Development and Application of PBPK Models to Support Transporter-mediated Drug-Drug Interaction (tDDI) Assessment

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Outline of Presentation

- Types of Interactions
- GastroPlus DDI Module Interface
- Overview of relevant regulatory guidance documents
  1. Investigation of Transporter Involvement in Drug Clearance
  2. Investigation of Transporter Inhibitory Potential
- PBPK Models of P-gp Substrates and tDDIs selected for today's presentation
  - Drug 1: Digoxin and DDIs with P-gp inhibitors
  - Drug 2: Fexofenadine and DDIs with P-gp inhibitors
- PBPK Models of OATP(1B1 & 1B3) Substrates and tDDIs selected for today's presentation
  - Drug 3: Rosuvastatin and DDIs with OATP(1B1 & 1B3) inhibitors
  - Drug 4: Pravastatin and DDIs with OATP(1B1 & 1B3) inhibitors
- Evaluation of predictive performance of *in silico* – based DDI

Types of Interactions

- Steady-state competitive inhibition
- Steady-state time-dependent inhibition
- Steady-state induction

may include metabolites effect with simulated perpetrator concentrations

- Dynamic competitive inhibition
- Dynamic time-dependent inhibition
- Dynamic induction

include effect of parent and/or metabolites; include enzymes and transporters

Dynamic Simulation – Equations

Competitive Inhibition

$$v = \frac{TransAct_0 \times V_{max} \times [S]_u}{K_m \left(1 + \sum_{j=1}^{N} \frac{[A]_{u,j}}{K_{m,j}} + \sum_{i=1}^{M} \frac{[I]_{u,i}}{K_{i,j}}\right) + [S]_u}$$

multiple substrates of given transporter

multiple inhibitors of given transporter

Time-Dependent Inhibition & Induction

$$v = \frac{TransAct_t \times V_{max} \times [S]_u}{K_m \left(1 + \sum_{j=1}^{N} \frac{[A]_{u,j}}{K_{m,j}} + \sum_{i=1}^{M} \frac{[I]_{u,i}}{K_{i,j}}\right) + [S]_u}$$

$$\frac{dTransAct}{dt} = -\left(\sum_{t=1}^{TDI} \frac{k_{inact,t} \times [I]_{u,t}}{K_{i,t}\left(1 + \sum_{\substack{n=1 \\ n \neq t}}^{TDI} \frac{[I]_{u,n}}{K_{i,n}}\right) + [I]_{u,t}}\right) \times TransAct + k(TransAct_0 - TransAct)$$

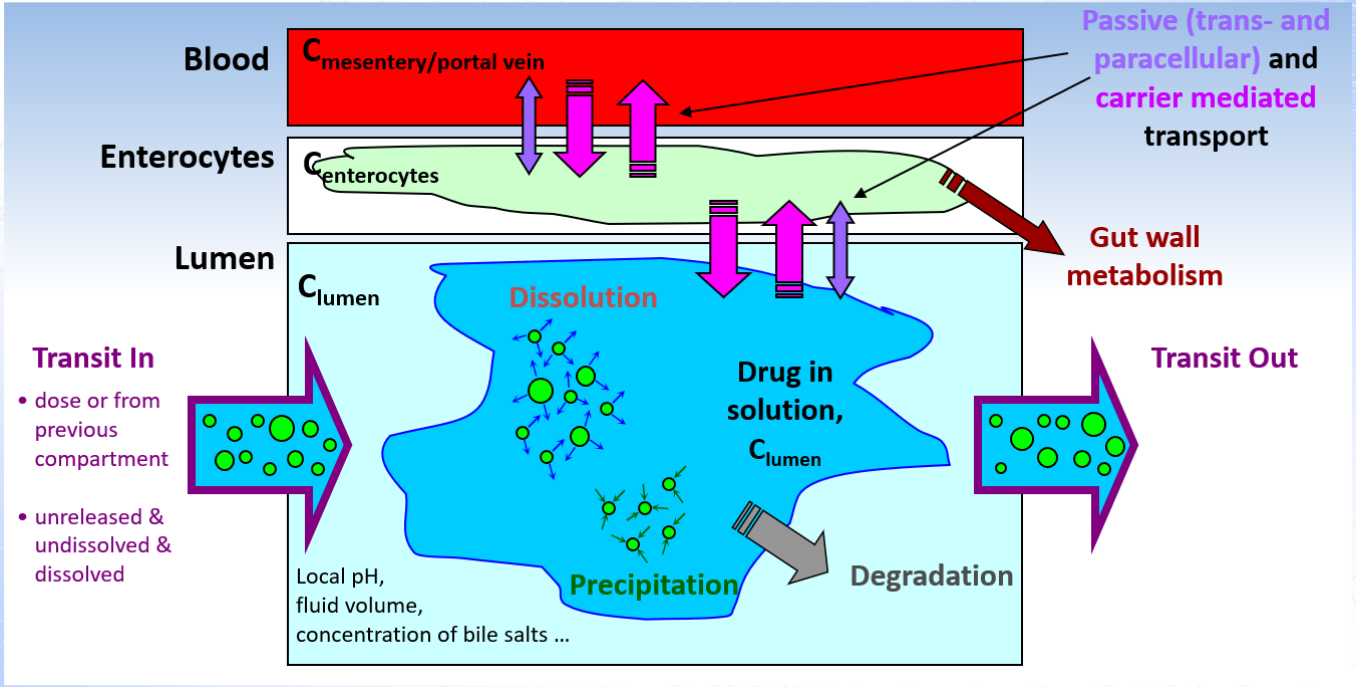
$$+ kdeg \times TransAct_0 \times \sum_{d=1}^{inducers} \frac{E_{max,\,d} \times [I]_u}{EC_{50,d} + [I]_u}$$

