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IMI – Oral Biopharmaceutics Tools project – Evaluation of Bottom-up PBPK Prediction Success Part 2: An Introduction to the Simulation Exercise and Overview of Results

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Graphical Abstract

Abstract

Orally administered drugs are subject to a number of barriers impacting bioavailability (F_{oral}), causing challenges during drug and formulation development. Physiologically-based pharmacokinetic (PBPK) modelling can help during drug and formulation development by providing quantitative predictions through a systems approach. The performance of three

available PBPK software packages (GI-Sim, Simcyp[®], and GastroPlus[™]) were evaluated by comparing simulated and observed pharmacokinetic (PK) parameters.

Since the availability of input parameters was heterogeneous and highly variable, caution is required when interpreting the results of this exercise. Additionally, this prospective simulation exercise may not be representative of prospective modelling in industry, as API information was limited to sparse details.

43 active pharmaceutical ingredients (APIs) from the OrBiTo database were selected for the exercise. Over 4,000 simulation output files were generated, representing over 2,550 study arm-institution-software combinations and approximately 600 human clinical study arms simulated with overlap. 84% of the simulated study arms represented administration of immediate release formulations, 11% prolonged or delayed release, and 5% intravenous (i.v.).

Higher percentages of i.v. predicted area under the curve (AUC) were within two-fold of observed (52.9%) compared to per oral (p.o.) (37.2%), however, F_{oral} and relative AUC (F_{rel}) between p.o. formulations and solutions were generally well predicted (64.7% and 75.0%).

Predictive performance declined progressing from i.v. to solution and immediate release tablet, indicating the compounding error with each layer of complexity. Overall performance was comparable to previous large-scale evaluations.

A general overprediction of AUC was observed with average fold error (AFE) of 1.56 over all simulations. AFE ranged from 0.0361 to 64.0 across the 43 APIs, with 25 showing overpredictions. Discrepancies between software packages were observed for a few APIs, the largest being 606, 171, and 81.7-fold differences in AFE between SimCYP and GI-Sim, however average performance was relatively consistent across the three software platforms.

Keywords:

Physiologically-based pharmacokinetics (PBPK); modelling and simulation (M&S);
absorption; oral bioavailability (F_{oral}); biopharmaceutics; drug database

Abbreviations:

API = Active pharmaceutical ingredient,

AUC = Area under the curve,

BCS = Biopharmaceutics classification system,

BE = Bioequivalence

BP = Blood-to-plasma ratio,

CL = Clearance,

CL/F = Apparent clearance

C_{max} = Maximum concentration,

D_o = Dose number,

f_a = Fraction absorbed,

FIM = First In Man

F_{oral} = Absolute oral bioavailability,

F_{rel} = Relative bioavailability,

f_{up} = fraction unbound in plasma,

IMI = Innovative Medicines Initiative,

i.v. = Intravenous,

IR = Immediate release,

LogP = Logarithm of the octanol/water partition coefficient,

LogD_{pH} = Logarithm of the octanol/water partition coefficient at a given pH,

MTD = Maximal Tolerated Dose

MW = Molecular weight,

PBPK = Physiologically-based pharmacokinetic,

PAC = Post Approval Changes

PK = Pharmacokinetic,

p.o. = per oral,

QbD = Quality by Design

SME = Small or Medium Enterprise

t_{max} = Time at maximum concentration,

V_d = Volume of distribution,

V_d/F = Apparent volume of distribution,

1. Introduction

The oral route is considered the most favourable for drug administration, however orally administered drugs are subject to a number of barriers that may result in variable oral bioavailability (F_{oral}) and systemic exposure. Such barriers include dissolution and potential precipitation in the gut lumen, chemical or enzymatic drug degradation, binding to constituents of the lumen, permeation through the gut wall, intestinal influx/efflux and intestinal and hepatic first-pass metabolism, to name the most crucial. These barriers have the potential to cause considerable challenges in drug and formulation development due to unexpected behaviour in oral drug exposure. Physiologically-based pharmacokinetic (PBPK) modelling and simulation (M&S) may aid biopharmaceutical development, in a manner similar to its role in the prediction of drug-drug interactions (Messier, 1960; Kostewicz et al., 2013). PBPK M&S can provide rationale and insight into the key underlying processes governing oral exposure and ultimately provide quantitative predictions of biopharmaceutic exposure through a systems approach *i.e.*, with the incorporation of *in vitro* and *in vivo* data (Huang et al., 2013; Thondre et al., 2013). This information is utilised throughout the drug development value chain within the pharmaceutical industry to make informed decisions around key milestones and, under certain conditions, to waive some *in vivo* human evaluations in late stage development.

Figure 1: The role of *in silico* modelling in the oral formulation and drug development value chain. BE = Bioequivalence; QbD = quality by design; PAC = post approval changes

At the time of drug pre-candidate selection, the ability of different compounds to be used commercially as pharmaceutical drugs can be assessed *in silico* to aid in selecting the best candidate to develop (Bueters et al., 2013). This decision is based on measured *in vivo* preclinical or *in vitro* drug efficacy, and predicted human *in vivo* absorption, first pass intestinal and hepatic extraction, distribution and elimination. It is also used to guide formulation scientists in the first in man (FIM) formulation choice and development, and alert them to possible limitations such as particle size, potential *in vivo* precipitation or degradation, or permeability limitations, in order to decide on the best formulation strategy and potential impact of certain excipients (Figure 1) (Willmann et al., 2010; Mitra et al., 2011; Chen et al., 2012).

Once human data have been generated the PBPK M&S models are refined with additional inputs, and can be used to define the best commercial formulation strategy. Throughout the

development value chain, *in silico* models can be used to assist the quality by design and drug product control strategies (Bredael et al., 2014; Yu et al., 2014; Kesisoglou et al., 2015). In particular, they can help justify the specifications and method used for dissolution testing of the drug product together with the specifications for particle size or the acceptance level of another polymorph in the formulation. During the approval process, modelling can be used to support responses to regulatory authorities and, in post approval, to justify changes that are beyond the current Scale Up and Post Approval Changes (SUPAC) guidances (Johnson et al., 2014; Pepin et al., 2016).

In the field of generic formulations or life cycle management, most of the human data are known and the inputs needed for PBPK M&S tools can be found in the literature or can be generated *in vitro*. The *in silico* tools can therefore be used at the ideation stage to define the best dose/release profile/formulation properties that could increase the medical value of the marketed drug (e.g. through reduction of side effects or increase in efficacy/robustness). The tools are also used to assist prototype selection and anticipate the outcome of bioequivalence studies (Figure 2).

Figure 2: The role of *in silico* modelling in late stage formulation development and generics.

(LCM = life cycle management; IVIVE = *in vitro* to *in vivo* extrapolation)

Today, such late stage use of PBPK M&S for oral absorption modelling are not routine in the industry or for the regulators and need to be justified/validated with *in vivo* data on similar formulations of the drug tested in humans. Examples of biowaivers based on *in silico* modelling for oral formulations are limited in the literature. The IMI (Innovative Medicines Initiative) OrBiTo (Oral Biopharmaceutical Tools) project attempts to address that gap through a holistic approach of data-sharing, refinement of *in vitro* and *in silico* tools and the integration of novel understanding of the gastrointestinal (GI) tract physiology (Lennernas et al., 2013).

In our previous work (Margolskee et al. – Part 1 – Submitted) we demonstrated the setup and characteristics of the OrBiTo database of APIs (active pharmaceutical ingredients), which contained drug and formulation-specific data along with preclinical and clinical data for 83

APIs, 43 of which were selected to be simulated in a large-scale performance evaluation of current state of the art *in silico* PBPK modelling platforms (see section 2.1 for selection criteria). This large-scale evaluation of current PBPK absorption models was seen as a first step toward identifying the strengths and weakness of current modelling software and approaches, and to guide further model development. Here we focus on the setup of this simulation exercise which involved the evaluation of three established software packages, GI-Sim (AstraZeneca, London, UK), Simcyp[®] Simulator (Certara, Sheffield, UK), and GastroPlus[™] (SimulationsPlus, Lancaster, CA), and we present an overview of the results.

2. Methods

2.1. API Selection

As discussed in our companion paper, the APIs chosen for the simulation set were selected based on availability of information from a set of 83 compounds comprising the OrBiTo API database. The simulation set contained 43 APIs, and were chosen based on having the following minimum criteria: molecular weight, logP or logD, at least one solubility point estimate or dissolution profile, *in vitro* permeability with reference compounds, any form of human *in vitro* clearance (e.g. measured in human liver microsomes, human hepatocytes, or recombinant CYP) or preclinical i.v. data (allowing for allometrically scaled clearance), and human fraction unbound in plasma (Margolskee et al. – Part 1 – Submitted). The 43 APIs chosen represented over 165 human studies, and approximately 600 clinical study arms.

