.DOSE5>0)THEN
   IG1 = sqrt(MIT1/(2*pi*CV1**2*(T-T5)**3))*exp(-(((T-T5)-MIT1)**2)/(2*CV1**2*MIT1*(T-T5)))
   IG2 = sqrt(MIT2/(2*pi*CV2**2*(T-T5)**3))*exp(-(((T-T5)-MIT2)**2)/(2*CV2**2*MIT2*(T-T5)))
   IG3 = sqrt(MIT3/(2*pi*CV3**2*(T-T5)**3))*exp(-(((T-T5)-MIT3)**2)/(2*CV3**2*MIT3*(T-T5)))
   IPT5 = DOSE5 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF(T>T6.AND.DOSE6>0)THEN
   IG1 = sqrt(MIT1/(2*pi*CV1**2*(T-T6)**3))*exp(-(((T-T6)-MIT1)**2)/(2*CV1**2*MIT1*(T-T6)))
   IG2 = sqrt(MIT2/(2*pi*CV2**2*(T-T6)**3))*exp(-(((T-T6)-MIT2)**2)/(2*CV2**2*MIT2*(T-T6)))
   IG3 = sqrt(MIT3/(2*pi*CV3**2*(T-T6)**3))*exp(-(((T-T6)-MIT3)**2)/(2*CV3**2*MIT3*(T-T6)))
   IPT6 = DOSE6 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF(T>T7.AND.DOSE7>0)THEN
   IG1 = sqrt(MIT1/(2*pi*CV1**2*(T-T7)**3))*exp(-(((T-T7)-MIT1)**2)/(2*CV1**2*MIT1*(T-T7)))
   IG2 = sqrt(MIT2/(2*pi*CV2**2*(T-T7)**3))*exp(-(((T-T7)-MIT2)**2)/(2*CV2**2*MIT2*(T-T7)))
   IG3 = sqrt(MIT3/(2*pi*CV3**2*(T-T7)**3))*exp(-(((T-T7)-MIT3)**2)/(2*CV3**2*MIT3*(T-T7)))
   IPT7 = DOSE7 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

IF(T>T8.AND.DOSE8>0)THEN
   IG1 = sqrt(MIT1/(2*pi*CV1**2*(T-T8)**3))*exp(-(((T-T8)-MIT1)**2)/(2*CV1**2*MIT1*(T-T8)))
   IG2 = sqrt(MIT2/(2*pi*CV2**2*(T-T8)**3))*exp(-(((T-T8)-MIT2)**2)/(2*CV2**2*MIT2*(T-T8)))
   IG3 = sqrt(MIT3/(2*pi*CV3**2*(T-T8)**3))*exp(-(((T-T8)-MIT3)**2)/(2*CV3**2*MIT3*(T-T8)))
   IPT8 = DOSE8 * (FR1*IG1 + FR2*IG2 + FR3*IG3)
ENDIF

INPT = IPT1*IV + (1-IV)*((IPT2 + IPT3 + IPT4 + IPT5 + IPT6 + IPT7 + IPT8)
   DADT(1) = BIO*INPT - A(1)*(K10+K12) + A(2)*K21
   DADT(2) = A(1)*K12 - A(2)*K21

; Residual error model
;----------------------------------------
$ERROR
   IPRED = F
   IF(F>0)LNIPRED = LOG(IPRED)
   Y = LNIPRED + EPS(1)

; Initial values of THETAs, OMEGAs and SIGMAs
;----------------------------------------
$THETA
$OMEGA
$SIGMA

; Computation of OFV: FO or FOCE
;----------------------------------------
$EST METHOD=0 POSTHOC MAX=0 NOABORT SIGL=9 NSIG=3 PRINT=10 ; FO
$EST METHOD=1 INTER MAX=0 NOABORT SIGL=9 NSIG=3 PRINT=10 ; FOCE-I

; Simulation method and outputs
;----------------------------------------
$SIM (123456) ONLYSIM
$TABLE ID TIME IPRED INPT AMT DOSENO ROUTE ONEHEADER NOPRINT FILE=sdtab
A1.6. References


Appendix A2: Supplementary material for Chapter 3
A2.1. Prediction of mavoglurant hepatic intrinsic clearance from recombinant human cytochrome P450 enzyme kinetic data