multiple time dependent inhibitors of given transporter

multiple inducers of given transporter

Let's not forget …

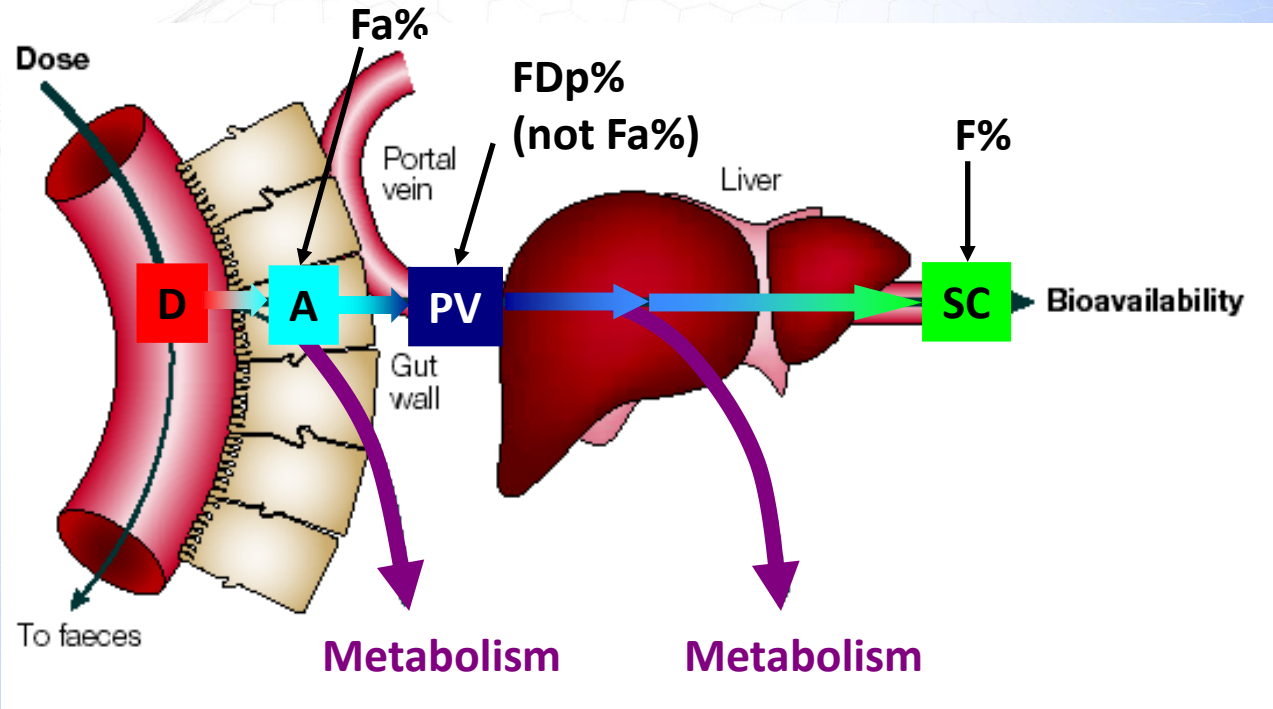
In GastroPlus™, the PBPK model is linked to the ACAT™ physiological intestinal model ….



These phenomena:

- are happening simultaneously
- are repeated in each of the compartments of the gastrointestinal tract

… and getting the correct dissolution/absorption is the prerequisite for getting correct PBPK & DDI predictions for oral dosage forms!