2.2. API allocation

Each API selected for this exercise was to be simulated by at least one institution in each of the three software packages. In order to test the impact of user differences, ten APIs (A0048, A1897, A2101, A3028, A3336, A3609, A4492, A6555, A9081, and A9606) were chosen to be simulated four times by different institutions in each of SimCYP[®] and GastroPlus[™]. Due to restricted availability of GI-Sim to participating institutions, each API could only be simulated once in this software package. Additionally, one API (A2733) was chosen to be simulated by all participating institutions in each software package available to them.

Each EFPIA partner was asked to randomly select API-software combinations to fulfil their resource allocation, while ensuring not to choose an API belonging to their own portfolio, in order to avoid introducing any unwelcomed bias that could arise from knowing the outcomes of the clinical trials. The remaining API-software combinations were distributed evenly among the academic institutions and commercial enterprises.

2.3. Simulation procedure

Each participating institution was asked to generate bottom-up predictions for every human study arm associated with their allocated APIs. Modellers were asked to simulate both population representative simulations as well as simulated trials (for GastroPlus and SimCYP) for each study arm. The simulated trials were set up using healthy volunteer populations built into the programs. The geometric mean age, height and weight of the observed trial were used to set both the population representative simulations and the simulated trial. The number of subjects for the simulated trials was set to match the observed trials. The modellers were asked to document their process through performing the simulations along with their selections for input parameters and simulation options. The modelling and simulation documentation and simulation output files coming directly from the

software programs were uploaded and frozen on a shared space for analysis. After simulation output upload was complete, the observed PK human data were unblinded in the OrBiTo database to allow for the analysis of predicted vs measured data.

Overall guidance was drafted on how to handle the estimation of any missing parameters allowed in the simulation set. For example, when blood-to-plasma ratio (BP) was missing, it was suggested to be taken as 1 for neutral and basic compounds, and 0.55 for acidic compounds. For allometric scaling of clearance, the recommended methods were the two best performing methods from Poulin et al. 2011 (referred to by them as TS_rat-dog and FCIM) or simple allometry. Due to lack of data on renal clearance, it was recommended at a minimum to use the fraction unbound times the glomerular filtration rate. For *in vitro* clearance data that were missing the unbound concentration in the incubation, it was recommended to use one of the various available estimation models (e.g. Austin et al. 2002, Halifax and Houston 2006; Turner et al. 2006).

However, due to the diversity of the parameters describing the APIs and how the *in vitro* data was obtained (in many cases data from multiple methods were available) along with software differences, it was decided against establishing standard operating procedures (SOPs) for selection and calculation of input parameters at the advantage of allowing the assessment of user differences between modellers and institutions. However, SimCYP and GI-Sim did create SOPs for entering the information into their software platforms, once the choice input parameters were decided upon. An overlap in the simulation study design between software platforms and modellers was implemented, giving the unique opportunity to enable the assessment of user and software differences in prediction success.

2.4. Data Collection

Automated extraction of input options, as selected by modellers, was carried out using Matlab[™] R2013a (Mathworks, Natick, MA). Input options that were not available from simulation output files were manually recorded by modellers in Microsoft Excel[®], 2010 (Microsoft, Redmond, WA). Following the completion of the simulation exercise, simulated plasma concentration-time profiles were extracted from simulation output files using Matlab and transferred into MS Excel templates which contained automated calculation of pharmacokinetic (PK) parameters. Following the unblinding of the clinical data, observed plasma concentration-time profiles were treated in the same manner as the simulated profiles.

2.5. Parameter Calculations

Quality indicators for the evaluation of prediction success were selected prior to the simulation exercise based on metrics used in the literature, specific to PK predictions. The common practice in the PK community is the comparison of predicted vs. observed pharmacokinetic parameters, such as: the area under the curve (AUC), the maximum concentration ($C_{\max}$) and half-life (Poulin et al., 2011b; Van den Bergh et al., 2011; Sjogren et al., 2013), as well as direct comparison of predicted vs. observed plasma concentration time profiles.

Table 1. Pharmacokinetic parameters to be evaluated.

All formulations	Oral formulations	i.v. formulations	Oral & i.v.	Combinations of studies and formulations
$AUC_{0-Tlast}$ AUC_{0-Inf} C_{max} T_{max} $T_{1/2}$	CL/F V_d/F MTT	CL V_d MRT	F_{oral} MAT	Relative F Relative C_{max} Relative AUC
$AUC_{0-Tlast}$ = Area under the curve from time zero to last measured time point, AUC_{0-Inf} = Area under the curve from time zero extrapolated to infinity, C_{max} = Maximum concentration, CL = Clearance, F = bioavailability, F_{oral} = oral bioavailability, i.v. = intravenous, MAT = Mean absorption time, MRT = Mean residence time, MTT = Mean transit time, t_{max} = Time of peak concentration, V_d = Volume of distribution				

Table 1 lists different PK parameters of interest, for different formulations, oral and i.v. study arms, and combinations of study arms and formulations. Each of the parameters for individual study arms was calculated for the observed plasma concentration profiles as well as the predicted profiles, using the same time points as the observed data.

Individual profiles, where available, were used to calculate all PK parameters, and the central tendency and deviation (Geometric Mean & SD) of these parameters were calculated for each particular study arm. This method of estimating the PK parameters was applied to all simulated trials, population representative simulations, as well as all observed study arms for which individual data were supplied. For observed study arms which did not report individual data, PK parameters were calculated on the mean profile, and compared with PK parameters calculated on the mean profile of simulated trials.

Table 2: Pharmacokinetic (PK) Parameter calculations

Name	Symbol	Calculation
Area under the curve from zero to the last time point	$AUC_{0-t_{last}}$	$\sum_{i=0}^N \frac{1}{2} (C(t_{i+1}) + C(t_i)) \cdot (t_{i+1} - t_i)$
Area under the curve from zero to infinity	AUC_{0-inf}	$AUC_{0-t_{last}} + \frac{C(t_{last})}{k}$
Maximum plasma concentration	C_{max}	$\max(C(t_i)) \text{ over all } t_i$
Time at maximum concentration	T_{max}	$t_i \text{ such that } C(t_i) = C_{max}$
Half life	$T_{1/2}$	$\frac{\ln(2)}{k}$
Clearance or apparent oral clearance	CL or CL/F	$\frac{Dose}{AUC_{0-inf}}$
Volume of distribution or apparent oral volume of distribution	V_d or V_d/F	$\frac{CL \text{ or } CL/F}{k}$
Area under the moment curve from zero to last time point	$AUMC_{0-t_{last}}$	$\sum_{i=0}^N \frac{1}{2} (t_{i+1} C(t_{i+1}) + t_i C(t_i)) \cdot (t_{i+1} - t_i)$
Area under the moment curve from zero to infinity	$AUMC_{0-inf}$	$AUMC_{0-t_{last}} + \frac{C(t_{last})}{k} \left(t_{last} + \frac{1}{k} \right)$
Mean residence time (for i.v. profiles) or mean transit time (for oral profiles)	MRT or MTT	$\frac{AUMC_{0-inf}}{AUC_{0-inf}}$
<p>$C(t)$ = plasma concentration at time t t_{last} = last observed time point k = terminal exponential decay rate estimated from an exponential fit ($C(t) = A e^{-kt}$) to the terminal plasma concentration profile following the adjusted R squared algorithm as detailed in Phoenix WinNonlin (Pharsight Corporation, CA).</p>		

2.6. Summary Statistics

The performance of the models to predict the PK parameters can be evaluated through different metrics such as Average Fold and Root Mean Squared Errors (Van den Bergh et al., 2011), as well as Absolute Average Fold Errors and correlation and Concordance Correlation

Coefficients (Poulin et al., 2011a). Each of these metrics provides a different measure for the prediction, each with its own interpretation, and provides a different perspective on the performance of the simulations. For example the percent of predictions within a certain fold error provides a measure of accuracy (i.e. the magnitude of the deviation from observed) while the average fold error measures the bias of the predictions (i.e. the central tendency of the deviation). Table 3 lists the metrics that were applied to analyse the success of prediction of PK parameters.