An *in vitro* experiment was performed at Novartis Pharma AG to gain understanding of the hepatic biotransformation of mavoglurant (MVG), i.e. to identify the enzymes involved in the metabolism process and quantify their contributions. Microsomes prepared from baculovirus-infected insect cells (BTI-TN-5B1-4) expressing numerous cytochrome P450 (CYP) isoenzymes were obtained from BD Biosciences (Woburn, MA, USA). MVG was incubated at concentrations of 0.5, 1, 2, 4, 8, 12, 16 and 20 μM in 400 μl of stock solution containing 20 pmol/ml of the recombinant human enzyme of interest. The results of the experiment suggested a major contribution of CYP 3A4, 2C8, 2C9 and 2C19 to MVG biotransformation. For the *i*th CYP enzyme, Michaelis-Menten kinetic parameters $V_{\text{max,rhCYP}_i}$ (maximum transformation rate achieved in the system) and $K_{\text{m,rhCYP}_i}$ (substrate concentration at which the rate is half $V_{\text{max,rhCYP}_i}$) were estimated using SigmaPlot version 8.0. The parameter estimates as well as other parameters used for extrapolation from the recombinant systems to human liver microsomes are presented in Table A2.1. For the *i*th isoenzyme, the intrinsic clearance in the recombinant system (CL_{int,rhCYP} in $\mu l/min/pmol$) was calculated as the ratio of $V_{\text{max,rhCYP}_i}$ (min$^{-1}$) to $K_{\text{m,rhCYP}_i}$ (μM) as in Eq. A2.1.

$$CL_{\text{int,rhCYP}_i} = \frac{V_{\text{max,rhCYP}_i}}{K_{\text{m,rhCYP}_i}}$$  \hspace{1cm} A2.1

Extrapolation of the intrinsic clearances from the recombinant human systems to human liver was done as follows [1]:

$$CL_{\text{int,hlmCYP}} = ISEF_i \cdot \frac{CL_{\text{int,rhCYP}_i}}{f_{\text{mic},i}} \cdot CYP_{\text{abundance}}$$  \hspace{1cm} A2.2

$$CL_{\text{int,LJ}} = \sum_{i=1}^{n} CL_{\text{int,hlmCYP}_i} \cdot MPPGL \cdot WT_{LJ} \cdot \left(\frac{60}{10^6}\right)$$
where $CL_{\text{int,hlmCYP}}$ is MVG intrinsic clearance in human liver microsomes ($\mu$l/mg/min) for the CYP enzyme $i$; $CL_{\text{int,LI}}$ is the hepatic intrinsic clearance (l/h); $ISEF_i$, $fu_{\text{mic},i}$ and $\text{CYP}abundance_i$ are the intersystem extrapolation factor, fraction unbound in microsomes and abundance (pmol/mg) in the liver of the CYP enzyme $i$, respectively; $MPPGL$ denotes the amount (mg) of microsomal protein per gram of liver [2]; $WT_{LI}$ is the weight (g) of the human liver [3]. The uncertainties in the $K_{m,rbCYP_i}$ and $V_{\text{max},rbCYP_i}$ estimates were used to calculate the uncertainty in $CL_{\text{int,LI}}$ by applying Fiellier’s theorem (Eq. A2.3) [4], as well as other basic properties of variances.

$$\text{var} \left( \frac{V_{\text{max},rbCYP}}{K_{m,rbCYP}} \right) = \left[ \frac{E(V_{\text{max},rbCYP})}{E(K_{m,rbCYP})} \right]^2 \times \left[ \frac{\text{var}(V_{\text{max},rbCYP})}{E(V_{\text{max},rbCYP})^2} + \frac{\text{var}(K_{m,rbCYP})}{E(K_{m,rbCYP})^2} \right]$$

A2.3

where $E(\cdot)$ and $\text{var}(\cdot)$ are the expectation and variance of a variable, respectively.

The variance of the sum $\sum_{i=1}^{n} CL_{\text{int,hlmCYP}}$ in Eq. A2.2 was calculated assuming no correlation between the variables.