* Modified from van de Waterbeemd, H, and Gifford, E. *ADMET In Silico Modelling: Towards Prediction Paradise?* Nat. Rev. Drug Disc. 2003, 2:192-204

Developing a DDI Qualification Matrix: PBPK Models of Probe Substrates, Inhibitors, and Inducers in Various Stages of Validation

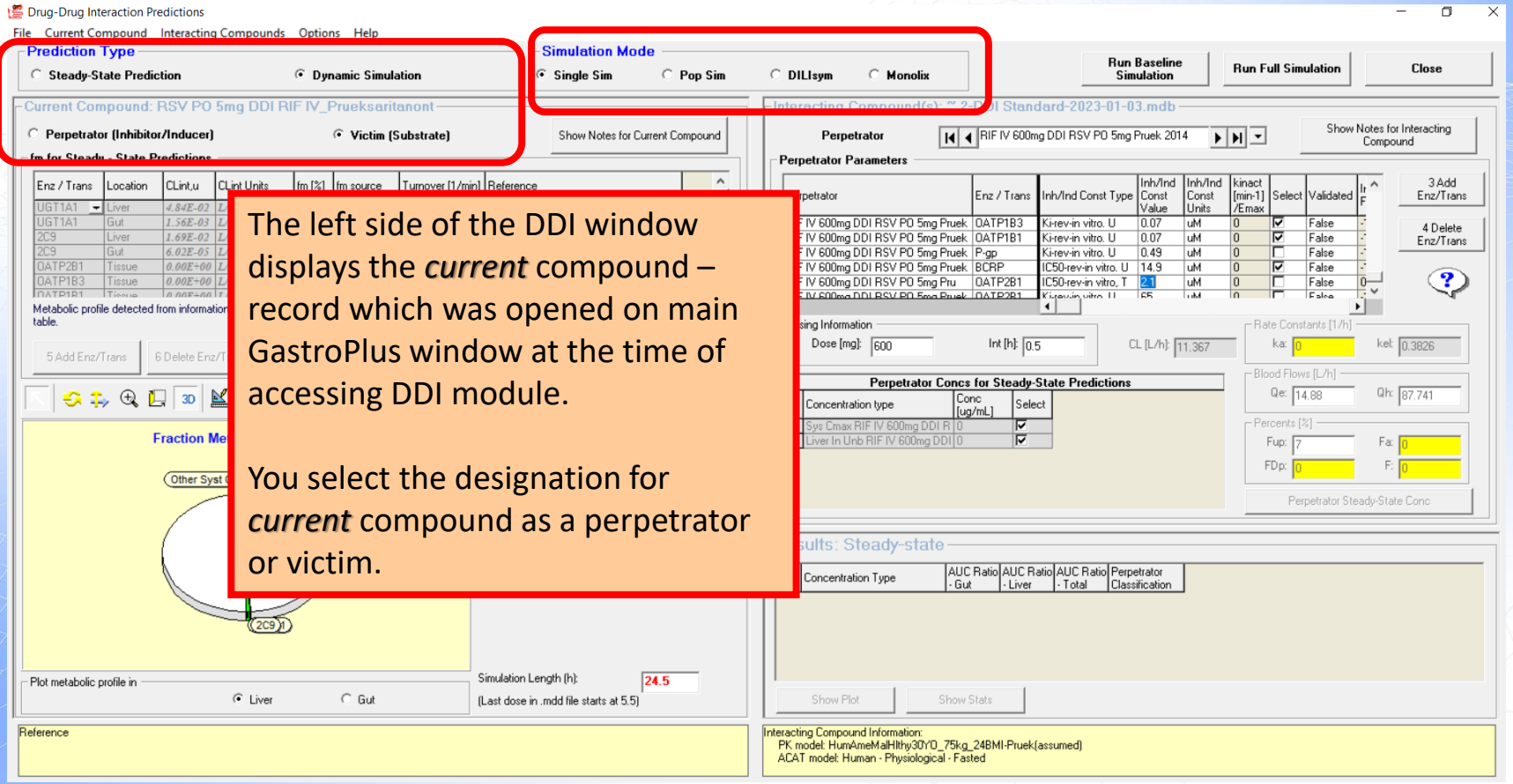
- The GastroDDIStandards database includes PBPK and compartmental PK models of many inhibitors, inducers, and probe substrates

Alfentanil	Dolutegravir	Metformin	Ranitidine	S-Warfarin
Atazanavir	Efavirenz	Midazolam	Rifampicin	
Atomoxetine	Fexofenadine	Omeprazole & Metabolites	Rivaroxaban	
Buproprion	Fluconazole	Phenytoin	Rosiglitazone	
Caffeine	Fluvoxamine	Posaconazole	Rosuvastatin	Atorvastatin
Cyclosporine	Gemfibrozil & glucuronide	Pravastatin	Theophylline	Simvastatin
Desipramine	Imipramine	Quinidine	Tolbutamide	
Digoxin	Itraconazole &Metabolites	Raltegravir & Metabolites	Triazolam	Verapamil
Diltiazem & Metabolites	Ketoconazole	Repaglinide	Clarithromycin	Voriconazole

- Periodically adding new models and updating previously built models
- Users can use any GastroPlus models that they have developed and verified