Table 3: Metrics for evaluating success of predictions

Name	Symbol	Formula	Description	References
% within 2-, 3-, 10-fold error		$freq \left(\max \left(\frac{Pred_i}{Obs_i}, \frac{Obs_i}{Pred_i} \right) < 2, 3, 10 \right)$	Gives an idea of the magnitude of the error; however meaningful cut-offs have not been rigorously defined.	(Takamatsu et al., 2001; Rodgers et al., 2005; Poulin et al., 2011a; Van den Bergh et al., 2011)
Average fold error	AFE	$10^{\frac{1}{n} \sum \log \left(\frac{Pred_i}{Obs_i} \right)}$ $= \prod_{i=1}^n \left(\frac{Pred_i}{Obs_i} \right)^{\frac{1}{n}}$	With the AFE, underpredictions and overpredictions cancel each other out, resulting in a measure of bias only.	(Takamatsu et al., 2001; Poulin et al., 2011a; Van den Bergh et al., 2011)
Absolute average fold error	AAFE	$10^{\frac{1}{n} \sum \left \log \left(\frac{Pred_i}{Obs_i} \right) \right }$ $= \prod_{i=1}^n \left(\max \left(\frac{Pred_i}{Obs_i}, \frac{Obs_i}{Pred_i} \right) \right)^{\frac{1}{n}}$	The AAFE converts negative log fold errors to positive values before averaging, measuring the spread of the predictions.	(Takamatsu et al., 2001; Poulin et al., 2011a)
Root mean squared log error	RMSLE	$\sqrt{\frac{1}{n} \sum (\log(Pred_i) - \log(Obs_i))^2}$ $= \sqrt{\frac{1}{n} \sum \left(\log \left(\frac{Pred_i}{Obs_i} \right) \right)^2}$	The RMSLE combines the variance and bias of an estimator. For an unbiased estimator, the RMSLE represents the standard deviation between the predicted and observed log data.	(Takamatsu et al., 2001)
Correlation coefficient	R	$\frac{s_{xy}}{\sqrt{s_x^2 s_y^2}}$	A measure of the degree of linear relationship between predicted and observed data.	(Poulin et al., 2011b)
Concordance correlation coefficient	CCC	$\frac{2s_{xy}}{s_x^2 + s_y^2 + (\bar{x} - \bar{y})^2}$ Where $s_{xy} = \frac{1}{n} \sum (x_i - \bar{x})(y_i - \bar{y})$ $s_x^2 = \frac{1}{n} \sum (x_i - \bar{x})^2$	The CCC is a measure of the degree of 1-to-1 correlation between predicted and observed data. It is based on the perpendicular deviation from the 45 degree line.	(Takamatsu et al., 2001; Winiwarter et al., 2003)

		$s_y^2 = \frac{1}{n} \sum (y_i - \bar{y})^2$	
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3. Results and Discussion

3.1. Overview

By the end of the simulation exercise, an endeavour set to last six months, over 4,000 simulation files had been generated by the participating institutions, representing over 2,550 study arm-institution-software combinations. Table 4 displays the breakdown of contribution by partner, detailing the number of study arms and APIs simulated in each software package. Figure 3 shows boxplots for fold error in $AUC_{0-t, last}$ and C_{max} predictions for all single dose, fasted state, i.v. and p.o. simulations, grouped by institution, institution type, modeller experience, and software package.

Table 4: Breakdown of contributed simulations by partner.

	Number of study arms simulated				Number of APIs simulated			
	Total software-study arm combinations	In GastroPlus	In SimCYP	In GI-Sim	Total software-API combinations	In GastroPlus	In SimCYP	In GI-Sim
UNIMAN	576	163	151	262	42	13	13	16
AstraZeneca	249	53	54	142	24	5	5	14
Sanofi	234	94	140	0	15	7	8	0
SimCYP	221	0	221	0	20	0	20	0
Orion	216	216	0	0	16	16	0	0
UGoethe	198	79	78	41	17	6	6	5
Novartis	197	99	98	0	14	7	7	0
AbbVie	160	80	80	0	10	5	5	0
SimulationsPlus	121	121	0	0	11	11	0	0
Pfizer	99	70	29	0	7	5	2	0
Merck	88	44	44	0	8	4	4	0
Janssen	85	41	44	0	6	3	3	0
GSK	72	36	36	0	8	4	4	0
UMainz	38	19	19	0	4	2	2	0
BMS	2	2	0	0	1	1	0	0

(UNIMAN = The University of Manchester; UGoethe = Goethe Universität Frankfurt; GSK = GlaxoSmithKline; UMainz = Johannes Gutenberg Universität Mainz; BMS = Bristol-Myers Squibb).

Average prediction performance was consistent across the three different institution types (Figure 3C-D), indicating no institution type performed better on average than the others. However, noticeably higher variability in fold error was observed for academia. This could be related to the level of experience of these modellers as this group tended to have less experience. While average prediction performance appeared to be consistent across different levels of experience, an increase in variability was displayed for modellers with less than 5 years of experience (Figure 3E-F).

In the context of this simulation exercise, each of industry, small-medium enterprises (SMEs) and academic partners had the same level of access to information and familiarity with the pharmaceuticals in question, a fact that was facilitated by the blinded nature of the exercise. In practice, these considerations could potentially give rise to a greater difference in performance outcome.

Within each API, variability was observed across different modelling groups and software package utilised. The largest discrepancy observed across the three software packages was for API A8791 where the fold difference between SimCYP and GI-Sim predicted AFEs in $AUC_{0-t, last}$ predictions was 606-fold, followed by A0799 with 171 fold difference, and A0633 with 81.7 fold difference between SimCYP and GI-Sim predictions (Figure 4A; Table 5). These three APIs followed the same trend in C_{max} predictions with 593, 87.2, and 60.5-fold difference between SimCYP and GI-Sim predicted AFEs in C_{max} (Figure 4B; Table 6). While

considerable software differences in $AUC_{0-t, last}$ predictions were observed for some APIs, the average prediction performance was relatively consistent across the three different software platforms evaluated in this exercise ($AUC_{0-t, last}$ AFE of 1.52, 1.32, and 0.992 for GastroPlus, SimCYP, and GI-Sim, respectively; Figure 3G).

The limited availability of information to inform the models, as well as limited complexity in that information could be masking potential differences in performance that level of experience or software package choice could have provided. As was discussed in the companion paper, the OrBiTo database and the simulation set of APIs expressed a high level of missing certain information (e.g. solubility in biorelevant media, dissolution and precipitation rates, *in vivo* preclinical PK), as well as a relatively limited complexity in formulations (only 7.1%, 1.6% and 0.5% of the administered formulations in the database represented prolonged release, delayed release, and colon targeted formulations, respectively) (Margolskee et al. – Part 1 – Submitted).

Figure 3A displays the fold error in $AUC_{0-t, last}$ predictions grouped by institution. The average prediction performance was fairly consistent across the different institutions, with a few deviations (e.g. institutions 9, 12, 13, 14). Within institution $AUC_{0-t, last}$ AFE ranged from 0.148 (or 6.77-fold underprediction; institution 13) to 3.67-fold overprediction (institution 12) while C_{max} AFE ranged from 0.129 (7.73-fold underprediction; institution 13) to 2.58-fold overprediction (institution 3). Further exploration of these differences may help to inform standard procedures in PBPK modelling approaches.

Figure 4 shows the overall FE in $AUC_{0-t, last}$ predictions for all single dose, fasted state, i.v. and p.o. simulations, grouped by API. On average, the observed $AUC_{0-t, last}$ values were slightly overpredicted, with a median FE for $AUC_{0-t, last}$ of 1.56 over all of the simulations, while median FE for C_{max} was slightly underpredicted at 0.875 (1.14-fold underprediction). The overall 5th and 95th percentiles of FE in $AUC_{0-t, last}$ prediction were 0.0283-fold (representing a 35.3-fold underprediction) and 21.6-fold, respectively. For C_{max} , the 5th and 95th percentiles of FE were 0.0183 (54.6-fold underprediction) and 7.58-fold overprediction. 25 of the 43 APIs showed overpredictions in $AUC_{0-t, last}$ on average ($AFE > 1$), while only 15 showed overpredictions in C_{max} . Prediction performance showed a high between API variability, with $AUC_{0-t, last}$ AFE ranging from 0.0361 (or 27.7-fold underprediction) to 64.0-fold overprediction, and C_{max} AFE ranging from 0.0235 (or 42.6-fold underprediction) to 4.54-fold overprediction (Figure 4).