**Table A2.1 Parameters used to calculate the isoenzyme-specific intrinsic clearances in human liver microsomes (Eq. A2.2)**

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>$K_{m,rbCYP}$</th>
<th>$V_{\text{max},rbCYP}$</th>
<th>$fu_{\text{mic}}$</th>
<th>ISEF</th>
<th>Hepatic abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 2C8</td>
<td>17.1 (1.3)</td>
<td>5.06 (0.19)</td>
<td>0.798</td>
<td>2.741</td>
<td>24</td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>2.49 (0.72)</td>
<td>1.15 (0.08)</td>
<td>0.805</td>
<td>1.892</td>
<td>73</td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>3.54 (0.34)</td>
<td>14.77 (0.4)</td>
<td>0.805</td>
<td>0.299</td>
<td>14</td>
</tr>
<tr>
<td>CYP 3A4</td>
<td>7.78 (2.64)</td>
<td>84.12 (6.4)</td>
<td>0.806</td>
<td>0.186</td>
<td>137</td>
</tr>
</tbody>
</table>

$a$ $K_{m,rbCYP}$ and $V_{\text{max},rbCYP}$ are given as estimate (standard error)

$b$ Values extracted from Rowland-Yeo et al [5]

ISEF: intersystem extrapolation factor (values were provided by BD Biosciences)
A2.2. Estimation of the formulation-specific dissolution constant $z$ from *in vitro* dissolution profiles

In the present analysis, the model from Hintz and Johnson was used to describe dissolution of solid drug in the gastro-intestinal lumen after oral administration of immediate-release formulations of MVG [6]. The dissolution rate was defined as follows:

\[
\frac{dA_{\text{und}}}{dt} = z \cdot \left( S - \frac{A_{\text{dis}}}{V} \right) \cdot A_{\text{und}}
\]

A2.4

where $A_{\text{und}}$ and $A_{\text{dis}}$ are the amount of undissolved and dissolved drug, respectively, $z$ is the dissolution constant, $S$ is drug solubility in the media, and $V$ is the volume of the media. Using the software NONMEM 7.3.0 (ICON Development Solutions, Hanover, Maryland, USA), the formulation-specific constant $z$ was estimating by fitting Eq. A2.4 to the *in vitro* dissolution profiles for the capsule and powder for oral suspension (POS) formulations of MVG [7,8]. For both the capsule (dosed with 100 mg of MVG) and suspension formulations (volume of 0.1 ml corresponding to 1 mg of MVG), the *in vitro* dissolution assay (Novartis Pharma AG) was performed in 900 ml (value of $V$) of fasted-state simulated intestinal fluid (FaSSIF). An in-house *in vitro* solubility experiment (internal unpublished results) provided an estimate of 0.037 mg/ml for MVG solubility in FaSSIF, which is independent of the pH of the media for a neutral compound. A proportional model was used for the residual error between model predictions and the observations. Goodness-of-fit plots are presented Figure A2.1. The parameters were estimated with good precision (relative standard errors less than 5%). The estimates are presented in Table 3.4.
A2.3. Prediction of mavoglurant effective permeability in human jejunum

An in vitro permeation experiment was performed at Novartis Pharma AG to determine MVG apparent permeability coefficient ($P_{\text{app}}$) in Caco-2 cell monolayers (pH 7.4). The results suggested an estimate of $P_{\text{app}}$ of $3.9 \times 10^{-6}$ and $6.7 \times 10^{-6}$ cm/s for propranolol (high permeability standard) and MVG, respectively (internal unpublished results). We used the model from Sun et al. to extrapolate MVG effective permeability from a Caco-2 cell system to human jejunum [9]. Prior to extrapolation, the MVG $P_{\text{app}}$ value was scaled from our in-house in vitro system to the Sun et al. system. The scaling factor was calculated as the ratio of their $P_{\text{app}}$ value for propranolol to our value. The Sun et al. $P_{\text{app}}$ value for propranolol ($64 \times 10^{-6}$ cm/s) was digitized from a plot in their manuscript using GetData Graph Digitizer version 2.26 (http://www.getdata-graph-digitizer.com/). Eq. A2.5 was then used to predict MVG effective permeability in human jejunum ($P_{\text{eff}}$) from the scaled $P_{\text{app}}$ value ($P_{\text{app, scaled}}$). The estimate of $P_{\text{eff}}$ is reported in Table 3.4.

$$\log(P_{\text{eff}}) = 0.6836 \cdot \log(P_{\text{app, scaled}}) - 0.5579$$  \hspace{1cm} \text{A2.5}
A2.4. Calculation of systemic bioavailability