DDI Module Interface

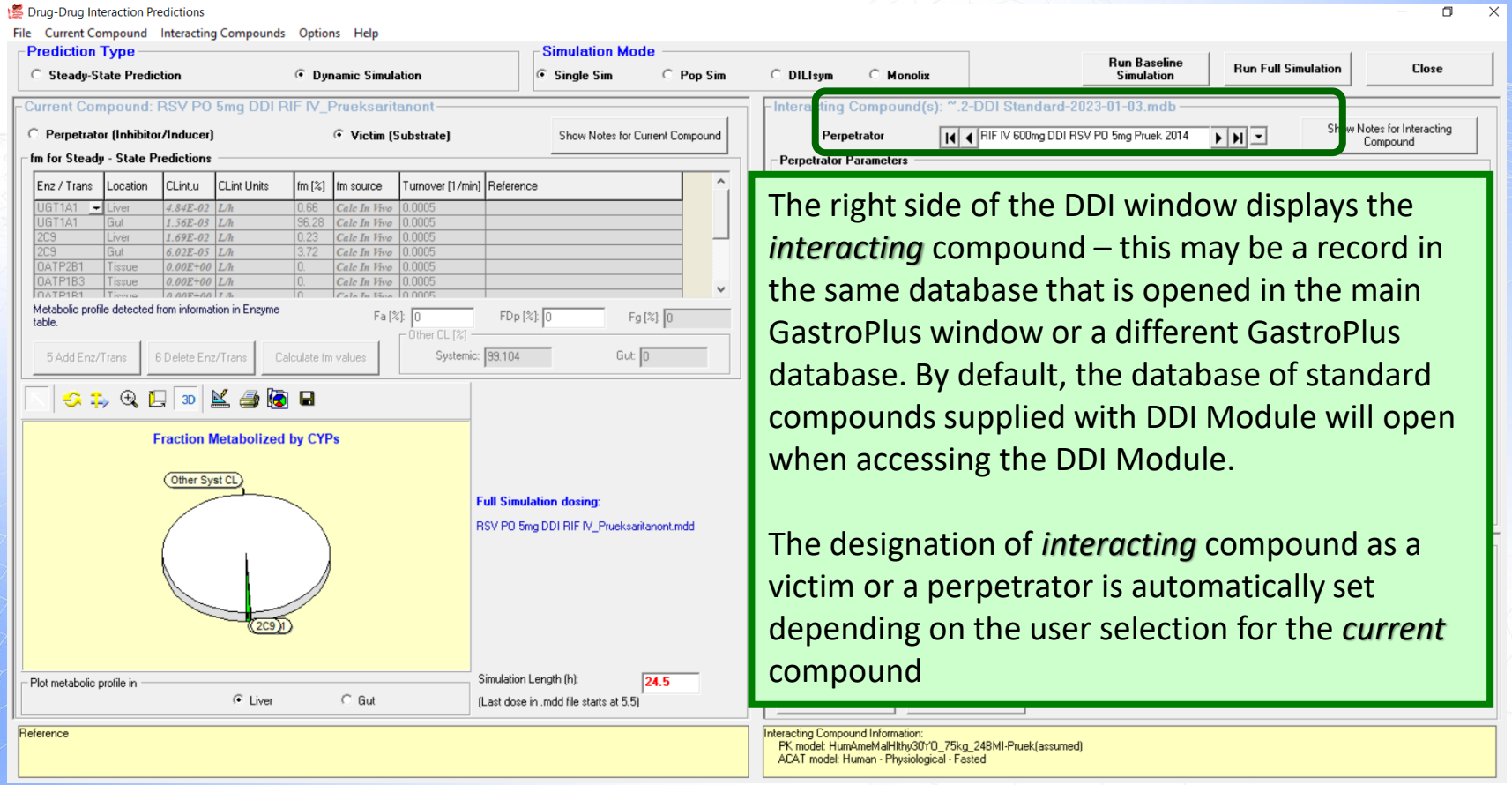
Basic Interface Layout



The left side of the DDI window displays the ***current*** compound – record which was opened on main GastroPlus window at the time of accessing DDI module.

You select the designation for ***current*** compound as a perpetrator or victim.