Figure 3: Boxplots for the fold error in $AUC_{0-t, last}$ (left panels) and C_{max} (right panels)

predictions for each studyarm-institution-software combination, grouped by **A-B**: institution (numbered 1 through 15), **C-D**: Institution type (industry, small-medium enterprise (SME), or academia), **E-F**: years' experience with modelling and simulation (M&S), and **G-H**: software package.

A

B

Figure 4. Fold error in **A**: $AUC_{0-tlast}$ and **B**: C_{max} predictions for each simulation generated in GastroPlus (blue circles), SimCYP (cyan squares) and GI-Sim (red triangles), grouped by API, along with the overall 5th and 95th percentile interval (grey region) and overall median (black dashed line).

Table 5: Average fold error (AFE) in $AUC_{0-t, last}$ predictions for each API overall and in each software package

APIcode	Overall AFE	GastroPlus AFE	SimCYP AFE	GI-Sim AFE
A0048	6.7543	6.8319	5.3956	12.6761
A0608	0.2219		0.3787	0.1225
A0619	1.4222	1.1453	2.7476	1.0044
A0633	0.9903	3.0249	5.1218	0.0627
A0765	5.3576	5.5983	4.9872	5.5083
A0799	0.4961	1.3229	0.0232	3.9777
A0855	3.1253	3.2876	2.9711	
A1260	5.8776	3.7359	8.1481	5.8274
A1897	1.4291	1.3449	1.7175	1.3405
A2101	2.1308	2.9359	1.9341	0.7907
A2276	1.0551	2.5185	0.1455	3.2061
A2352	0.0902	0.0333	0.1452	0.1518
A2437	0.4488	0.3849	0.3387	0.6935
A2733	1.6738	2.079	1.5741	0.8235
A2771	1.519	1.8844	2.0469	0.9087
A2853	0.8215	0.9945	0.6651	
A3028	4.7808	6.8915	4.629	1.0741
A3078	2.1982	2.1429	2.2549	
A3336	3.8644	6.4545	2.5162	1.6538
A3427	2.5545	3.1029	1.6377	
A3609	1.1578	0.8301	1.7782	1.2095
A3837	0.8262	0.5465	1.104	0.5367
A4356	0.0361	0.1285	0.0101	
A4460	0.9534		0.5616	1.6185
A4492	0.1353	0.0967	0.1995	0.1254
A5262	2.3306	3.4974	1.5531	
A5637	27.9434	73.6427	15.9327	18.5959
A6099	2.4249	2.9093	2.0211	
A6135	63.9694	46.1723	93.9698	60.3319
A6555	0.1391	0.1558	0.1674	0.0104
A6598	7.2007	8.4175	6.1509	7.2489
A6882	1.0778	1.1835	0.9815	
A6939	0.4958	1.4499	0.1695	
A7294	1.381	0.6496	5.4808	0.7397
A7415	0.7766	1.0422	1.1228	0.4003
A7513	0.8864	0.6733	1.1876	0.8709
A7597	0.1138	0.1752	0.1574	0.0479
A7651	0.5155	0.5853	0.4681	0.5088
A8791	0.5038	0.9736	2.6648	0.0044

A8942	0.7461	0.6338	0.8615	
A9081	6.0196	6.1116	6.1725	3.847
A9606	3.636	4.3199	2.3232	13.0719
A9995	8.0456	0.8038	34.0971	19.0014

Table 6: Average fold error (AFE) in $C_{\max}$ predictions for each API overall and in each software package

APIcode	Overall AFE	GastroPlus AFE	SimCYP AFE	GI-Sim AFE
A0048	2.0674	2.139	1.431	5.264
A0608	0.04473		0.1421	0.01254
A0619	1.554	3.558	3.191	0.3613
A0633	0.5058	1.331	2.525	0.0417
A0765	0.9810	1.126	0.9203	0.9113
A0799	0.5855	3.507	0.04024	1.422
A0855	0.6337	0.8254	0.4865	
A1260	0.1344	0.3212	0.1952	0.05082
A1897	2.929	3.250	2.956	1.708
A2101	0.7184	0.9099	0.8518	0.1674
A2276	0.6693	2.608	0.1945	0.5910
A2352	0.02611	0.02454	0.02614	0.02774
A2437	0.6479	1.250	0.3486	0.6239
A2733	0.8034	0.9812	0.8973	0.2113
A2771	0.9234	1.120	1.023	0.6871
A2853	0.7228	0.7161	0.7302	
A3028	1.870	3.304	1.502	0.3317
A3078	0.3758	0.2966	0.4762	
A3336	0.5083	1.007	0.2907	0.2563
A3427	2.123	3.370	0.7600	
A3609	0.8739	0.8417	1.268	0.3325
A3837	2.149	2.162	2.141	2.155
A4356	0.2031	0.7702	0.05354	
A4460	1.120		0.4950	2.536
A4492	0.1574	0.1282	0.1726	0.2501
A5262	0.9425	1.063	0.8355	
A5637	1.442	2.729	1.432	0.7672
A6099	2.752	3.136	2.415	
A6135	4.537	4.046	4.327	5.387
A6555	0.06320	0.07080	0.06880	0.009279
A6598	2.045	3.526	2.675	0.8995
A6882	0.4183	0.5275	0.3318	
A6939	0.02347	0.07342	0.007500	

A7294	0.1950	0.1044	1.002	0.07086
A7415	0.2528	0.4711	0.3401	0.1009
A7513	1.760	0.4121	4.002	3.306
A7597	0.04496	0.05762	0.1036	0.01308
A7651	0.1067	0.1006	0.1232	0.09692
A8791	0.5090	0.9532	1.733	0.002921
A8942	0.2549	0.1863	0.3329	
A9081	2.639	2.713	2.605	1.246
A9606	1.472	1.153	1.412	5.521
A9995	3.910	0.9733	7.185	8.549

3.2. Observed vs. predicted regression lines

In order to gauge the overall predictive performance of the OrBiTo simulations for each of the 43 APIs in the simulation set, predicted and observed, absolute and relative bioavailability, and $AUC_{0-t, last}$ averaged by geometric mean over the study arms for each API were examined (Figure 5; Table A4). For all oral simulations (n APIs = 43), the correlation between predicted and observed $\log_{10}(AUC_{0-t})$ gave an R of 0.766 and a CCC of 0.759 (Figure 5A), or 0.103 and 0.0969, respectively, for the linear scale. The examination of i.v. only simulations (n APIs=17) gave slightly better regression coefficients (R: 0.908 and CCC: 0.870, or R: 0.500 and CCC: 0.438 for the linear scale) (Figure 5B). The overall trend in oral bioavailability (F_{oral} ; n APIs=17) showed lower correlations between predicted and observed, with an R of 0.346 and CCC of 0.345 (Figure 5C). However, these correlations were better than those for the linear scaled data for oral formulations. The overall ability to predict relative bioavailability between oral formulations and solutions gave an R of 0.443 and CCC of 0.389 between observed and predicted data (Figure 5D). For more information on the types of oral formulations available in the database, see our companion paper (Margolskee et al. – Part 1 – Submitted).

Figure 5: Overall predictive performance of the OrBiTo simulation dataset, displaying the geometric mean observed against predicted data for **A:** Log₁₀(AUC_{0-t,last}) for observed against predicted oral data only, **B:** Log₁₀(AUC_{0-t,last}) for observed against predicted i.v.data only, **C:** Observed vs. predicted oral bioavailability (F_{oral}), **D:** Observed vs. predicted relative bioavailability (F_{rel}) for per oral formulations as compared to immediate-release formulations. R=Pearson regression coefficient, CCC=concordance correlation coefficient.

3.3. Overview by different formulations

A total of 594 human study arms were simulated by the different institutions, of which 445 were both single dose and in the fasted state. Of these 445 study arms, 22 had administered

i.v. formulations, 376 immediate-release (IR) formulations, 32 prolonged release, 3 delayed release and 12 enterion coated (Table 7).

Table 7. Number of simulated study arms and APIs administering different formulations types.

Formulation Type	Number of Study Arms	Number of APIs
i.v. Bolus	9	7
i.v. Infusion	13	10
Solution	131	28
IR Suspension	29	10
IR Tablet	102	31
IR Capsule	100	16
IR Solid dispersion	7	1
IR Nano-suspension	7	1
Prolonged Release Tablet	31	5
Prolonged Release Capsule	1	1
Delayed release (time based) Capsule	3	1
Enterion	12	7

Progressing from i.v. administration to oral solution, IR tablet and all p.o. formulations, there was a reduction in $AUC_{0-t, last}$ prediction performance as measured by percent within 2, 3, and 10-fold error and correlation coefficients, R and CCC. This is indicative of the compounding error that occurs with each layer of model complexity, highlighting the challenges of predicting oral bioavailability and the need for clinically informed i.v. disposition for the proper investigation of intestinal model performance. Examining the predictive performance of bioavailability can partially account for these confounding errors however F_{oral} calculations were limited to 17 APIs in the OrBiTo simulation dataset. Many of the APIs were lacking i.v. data; however, it was possible to compare relative bioavailability between oral formulations (Figure 6; Table A4).