The absorption model proposed to describe MVG pharmacokinetics following oral administration (see Fig. 3 in Chapter 3 for the structure of the model) is mechanistic enough to calculate the systemic bioavailability ($F$) from the capsule or the powder for oral suspension (POS) formulation, while distinguishing the fraction absorbed ($F_a$) from the fraction escaping gut wall metabolism ($F_g$) and the fraction escaping hepatic extraction ($F_h$). The relationship between $F$ and its mechanistic components is given in the equation below:

$$F = F_a \cdot F_g \cdot F_h$$  \hspace{1cm} \text{(A2.6)}

$F_a$ was derived from the fraction that was not absorbed into the small intestine enterocytes. The latter was calculated by simply normalising by the dose the sum of the maximal amounts in the two colonic compartments. To calculate $F_g$, we added an extra compartment into the model that collects the amount of drug metabolised in the small intestine enterocytes. $F_g$ was then derived as follows:

$$F_g = 1 - \frac{A_{\text{mer.max}}}{D \cdot F_a}$$  \hspace{1cm} \text{(A2.7)}

where $A_{\text{mer.max}}$ is the maximal amount metabolised in the enterocytes and $D$ is the administered dose. $F_h$ was derived from the bioavailability $F$ and $F_a$ using Eq. A2.6. $F$ was computed as follows:

$$F = \frac{AUC_{po}}{AUC_{IV}}$$  \hspace{1cm} \text{(A2.8)}

where $AUC_{po}$ and $AUC_{IV}$ are the area under the concentration-time curve (AUC) produced by oral (dose in the stomach compartment) and intravenous (dose in the venous blood compartment) administration, respectively. The AUC values were calculated using the trapezoidal method.
A2.5. Drug-drug interaction between mavoglurant and ketoconazole in adult subjects

Table A2.2 Simcyp default values of the inhibition-related ketoconazole parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{up}$</td>
<td>Fraction unbound in plasma</td>
<td>0.029</td>
</tr>
<tr>
<td>$f_{u_{ENT}}$</td>
<td>Fraction unbound in enterocytes</td>
<td>0.060</td>
</tr>
<tr>
<td>$K_{p,li}$</td>
<td>Liver-to-plasma partition coefficient</td>
<td>1</td>
</tr>
<tr>
<td>$K_i$ (µM)</td>
<td>Inhibition constant</td>
<td></td>
</tr>
<tr>
<td>CYP2C8</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>CYP2C9</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>$f_{u_{mic}}$</td>
<td>Fraction unbound to microsomes</td>
<td></td>
</tr>
<tr>
<td>CYP2C8</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>CYP2C9</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td>0.97</td>
</tr>
</tbody>
</table>
Figure A2.2 Ketoconazole mean concentrations in the liver (blue open circles) and portal vein (red open circles) plotted against time. The profiles were simulated using the minimal physiologically-based pharmacokinetic model for ketoconazole implemented in Simcyp. The concentration-time data on the 5th day (from 96 h post-dose) were used as forcing function for the drug-drug interaction mechanism in the physiologically-based pharmacokinetic model for mavoglurant.
A2.6. Results of the Bayesian analysis of Study 1 data when estimating all drug-specific parameters

Figure A2.3 Trace-plots of the three Markov chains run for the Bayesian population analysis of Study 1 data. Log-transformed parameter values (except for $\sigma^2$ and $\omega^2_{CL_{int,LI}}$) are plotted against the number of iteration ($\times10^5$) of the MCMC simulation. See Chapter 3 for definition of symbols. These results show that the marginal distributions of the parameters that have negligible influence on the plasma response (e.g., $K_{b,ki}$ or $K_{b,LU}$ as shown in Fig. 3.4) are not converging which suggests that the model is numerically unidentifiable.