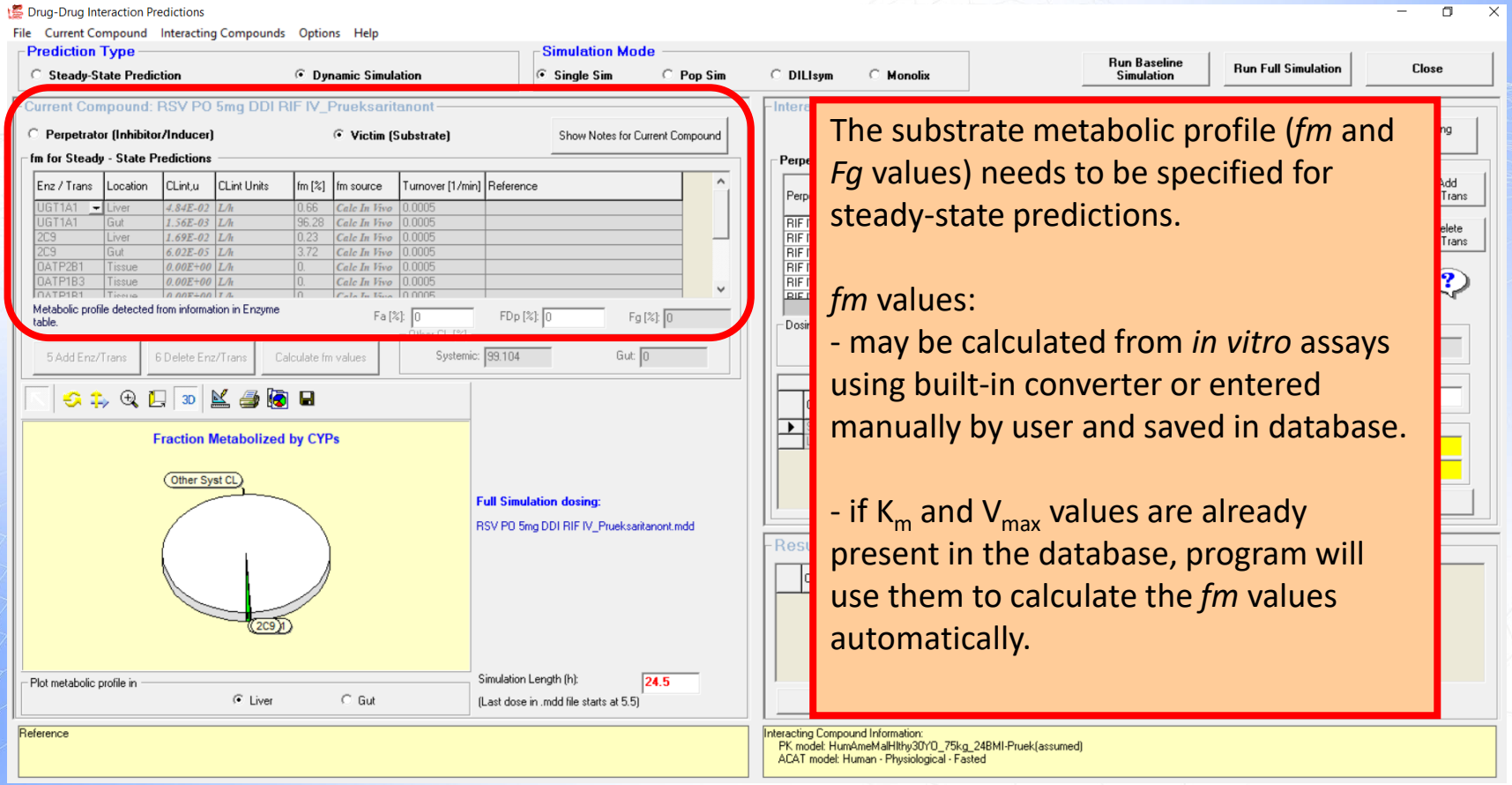
Basic Interface Layout



The right side of the DDI window displays the ***interacting*** compound – this may be a record in the same database that is opened in the main GastroPlus window or a different GastroPlus database. By default, the database of standard compounds supplied with DDI Module will open when accessing the DDI Module.

The designation of ***interacting*** compound as a victim or a perpetrator is automatically set depending on the user selection for the ***current*** compound