A general trend of overprediction of exposure was observed for p.o. and i.v. simulations as compared to observed data with a calculated AFE of 1.30 and 1.64, respectively (Figure 6B; Table A4). Overprediction for solutions was even higher with an AFE of 1.97. The variability in FEs was slightly higher for p.o. and solution simulations with AAFE above 3 (3.21 and 3.04) as compared with i.v. FEs, displaying an AAFE of 2.51. Similarly, a higher percentage of i.v. simulations were predicted to within two and three-fold (52.9% and 70.6%) as compared with all p.o. simulations (37.2% and 55.8%; Figure 6A).

F_{oral} was generally well predicted, with 64.7%, 53.8% and 70.0% within two-fold for all p.o. formulations (n=17), for IR tablets (n=13), and solutions (n=10), and displaying AFE of 1.00, 0.89 (a 1.12-fold underprediction), and 1.10 (Figure 6D and E; Table A4). However, poor correlation between observed and predicted was exhibited for bioavailability of solutions ($R = 0.111$; CCC -0.106) (Figure 6F).

The poor correlation between observed and predicted solution F_{oral} ($R = -0.111$ and CCC -0.106; Figure 6F) raises some concerns about the consistency in predictions of permeation, as other formulations showed slightly better correlation between predicted and observed bioavailability ($R = 0.35$ and 0.178 for p.o. and IR tablet, respectively), and F_{rel} between p.o. formulations and solution showed even greater improvement (R of 0.44 and 0.6 for p.o. formulations and IR tablet; Figure 6I). One might expect the prediction of F_{oral} for solutions to have better correlation than for other formulations since permeation is not generally thought to be as big of a hurdle to overcome as solubility. However, these results could be interpreted as permeability and first pass effects not being as well characterised, or the input parameters not as informative as those for solubility and dissolution.

The focus of the OrBiTo project is on oral bioavailability and biopharmaceutics, thus the key interest of the simulation exercise is the predictive ability of the *in silico* methods to predict release, dissolution, permeation, and intestinal first pass. Pure bottom-up prediction of p.o. study arms can be confounded by errors in prediction of disposition. Here we attempted to account for these errors by looking at relative AUC between i.v. and p.o. simulations, however this could only be performed for the subset of APIs for which i.v. data was available. Similarly, relative AUC between p.o. formulations and oral solutions attempted to untangle the prediction of permeation and first pass extraction from that of luminal release, dissolution and potential precipitation of drug product.

Predictions of relative AUC between all p.o. formulations (other than solution) and solutions (F_{rel}) performed very well, with 75.0%, 79.2% and 100% within 2-, 3- and 10-fold. Similarly predictions for F_{rel} between IR tablet and solution achieved 75.0%, 87.5% and 100% within 2-, 3-, and 10-fold. A general trend of underprediction was seen for both categories of F_{rel} , where the AFE was 0.744 and 0.708 for p.o. vs. solution and IR tablet vs. solution, equating to 1.35 and 1.41-fold underpredictions. Correlation between predicted and observed appeared to be an improvement compared to those for F_{oral} , with R of 0.443 and 0.601 and CCC of 0.389 and 0.504 for F_{rel} of p.o. and IR tablet, respectively, vs. solution (Figure 6G-I).

The general trend of underprediction for relative AUC between p.o. and solutions points to an underestimation of the extent of dissolution and/or release. However, this was a very slight negative bias on average, and the correlations between predicted and observed F_{rel} were in general very good, especially compared to that for F_{oral} . This suggests the models for dissolution and/or release perform well and consistently across different extents of dissolution.

There was a debate prior to the start of the simulation exercise as to whether the clinical i.v. study arms should be unblinded. This could have allowed for the use of observed elimination and distribution to inform disposition characteristics, shifting the focus of the performance of predictions to the behaviour of oral formulations along the intestine (i.e. release, dissolution, and permeation). However, there was also an interest in evaluating the performance of completely prospective predictions as one might do in practice, particularly in FIM study designs, necessitating the i.v. profiles remain blinded throughout the simulation exercise. Alternatively, a stepwise approach could have been adopted whereby all i.v. predictions were performed in one step, followed by an unblinding of the i.v. data with p.o. predictions following. However, given that there was a time constraint due to the urgency in opening up the database for use with other tasks within the OrBiTo, this was not undertaken.

Figure 6. Prediction metrics for $AUC_{0-t,last}$ (A-C), F_{oral} between p.o. and i.v. simulations (D-F) and F_{rel} between p.o. and solution simulations (G-I); <n-fold = % APIs within n-fold;

3.4. Comparison to literature exercises

The overall performance of the OrBiTo simulation exercise was comparable to previous large-scale PBPK model evaluations (Tannergren et al., 2004; Jones et al., 2011; Poulin et al.,

2011b). However, i.v. predictions tended to perform slightly worse and oral predictions slightly better than those in the literature (Figure 7A,B), with both i.v. and p.o. overpredicting $AUC_{0-t, last}$. This overprediction in exposure can be attributed to general underpredictions in i.v. clearance (Figure 7C,D), as the overall bias in predictions of i.v. V_{ss} and oral V_d/F were comparable to, if not better than, the other exercises (Figure 7E,F). As additional evidence, F_{oral} was generally well predicted (Figure 6D-F) compared to the trend of overpredictions for absolute $AUC_{0-t, last}$ (Figure 6A-C), pointing again to underpredictions of CL or V_{ss} as a potential source for the overprediction of drug exposure. An underprediction of CL may also contribute to an overprediction of F_{oral} , through an underprediction of hepatic and intestinal first pass metabolism. However, it was difficult to assess this due to general lack of information regarding extent of intestinal metabolism with only 2/43 APIs reporting information on individual CYPs contributing to the overall clearance based on recombinant CYP *in vitro* data.

On average i.v. $AUC_{0-t, last}$ was slightly overpredicted with an AFE of 1.64, vs. AFE of 0.72 and 0.92 for Poulin 2011 (V_{ss} : JA, CL: FCIM) and (V_{ss} : TCK_{pu} , CL: IVIVE), respectively (Figure 7; Table A1). The percentage of predictions falling within two-fold of observed data was slightly lower as compared to Poulin 2011, with 53% for OrBiTo vs. 67% and 71% for Poulin (V_{ss} : JA, CL: FCIM) and (V_{ss} : TCK_{pu} , CL: IVIVE) (Poulin et al., 2011b). Oral $AUC_{0-t, last}$ was slightly overpredicted on average with AFE 1.30, however this outperformed most comparative PBPK M&S studies, where Poulin 2011: AFE ranging from 0.14 to 0.41 for different absorption and solubility estimates and De Buck 2007: method 1 AFE 5.09 (*in silico* volume of distribution and traditional IVIVE of metabolic clearance). The OrBiTo simulations underperformed compared to De Buck 2007 method 2 which displayed AFE of

1.06 (Rat K_p 's scaled to human, and metabolic clearance using IVIVE while ignoring effects of f_{up}/BP and $f_{u_{inc}}$) (Figure 7A,B; Table A1).

Intravenous CL was in general underpredicted for OrBiTo simulations (AFE 0.48, or a 2.08 fold underprediction), with only De Buck Method 1 underperforming compared to OrBiTo (AFE of 0.415, or 2.41-fold underprediction). Further, CL predictions for OrBiTo displayed a high degree of bias between predicted and observed data (AAFE of 2.97), comparable to that of De Buck Method 1 (AAFE 2.60), but higher than most other comparable exercises which displayed AAFE of 1.5 to 1.9 (Figure 7C,D; Table A2). It has been documented that *in vitro* systems tend to underpredict *in vivo* CL (Takeuchi et al., 1998). This is in agreement with the noticeable underpredictions of the IVIVE methods of Poulin et al. (2011) and De Buck et al. (2007) Method 1, and the underpredictions of CL in the current exercise, as the majority were informed by *in vitro* CL measurements.