A2.7. References


Appendix A3: Supplementary material for Chapter 4
### Table A3.1 Prior and posterior distributions of the drug-specific parameter values for mavoglurant reduced PBPK model (STATMOD 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prior distribution</th>
<th>Posterior distribution</th>
<th>Iterations ($\times 10^4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CL}_{\text{int},L}$ (l/h)</td>
<td>2017 (1.30)</td>
<td>1688 (1.04)</td>
<td>1.00 1.00 1.00</td>
</tr>
<tr>
<td>$k_{\text{out},LUM}$ (h$^{-1}$)</td>
<td>19.72 (1.22)</td>
<td>7.16 (1.11)</td>
<td>1.20 1.05 1.01</td>
</tr>
<tr>
<td>$K_{B,\text{BR}}$</td>
<td>3.04 (1.35)</td>
<td>4.34 (1.25)</td>
<td>1.73 1.09 1.01</td>
</tr>
<tr>
<td>$K_{B,\text{MU}}$</td>
<td>1.38 (1.35)</td>
<td>2.30 (1.07)</td>
<td>1.08 1.02 1.00</td>
</tr>
<tr>
<td>$K_{B,\text{AD}}$</td>
<td>7.43 (1.35)</td>
<td>10.54 (1.06)</td>
<td>1.06 1.02 1.00</td>
</tr>
<tr>
<td>$k_{\text{out},\text{SPL}}$ (h$^{-1}$)</td>
<td>26.13 (1.17)</td>
<td>19.60 (1.41)</td>
<td>4.47 1.03 1.03</td>
</tr>
<tr>
<td>$K_{B,\text{LI}}$</td>
<td>5.82 (1.35)</td>
<td>8.98 (1.27)</td>
<td>3.07 1.05 1.02</td>
</tr>
<tr>
<td>$\omega^2_{\text{CL}_{\text{int},L}}$</td>
<td>0.1 ($\nu = 1$)</td>
<td>0.173 (0.0247)</td>
<td>1.00 1.00 1.00</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>-</td>
<td>0.0765 (0.0029)</td>
<td>1.03 1.01 1.00</td>
</tr>
</tbody>
</table>

$\hat{R}$ is the potential scale reduction statistic

No prior information was considered for the residual variance $\sigma^2$

Prior distribution was assumed multivariate log-normal for the population median parameters and inverse-Wishart for the variance $\omega^2_{\text{CL}_{\text{int},L}}$

For the population median parameters, both prior and posterior marginal distributions are expressed as geometric mean (geometric standard deviation)

For the variance $\omega^2_{\text{CL}_{\text{int},L}}$, the prior marginal distribution is expressed as expected value (degrees of freedom of the inverse-Wishart distribution $\nu$) and the posterior marginal distribution as arithmetic mean (standard deviation)
Table A3.2 Estimated effective sample size of the posterior sequences obtained with STATMOD 1 and STATMOD 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effective sample size</th>
<th>STATMOD 1</th>
<th>STATMOD 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_{int,LI}$</td>
<td>3230</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>$k_{out,LUM}$</td>
<td>217</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>$K_{b,BR}$</td>
<td>64</td>
<td>2560</td>
<td></td>
</tr>
<tr>
<td>$K_{b,MU}$</td>
<td>608</td>
<td>3180</td>
<td></td>
</tr>
<tr>
<td>$K_{b,AD}$</td>
<td>758</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>$k_{out,SPL}$</td>
<td>24</td>
<td>2040</td>
<td></td>
</tr>
<tr>
<td>$K_{b,LI}$</td>
<td>46</td>
<td>2250</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{CL_{int,LI}}$</td>
<td>3000</td>
<td>2870</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{k_{out,LUM}}$</td>
<td>-</td>
<td>2550</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{K_b,BR}$</td>
<td>-</td>
<td>3170</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{K_b,MU}$</td>
<td>-</td>
<td>2770</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{K_b,AD}$</td>
<td>-</td>
<td>2890</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{k_{out,SPL}}$</td>
<td>-</td>
<td>2900</td>
<td></td>
</tr>
<tr>
<td>$\omega^2_{K_b,LI}$</td>
<td>-</td>
<td>3160</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>2860</td>
<td>3110</td>
<td></td>
</tr>
</tbody>
</table>

Calculated on a total of 3,000 samples obtained by discarding the first 360,000 for STATMOD 1 and the first 8,000 iterations for STATMOD 2, then thinning each of the 3 Markov chains to keep 1,000 samples and pooling the chains together.

When the MCMC samples are not auto-correlated, the estimate of the effective sample size should be close to the total sample size (3,000 samples).

The smaller effective sample size estimate the higher uncertainty in the posterior quantities (e.g. means and variances).
A3.2. Supplementary figures

Figure A3.1 Trace-plots of the three Markov chains run for the Bayesian analysis of mavoglurant clinical data (STATMOD 1). Parameter values (log-transformed for the fixed-effects) are plotted against the number of iterations (×10^5) of the MCMC simulation. See Chapter 4 for definition of symbols.
Figure A3.2 Posterior (solid lines) and prior (dashed lines) marginal densities of the log-transformed population parameters estimated during the Bayesian analysis of mavoglurant clinical data (STATMOD 2). See Chapter 4 for definition of symbols.
Figure A3.3 Plots of the confidence intervals (90%) around mavoglurant median kinetic profiles in the adipose (solid lines) and muscle (dashed lines) tissues simulated every 0.1 h for 48 h with the original whole-body PBPK model after an intravenous infusion (10 min) of 50 mg, for 1000 parameter sets sampled from the prior distribution.
Appendix A4: Supplementary material for Chapter 5
A4.1. Stan code for the tumour size time-series model