Substrate Settings



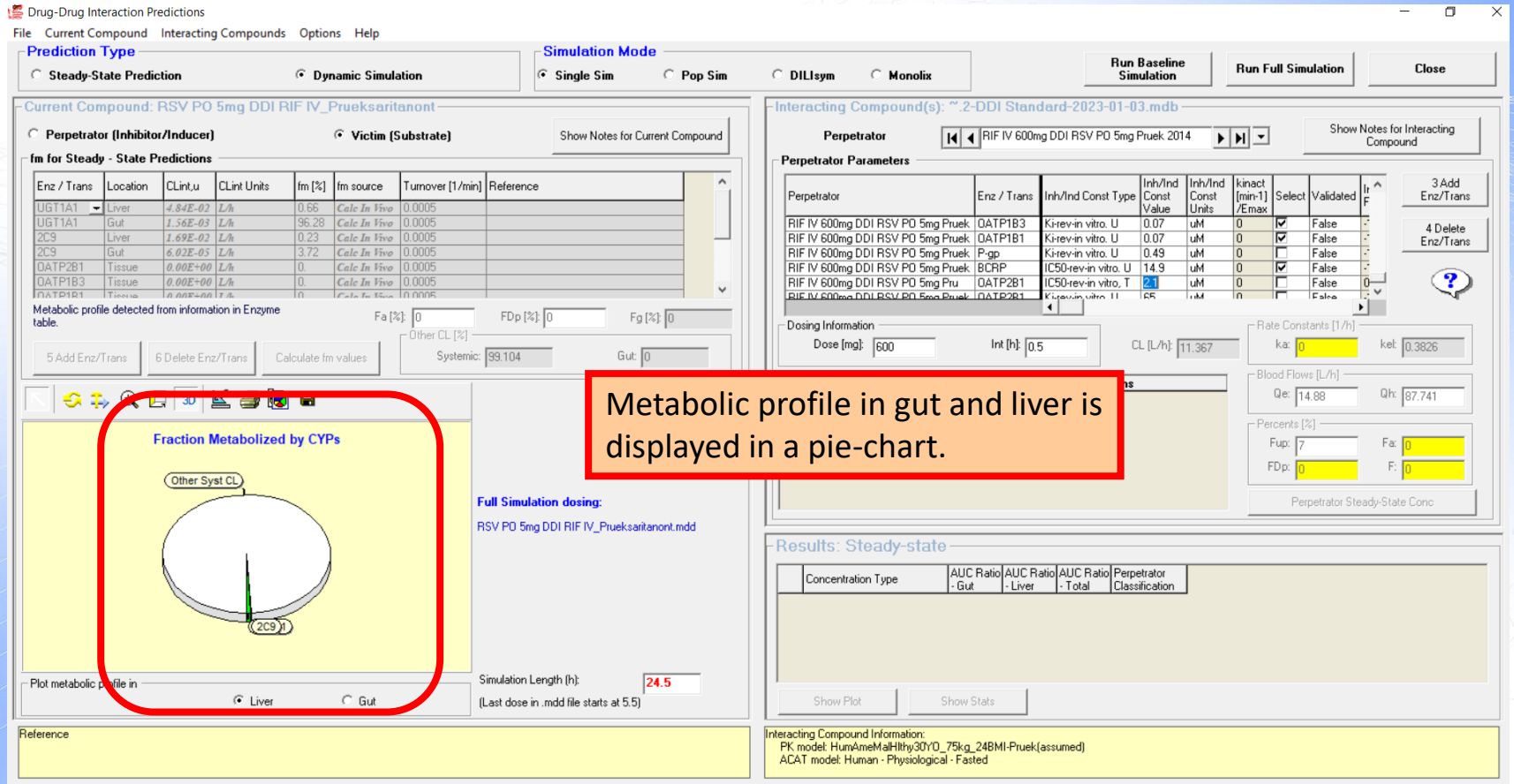
The substrate metabolic profile (*fm* and *Fg* values) needs to be specified for steady-state predictions.

fm values:

- may be calculated from *in vitro* assays using built-in converter or entered manually by user and saved in database.

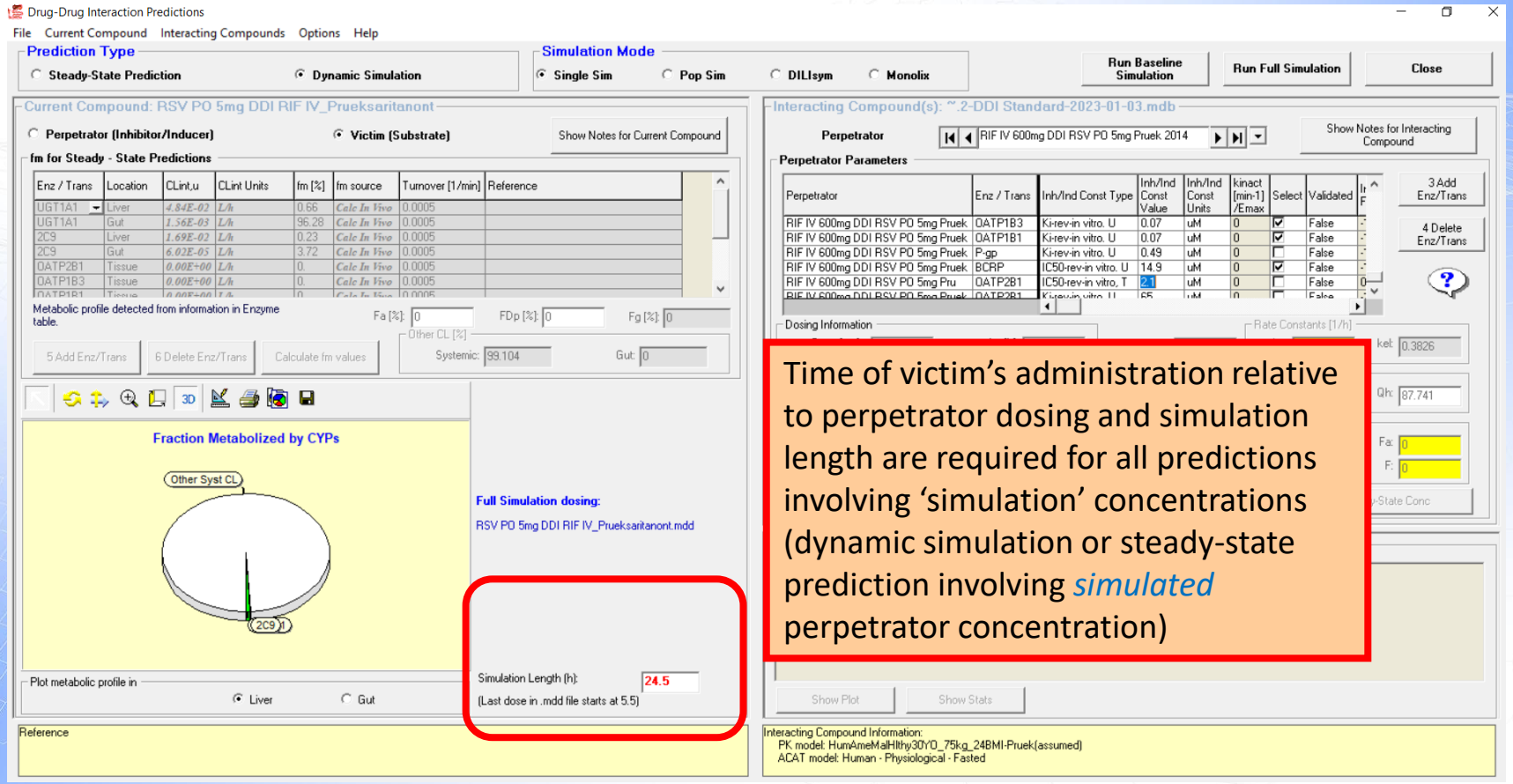
- if K_m and V_{max} values are already present in the database, program will use them to calculate the *fm* values automatically.