Predictions of V_{ss} for OrBiTo simulations showed a slight overprediction with an AFE of 1.28 (n APIs=17), performing better compared to most other studies (AFE 2 to 2.1), except for De Buck V_d Method 2 (based on preclinical tissue:plasma partition coefficients) which performed better with a reported AFE of 1.14. Predictions of oral V_d/F also showed a slight overprediction on average, with AFE of 1.3, however performed better than De Buck Methods 1 and 2, with reported AFE of 1.38 and 0.0758 (1.32-fold underprediction), respectively. Despite the overall average trend performing relatively well compared with other literature exercises, the predictions of intravenous V_{ss} and oral V_d/F showed high variability for OrBiTo simulations (AAFE of 2.66 and 2.9), with only De Buck V_d method 1 showing higher variability (AAFE 3.50 and 4.79, for V_{ss} and V_d/F ; Figure 7E,F; Table A3).

Figure 7. Prediction metrics for i.v. and p.o. AUC_{0-t,last} (A, B), i.v. CL (C,D) and i.v. V_{ss} or p.o. V_d/F (E,F), compared with other physiologically-based pharmacokinetic (PBPK) simulation studies in the literature (Tannergren et al., 2004; Jones et al., 2011; Poulin et al., 2011b). ^a(V_{d1}, CL₁)= Prediction of V_{ss} using Poulin and Theil method, prediction of hepatic CL using IVIVE from human liver microsomes or hepatocytes alternatively using glomerular filtration rate ratio to predict renal CL; ^b(V_{d2}, CL₂) = Predicting V_{ss} from rat tissue:plasma partition coefficients obtained experimentally or if not available calculated via tissue composition-based Rodgers and Rowland method, predicting CL using in vitro-in vivo extrapolation (IVIVE) not considering binding; ^cCL_R = renal clearance; ^dPBPK(GastroPlus) = using GastroPlus ACAT model coupled with full PBPK distribution model; ^e1 comp model = using one-compartment approach; ^fPBPK (V_{ss}, JA; CL, FCIM) = Prediction of tissue:plasma partition coefficients (K_p's) from rat volume of distribution at steady state following i.v. infusion (V_{ss}) and lipophilicity (JA), scaling of

clearance (CL) using fraction unbound (f_u) correlated intercept method (FCIM) allometry; g PBPK(V_{ss} , TCK_{pu} ; CL, IVIVE) = Predicting V_{ss} using Rodgers and Rowland model, *in vitro-in vivo* extrapolation of CL; $^h(k_a, F_{abs1}) = k_a$ and f_a from average observed *in vivo* preclinical k_a and f_a estimated from F_{oral} and CL; $^i(k_a, F_{abs2}) = k_a$ from average observed *in vivo* preclinical k_a and f_a from average *in vivo* preclinical F_{oral} and CL; j (est Solfassif) = using estimated solubility in fasted simulated small intestinal fluid (FaSSIF); k (meas Solfassif) = using measured FaSSIF.

Zhang et al. recently reviewed prospective predictions of human pharmacokinetics for 18 Novartis compounds (Chang and Leblond, 1971). Their analysis revealed that high prediction accuracy in human PK parameters can be achieved when observed *in vivo* preclinical i.v. data were used to predict human i.v. profiles, which were then combined with mechanism-based absorption modelling to predict human PK for orally dosed compounds. The *a priori* predictions were within twofold of observed for 80% (C_{max}), 65% (AUC), 65% (CL/F), and 80% (V_{ss}/F) of the compounds. This implies that inclusion of animal PK data can significantly improve the prediction of human PK parameters and thus prediction of human oral drug exposure. The data available for the OrBiTo exercise was sparse in this area, with approximately 60% of the APIs having preclinical i.v. data available for allometric scaling, and approximately 50% having data for multiple species (Margolskee et al. – Part 1 – Submitted). Additionally, since one of the interests was to test modeller differences, modellers were given the option of which information to use, thus there was no guarantee that preclinical data were utilised for CL predictions if *in vitro* CLint information was available, or that preclinical data were used to inform distribution parameters.

3.5. Industry perspective

An important consideration of this study relied on the modellers to extract what they considered relevant compound information from the database. To a degree, this was limited by the sparse details provided through the interface of the database. Consequently, the form of this simulation study is not necessarily representative of how prospective modelling is carried out in the pharmaceutical industry, but may be considered a 'worst-case' scenario. Although communication was allowed between PBPK modellers and API owners through an online query system, the task suffered from poor, delayed, and sometimes absent responses. In addition, modellers carried out the task separately with limited communication with other modellers and a general absence of cross-checking, contrary to common industry practice. The overall lack of responses or data update prior to the simulation exercise did not prevent the analysis but restricted the breadth of the selected compounds. The effort needed to upload datasets in the OrBiTo database precluded EFPIA partners to generate new data in time for the simulation exercise. From the analysis of missing information, there is room for improvement and the EFPIA partners were asked to complete their datasets with missing information to inform and evaluate model improvements.

Pharmaceutical industry generally employs a learn-and-confirm approach for PBPK model development in drug discovery. In short, this entails an initial modelling phase followed by validation and if unacceptable performance is observed, further parameter data can be experimentally generated in order to improve the model. At this stage pre-clinical data can serve as an initial validation set prior to supplementing with additional information to predict human exposure. This approach has been shown to display better accuracy than simple allometric scaling. Further, the pharmaceutical industry has the advantage of the ability to

utilise information from compounds of the same drug class or chemical structural analogues (Jones et al., 2009; Salles Junior et al., 2013; Aulinger et al., 2014). Similarly, model refinement can occur based on clinical pharmacokinetic data prior to biopharmaceutical development.

The OrBiTo simulation task did not allow for this approach due to rules setup for blinding data and general lack of supplementation of novel parameter information. Due to the blinded nature of the exercise, modellers were asked to run bottom-up anticipation of all human PK profiles with very limited time to familiarise themselves with the drug substance and drug product properties, and no time to discuss the *in vitro* or preclinical *in vivo* performance with the experts. While the setup of the database enabled for anonymous contribution and maintenance of data, and a means to facilitate unbiased evaluation, it also prevented important quality control mechanisms from being implemented efficiently. Whenever a user had a doubt about the validity of a measurement, the anonymous messaging system was the only route for resolving the issue, since identifying characteristics of the APIs were intentionally blinded or systematically altered (e.g. rounding of MW to the nearest ten). The blinded nature of the database also made it impossible to use pre-existing knowledge and information of similar compounds in model building, which is often done in industry. Importantly, the modellers conducted the simulations based on “their” selection of what they considered to be appropriate data contained within the database. Any missing information was completed using *in silico* tools, whereas in the industry this would have led to discussion and generation of additional *in vitro* and *in vivo* preclinical data, supported by sensitivity analysis to inform which data are critical for improving model quality. The results of this exercise should therefore not be taken out of context, i.e. be seen as an evaluation of the PBPK approach in drug discovery or biopharmaceutical development, but rather as an

evaluation of the software prediction performance with respect to input parameters. In our companion paper we discuss the influence of compound specific properties and the identification of areas of improvement in the models and inputs (Darwich et al. – Part 3 – Submitted).

Also depending on the timing of the exercise, the aim of PBPK modelling may vary in the industry and could allow for the use of more or less precise models. For example, prior to FIM testing, the pharmaceutical industry is concerned with predicting oral performance and dose-response characteristics of the clinical intended formulations to aid in the choice of that formulation. The usual target formulation for FIM is sought to achieve linear dose response up to roughly twice the predicted human pharmacological exposure in order to demonstrate human drug safety using a given formulation prior to larger scale human studies and, ideally, to demonstrate that the therapeutic dose is lower than the maximum tolerated dose (MTD) (Figure 8). In this respect, rather than the absolute pharmacokinetic profiles, the prediction of the relative performance of standard vs enhanced formulations is explored. Typically the bottom-up exercises performed at this stage are to evaluate dose linearity and relative exposure between different types of formulations so as to guide the choice of FIM formulation development. The FDA recommended method for choosing the maximum recommended starting human dose is primarily based on preclinical safety, scaling factors and application of safety margins (Karam et al., 2003), though some companies use PBPK modelling in combination with target potency and animal pharmacology to select this sub-
efficacious starting dose. The lower dose of the FDA or the mechanistic approaches is then selected for optimal safety.

Figure 8: Evaluating relative exposure between different types of formulations can help guide choice of first time in man (FIM) formulation development. In order to explore the threshold for human safety, the target formulation for FIM is sought to achieve linear dose-response up to roughly twice the anticipated level of therapeutic response, ideally to be able to demonstrate that the therapeutic dose is lower than the maximal tolerated dose (MTD).