data {
  int<lower=0> Nsub;              // number of subjects
  int<lower=0> Nobs;              // total number of observations
  int<lower=0> ID[Nobs];          // subject mapping at each record
  vector<lower=0>[Nobs] TIME;     // observation times
  vector[Nobs] log_TS;            // log-transformed observations
}

parameters {
  vector<lower=-7,upper=6>[3] theta;     // population means
  vector[3] eta[Nsub];                   // variability random effects
  vector<lower=0,upper=5>[3] omega;      // population SDs
  real<lower=0> sigma;                   // residual SD
}

transformed parameters {
  vector[Nsub] BASE;
  vector[Nsub] SR;
  vector[Nsub] PR;
  vector[Nobs] log_f;   // log-transformed individual predictions
  for(i in 1:Nsub){
  }
  for(j in 1:Nobs){
    int ID_j;
    real f;
    ID_j <- ID[j];
    f <- BASE[ID_j] * exp(-SR[ID_j] * TIME[j]) + PR[ID_j] * TIME[j];
    log_f[j] <- log(f);
  }
}

model {
  // Likelihood
  log_TS ~ normal(log_f, sigma);

  // Population variability
  for(i in 1:Nsub)
    eta[i] ~ normal(0, 1);

  // Priors
  theta ~ normal(0, 1000);
  omega ~ cauchy(0, 5);
  sigma ~ cauchy(0, 5);
}

generated quantities {
  vector[Nobs] log_lik;
  for(n in 1:Nobs)
    log_lik[n] <- normal_log(log_TS[n], log_f[n], sigma);
}
A4.2. Stan code for the Weibull accelerated failure time model

```stan
/* Variable naming:
obs = observed
cen = (right) censored
N = number of samples
M = number of covariates
T = time to event
X = covariates */

data {
  int<lower=0> Nsub;
  int<lower=0> Nobs;
  int<lower=0> Ncen;
  int<lower=0> M;
  vector[Nobs] Tobs;
  vector[Ncen] Tcen;
  matrix[Nobs,M] Xobs;
  matrix[Ncen,M] Xcen;
}

parameters {
  real<lower=0> alpha;          // shape
  real theta0;                  // typical value of log(sigma)
  vector[M] theta;              // covariate effects
}

transformed parameters {
  vector<lower=0>[Nobs] sigma_obs;
  vector<lower=0>[Ncen] sigma_cen;
  sigma_obs <- exp( theta0 + Xobs * theta);
  sigma_cen <- exp( theta0 + Xcen * theta);
}

model {
  // Likelihood
  Tobs ~ weibull(alpha, sigma_obs);
  increment_log_prob(weibull_ccdf_log(Tcen, alpha, sigma_cen));

  // Priors
  alpha ~ lognormal(1,1000);
  theta0 ~ normal(0,1000);
  theta ~ normal(0,1000);
}

generated quantities {
  vector[Nobs] LL_obs;
  vector[Ncen] LL_cen;
  vector[Nsub] log_lik;

  // Log-likelihood to estimate LOO and WAIC
  for(n in 1:Nobs)
    LL_obs[n] <- weibull_log(Tobs[n], alpha, sigma_obs[n]);
  for(n in 1:Ncen)
    LL_cen[n] <- weibull_ccdf_log(Tcen[n], alpha, sigma_cen[n]);
  log_lik <- append_row(LL_obs, LL_cen);
}
A4.3. Supplementary figures

**Figure A4.1** A visual evaluation of the ability of the tumour size time-series model to describe the training and validation data. The *black open circles* are the observations, the *red lines* are the medians of the simulations and the *grey areas* are 90% prediction intervals.

**Figure A4.2** A visual evaluation of the ability of the lognormal and Weibull accelerated failure time models to describe the survival data in the reduced-training set. The observed median survival curves (*solid black lines*) are plotted along with their 95% confidence intervals (*dashed black lines*) as well as with the simulated median survival curves (*solid blue lines*) and their 95% credible intervals (*blue areas*)
Figure A4.3 Scaled Schoenfeld residuals (open circles) plotted against time for the two variables retained in the COX₁ model, namely TS₀ and PTR_{max}. The solid lines represent fitted natural splines and the dashed lines their 95% confidence interval.