Substrate Settings



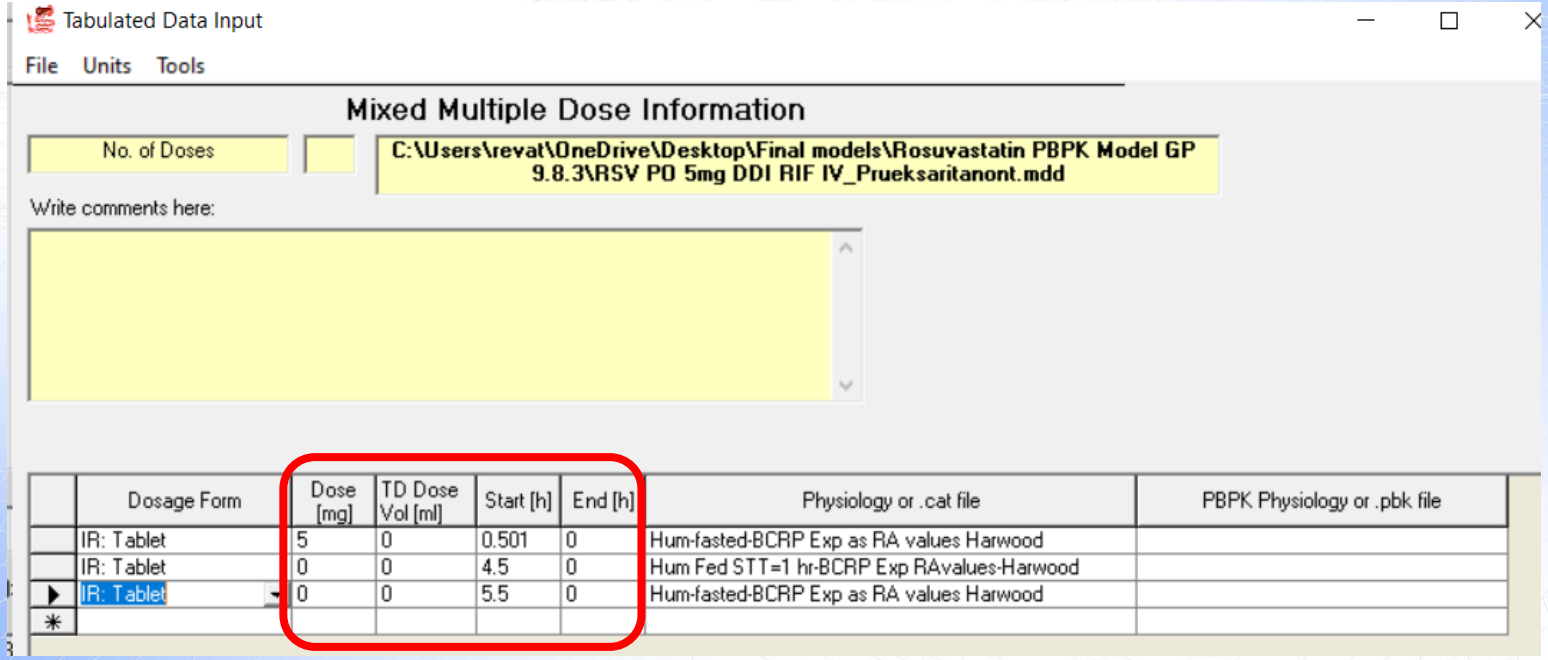
Metabolic profile in gut and liver is displayed in a pie-chart.