Due to errors in predicting an accurate V_d or CL in man (as was highlighted in this exercise) FIM data are generally utilised to inform the PBPK models and make adjustments to the clearance and distribution parameters used as inputs into the models. In parallel approaches, human i.v. PK profile measurements using microdosing, though uncommon, are also employed during phase I with oral co-administration or prior to phase I to avoid the pitfalls of predicting human clearance and distribution volumes using a full PBPK approach. In conclusion, pure bottom-up anticipation in the industry is used prior to human testing to

enable phase I clinical formulation choice where relative changes or linearity in dose exposure is determined. Once human data are obtained a mixed approach is employed where human data at one dose are used to predict the exposure at the next dose level or where fasted state data are used to predict fed state exposures.

4. Conclusion

In this study we present the largest effort to date in evaluating physiologically-based pharmacokinetic absorption models in a biopharmaceutics context. The work carried out in setting up the database, populating API files and modelling and simulation of compounds was a joint effort between European pharmaceutical industry, commercial enterprises and involved academic institutions. A modelling exercise that tests the differences in user and software selections based on the same available information has not been attempted previously. While the average predictive performance did not seem related to institution type, level of experience, or software package, there was a high level of variability in predictions for some individual APIs. The greatest variability in average prediction performance was observed across different APIs. The reasons for these API-specific differences could be related to compound specific properties, which will be explored in the companion paper (Darwich et al. – Part 3 – Submitted), the quality and availability of information for building each model, or errors in scaling from *in vitro* and preclinical *in vivo* data to human *in vivo* behaviour.

The overall predictive performance of the simulations indicated a slight overprediction of exposure on average, with 25 of the 43 simulated APIs producing overpredictions on average. Predictive performance declined progressing from i.v. to solution to p.o. formulations, indicating the compounding error with increased model complexity. However, predictive

performance of F_{oral} was relatively consistent, thus better predictions of CL and V_d and/or availability of i.v. data are important for the ability to model oral formulations effectively.

The overall success of predicting i.v. and p.o. data is also highly dependent upon the data used for simulations. The limitations of data availability and individual approaches to modelling may have contributed to decreased prediction performance as compared to the process in industry which generally involves open discussion, a learn and confirm approach to model building, and additional data collection for model improvement and validation.

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Name	Affiliation(s)	Contributions(s)
Leon Aarons	University of Manchester	PI for University of Manchester
Adam S. Darwich	University of Manchester	Performed gap analysis on database, performed simulations, analysed results
Aleksandra Galetin	University of Manchester	PI for University of Manchester
Alison Margolskee	University of Manchester	Performed gap analysis on database, performed simulations, analysed results
Amin Rostami-Hodjegan	University of Manchester/SimCYP	Work package co-leader, PI for University of Manchester, PI for SimCYP
Sara Carlert	AstraZeneca	Performed simulations, analysed results
Maria Hammarberg	AstraZeneca	Performed simulations
Constanze Hilgendorf	AstraZeneca	Performed simulations
Pernilla Johansson	AstraZeneca	Performed simulations
Eva Karlsson	AstraZeneca	Performed simulations
Donal Murphy	AstraZeneca	Performed simulations

Christer Tannergren	AstraZeneca	Performed simulations, PI for AstraZeneca
Helena Thörn	AstraZeneca	Performed simulations
Mohammed Yasin	AstraZeneca	Performed simulations
Florent Mazuir	Sanofi	Performed simulations
Olivier Nicolas	Sanofi	Performed simulations, analysed results
Xavier Pepin	Sanofi/AstraZeneca	Work package co-leader, PI for Sanofi until March 2015, performed gap analysis on database, performed simulations, analysed results
Sergej Ramusovic	Sanofi	Performed simulations
Christine Xu	Sanofi	Performed simulations
Shriram Pathak	SimCYP	Performed gap analysis on database, performed simulations, analysed results
Timo Korjamo	Orion Pharma	Performed simulations, analysed results
Johanna Laru	Orion Pharma	Performed simulations
Jussi Malkki	Orion Pharma	Performed simulations, analysed results
Sari Pappinen	Orion Pharma	Analysed results
Johanna Tuunainen	Orion Pharma	Analysed results
Jennifer Dressman	Goethe University	PI for Goethe University
Carmen Gött	Goethe University	Analysed results
Simone Hansmann	Goethe University	Performed simulations, analysed results
Edmund Kostewicz	Goethe University	PI for Goethe University
Handan He	Novartis	Performed simulations, analysed results
Tycho Heimbach	Novartis	Performed simulations, analysed results
Fan Wu	Novartis	Performed simulations, analysed results
Carolin Hoft	AbbVie	Performed simulations
Loic Laplanche	AbbVie	PI for Abbvie, analysed results
Yan Pang	AbbVie	Performed simulations
Michael B. Bolger	Simulations Plus	PI for Simulations Plus, lead for analysis of impact of solubility and dissolution
John DiBella	Simulations Plus	Financial and time accounting for Simulations Plus
Eva Huehn	Simulations Plus	Performed gap analysis on database, performed simulations
Viera Lukacova	Simulations Plus	Co-PI for Simulations Plus
James M. Mullin	Simulations Plus	Performed gap analysis on database, performed simulations
Ke X. Szeto	Simulations Plus	Performed gap analysis on database, performed simulations
Chester Costales	Pfizer	Performed simulations
Jian Lin	Pfizer	Performed simulations
Mark McAllister	Pfizer	Performed simulations
Sweta Modi	Pfizer	Performed simulations
Charles Rotter	Pfizer	Performed simulations
Manthena Varma	Pfizer	Performed simulations
Mei Wong	Pfizer	Performed simulations
Amitava Mitra	Merck Sharp & Dohme (MSD)	Performed simulations, analysed results
Jan Bevernage	Janssen	Performed simulations

Jeike Biewenga	Janssen	Performed simulations
Achiel Van Peer	Janssen	Performed simulations
Richard Lloyd	GlaxoSmithKline	Performed simulations, analysed results
Carole Shardlow	GlaxoSmithKline	Performed simulations, analysed results
Peter Langguth	University of Mainz	PI for University of Mainz
Irina Mishenzon	University of Mainz	Performed simulations
Mai Anh Nguyen	University of Mainz	Performed simulations
Jonathan Brown	Bristol-Myers Squibb	Performed simulations

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Appendix

Comparators

Table A1: Summary statistics for AUC i.v. and p.o. compared with other simulation exercises from the literature.

	n	% within 2 fold	% within 3 fold	% within 10 fold	AFE	AAFE	R	CCC (of log data)
AUC, i.v.								
Poulin 2011 ^f	15	67	87	93	0.72	1.9	0.99	0.93
Poulin 2011 ^g	14	71	93	100	0.92	1.8	0.52	0.92
OrBiTo	17	53	71	94	1.64	2.51	0.500	0.870

AUC, p.o.								
De Buck 2007 ^a	23	34.8	43.5	69.6*	5.09	5.34*	0.984*	0.577*
De Buck 2007 ^b	23	73.9	87	100*	1.06	1.83*	0.997*	0.910*
Jones 2011 ^d		50	72			2.7		
Jones 2011 ^e		33	56			3.9		
Poulin 2011 ^{f,h}	84	25	44	74	0.17	11	0.88	0.45
Poulin 2011 ^{f,i}	82	28	45	84	0.41	4.2	0.87	0.75
Poulin 2011 ^{g,j}	33	21	30	64	0.37	7.8	0.47	0.58
Poulin 2011 ^{g,k}	18	11	28	61	0.14	11.1	0.96	0.38
OrBiTo	43	37	56	88	1.30	3.2	0.103	0.759
^a (Vd1, CL1), ^b (Vd2, CL2), ^c CLR, ^d PBPK (Gastroplus), ^e 1 comp model, ^f PBPK (Vss, JA; CL, FCIM), ^g PBPK (Vss, TCKpu ; CL, IVIVE), ^h (ka, Fabs1), ⁱ (ka, Fabs2), ^j (est Solfassif), ^k (meas Solfassif), *calculated based on digitized data.								

Table A2: Summary statistics for CL i.v. compared with other simulation exercises in the literature

	n	% within 2 fold	% within 3 fold	% within 10 fold	AFE	AAFE	R	CCC (of log data)
CL, i.v.								
De Buck 2007 ^a	19	52.6	68.4	94.7*	0.415	2.60*	0.443*	0.607*
De Buck 2007 ^b	19	73.7	89.5	100*	1.10	1.61*	0.899*	0.996*
De Buck 2007 ^c	4	100	100	100	1.09			
Jones 2011 ^d		80	85			1.6		
Jones 2011 ^e		80	85			1.6		
Poulin 2011 ^f	17*	76*	88*	100*	0.753*	1.66*		
Poulin 2011 ^g	14*	57*	64*	71*	0.383*	3.27*		
OrBiTo	17	35	59	94	0.48	2.97	0.8	0.441
^a (Vd1, CL1), ^b (Vd2, CL2), ^c CLR, ^d PBPK (Gastroplus), ^e 1 comp model, ^f PBPK (Vss, JA; CL, FCIM), ^g PBPK (Vss, TCKpu ; CL, IVIVE), ^h (ka, Fabs1), ⁱ (ka, Fabs2), ^j (est Solfassif), ^k (meas Solfassif), *calculated based on digitized data.								

Table A3: Summary statistics for Vss i.v. and Vd/F p.o. compared with other simulation exercises from the literature

	n	% within 2 fold	% within 3 fold	% within 10 fold	AFE	AAFE	R	CCC (of log data)
Vss, i.v.								
De Buck 2007 ^a	19	31.6	52.6	94.7*	2.10	3.50*	0.0356*	0.272*
De Buck 2007 ^b	19	84.2	94.7	100*	1.14	1.45*	0.811*	0.881*

Jones 2011 ^d		90	100	100		1.4		
Jones 2011 ^e		75	85			1.6		
Poulin 2011 ^f	15*	53*	73*	100*	2.09*	2.24*		
Poulin 2011 ^g	17*	47*	76*	82*	1.81*	3.07*		
OrBiTo	17	35	65	94	1.28	2.66	0.49	0.636
Vd/F, p.o.								
De Buck 2007 ^h	23	21.7	39.1	82.6*	1.38	4.79*	-0.103*	0.203*
De Buck 2007 ⁱ	23	69.6	82.6	100*	0.758	1.73*	0.791*	0.863*
OrBiTo	43	37	63	93	1.3	2.9	0.598	0.648
^a (Vd1, CL1), ^b (Vd2, CL2), ^c CLR, ^d PBPK (Gastroplus), ^e 1 comp model, ^f PBPK (Vss, JA; CL, FCIM), ^g PBPK (Vss, TCKpu ; CL, IVIVE), ^h (ka, Fabs1), ⁱ (ka, Fabs2), ^j (est Solfassif), ^k (meas Solfassif), *calculated based on digitized data.								

Formulations

Table A4: Summary statistics for PK Parameter, Foral, and Frel predictions grouped by Formulations

	No. APIs	% within 2 fold	% within 3 fold	% within 10 fold	AFE	AAFE	R	CCC	R (of log data)	CCC (of log data)
AUClast										
All	43	37.2	55.8	90.7	1.30	3.13	0.106	0.102	0.762	0.756
p.o.	43	37.2	55.8	88.4	1.30	3.21	0.103	0.0969	0.766	0.759
IR tablet	27	40.7	66.7	88.9	0.93	2.74	0.339	0.280	0.793	0.783
solution	28	39.3	50.0	92.9	1.97	3.04	0.530	0.299	0.867	0.836
i.v.	17	52.9	70.6	94.1	1.64	2.51	0.500	0.438	0.908	0.870
AUCinf										
All	43	32.6	44.2	86.0	1.43	3.65	0.116	0.0893	0.726	0.712
p.o.	43	34.9	46.5	83.7	1.40	3.65	0.119	0.0859	0.739	0.726
IR tablet	27	37.0	59.3	81.5	1.10	3.21	0.200	0.177	0.744	0.743
solution	28	28.6	42.9	85.7	2.20	3.65	0.431	0.147	0.831	0.789
i.v.	17	35.3	58.8	94.1	2.07	2.97	0.472	0.388	0.886	0.836
Cmax										
p.o.	43	39.5	62.8	86.0	0.616	3.19	0.453	0.452	0.787	0.756
IR tablet	27	48.1	74.1	88.9	0.569	2.63	0.426	0.383	0.812	0.780
solution	28	42.9	67.9	89.3	0.745	2.9	0.451	0.375	0.855	0.845
Tmax										
p.o.	43	53.5	76.7	95.3	1.30	2.24	-0.0511	-0.0462	-0.071	-0.0663
IR tablet	27	59.3	70.4	96.3	1.10	2.17	0.0432	0.0393	0.0483	0.0475
solution	28	39.3	67.9	96.4	1.60	2.63	-0.108	-0.0954	-0.0823	-0.0704
CL or CL/F										
All	43	32.6	44.2	88.4	0.675	3.67	0.265	0.0858	0.523	0.497
p.o.	43	34.9	46.5	83.7	0.694	3.63	0.404	0.235	0.580	0.556
IR tablet	27	37.0	59.3	81.5	0.914	3.19	0.918	0.910	0.573	0.564
solution	28	28.6	42.9	85.7	0.441	3.68	0.656	0.089	0.617	0.547
i.v.	17	35.3	58.8	94.1	0.483	2.97	0.796	0.751	0.576	0.441
T1/2										
All	43	53.5	60.5	93.0	1.91	2.67	0.680	0.318	0.525	0.438

p.o.	43	53.5	60.5	95.3	1.87	2.61	0.702	0.284	0.550	0.459
IR tablet	27	59.3	66.7	100	1.62	2.51	0.635	0.170	0.413	0.355
solution	28	32.1	46.4	89.3	2.66	3.60	0.544	0.308	0.329	0.232
i.v.	17	35.3	64.7	94.1	1.88	2.89	0.963	0.901	0.543	0.484
Vd or Vd/F										
All	43	34.9	62.8	93.0	1.29	2.86	0.173	0.163	0.631	0.617
p.o.	43	37.2	62.8	93.0	1.30	2.90	0.598	0.592	0.661	0.648
IR tablet	27	51.9	77.8	96.3	1.48	2.23	0.753	0.735	0.762	0.731
solution	28	39.3	57.1	85.7	1.17	3.15	0.213	0.119	0.554	0.551
i.v.	17	35.3	52.9	94.1	0.909	3.07	0.412	0.253	0.640	0.636
AUMC0-t										
All	43	18.6	41.9	76.7	2.47	5.90	-0.00234	-0.00113	0.662	0.621
p.o.	43	18.6	41.9	72.1	2.49	6.01	-0.00327	-0.00153	0.666	0.624
IR tablet	27	25.9	40.7	77.8	1.35	4.33	0.245	0.234	0.698	0.674
solution	28	25.0	46.4	60.7	5.21	6.70	0.367	0.0825	0.776	0.658
i.v.	17	29.4	47.1	70.6	3.24	5.20	-0.0006	-0.00038	0.772	0.707
AUMCinf										
All	43	20.9	32.6	62.8	3.27	8.30	0.0308	0.00517	0.563	0.505
p.o.	43	20.9	32.6	60.5	3.13	8.06	0.0327	0.00524	0.595	0.539
IR tablet	27	25.9	33.3	66.7	2.05	6.98	0.0357	0.00657	0.569	0.546
solution	28	17.9	28.6	57.1	6.95	10.5	0.0696	0.0134	0.650	0.518
i.v.	17	23.5	35.3	58.8	5.50	8.39	0.00665	0.00321	0.692	0.587
MRT or MTT										
All	43	32.6	60.5	88.4	2.29	2.94	0.645	0.199	0.485	0.358
p.o.	43	34.9	60.5	88.4	2.23	2.84	0.643	0.180	0.501	0.370
IR tablet	27	40.7	66.7	100	1.87	2.5	0.682	0.182	0.483	0.384
solution	28	14.3	42.9	85.7	3.16	4.00	0.537	0.231	0.351	0.220
i.v.	17	23.5	52.9	82.4	2.65	3.73	0.968	0.616	0.524	0.407
Foral (p.o. vs. i.v.)										
p.o.	17	64.7	82.4	94.1	1.00	2.05	0.346	0.345	0.383	0.380
IR tablet	13	53.8	53.8	92.3	0.893	2.69	0.178	0.176	0.150	0.149
solution	10	70.0	70.0	90.0	1.10	2.13	-0.111	-0.106	-0.0272	-0.0269
Frel (p.o. vs. solution)										
p.o. vs. solution	24	75.0	79.2	100	0.744	1.67	0.443	0.389	0.333	0.269
tablet vs. solution	16	75.0	87.5	100	0.708	1.53	0.601	0.504	0.472	0.334