Substrate Settings



Time of victim's administration relative to perpetrator dosing and simulation length are required for all predictions involving 'simulation' concentrations (dynamic simulation or steady-state prediction involving *simulated* perpetrator concentration)

Substrate Settings



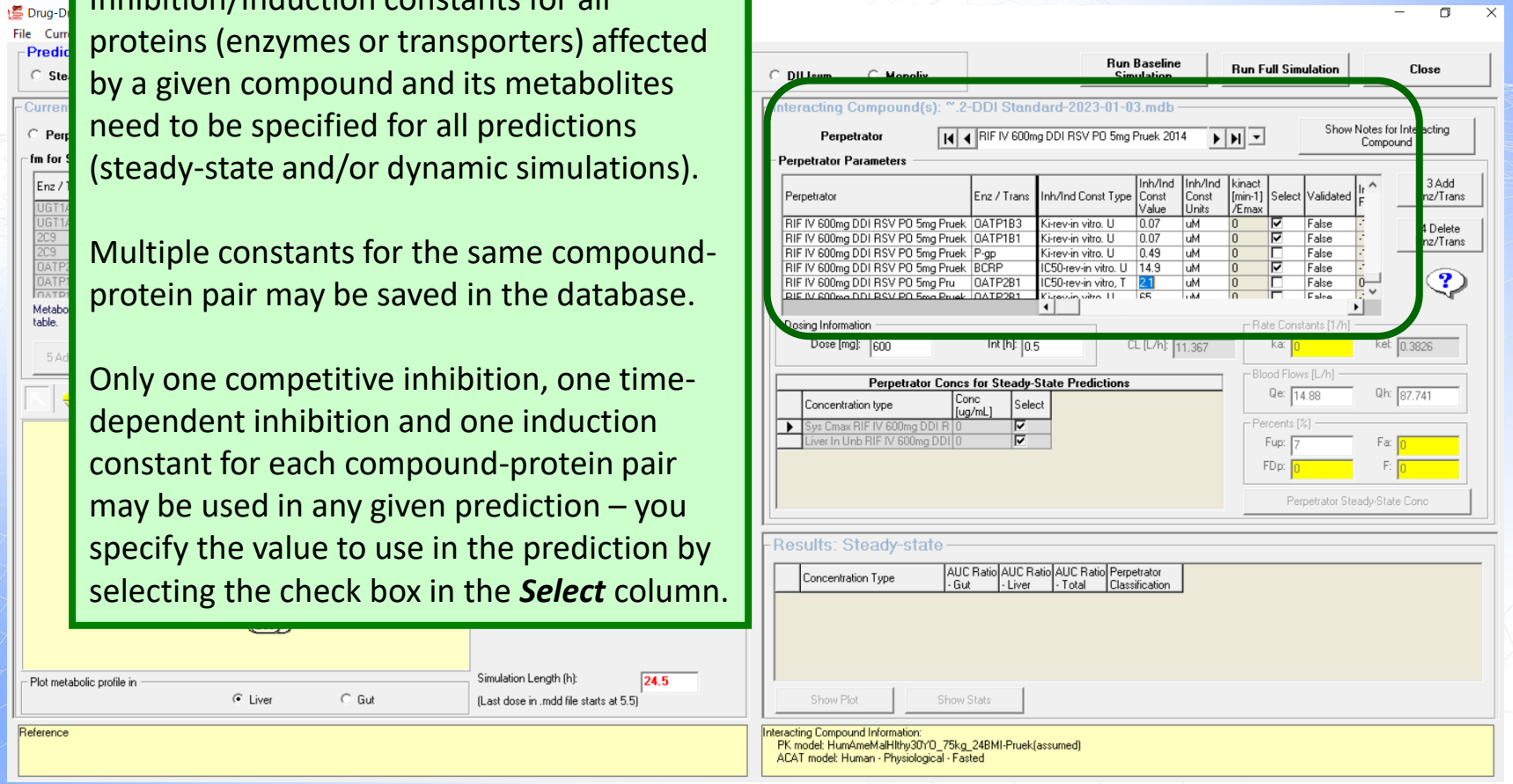
Dosage Form	Dose [mg]	TD Dose Vol [ml]	Start [h]	End [h]	Physiology or .cat file	PBPK Physiology or .pbk file
IR: Tablet	5	0	0.501	0	Hum-fasted-BCRP Exp as RA values Harwood	
IR: Tablet	0	0	4.5	0	Hum Fed STT=1 hr-BCRP Exp RAvalues-Harwood	
IR: Tablet	0	0	5.5	0	Hum-fasted-BCRP Exp as RA values Harwood	

Perpetrator Settings

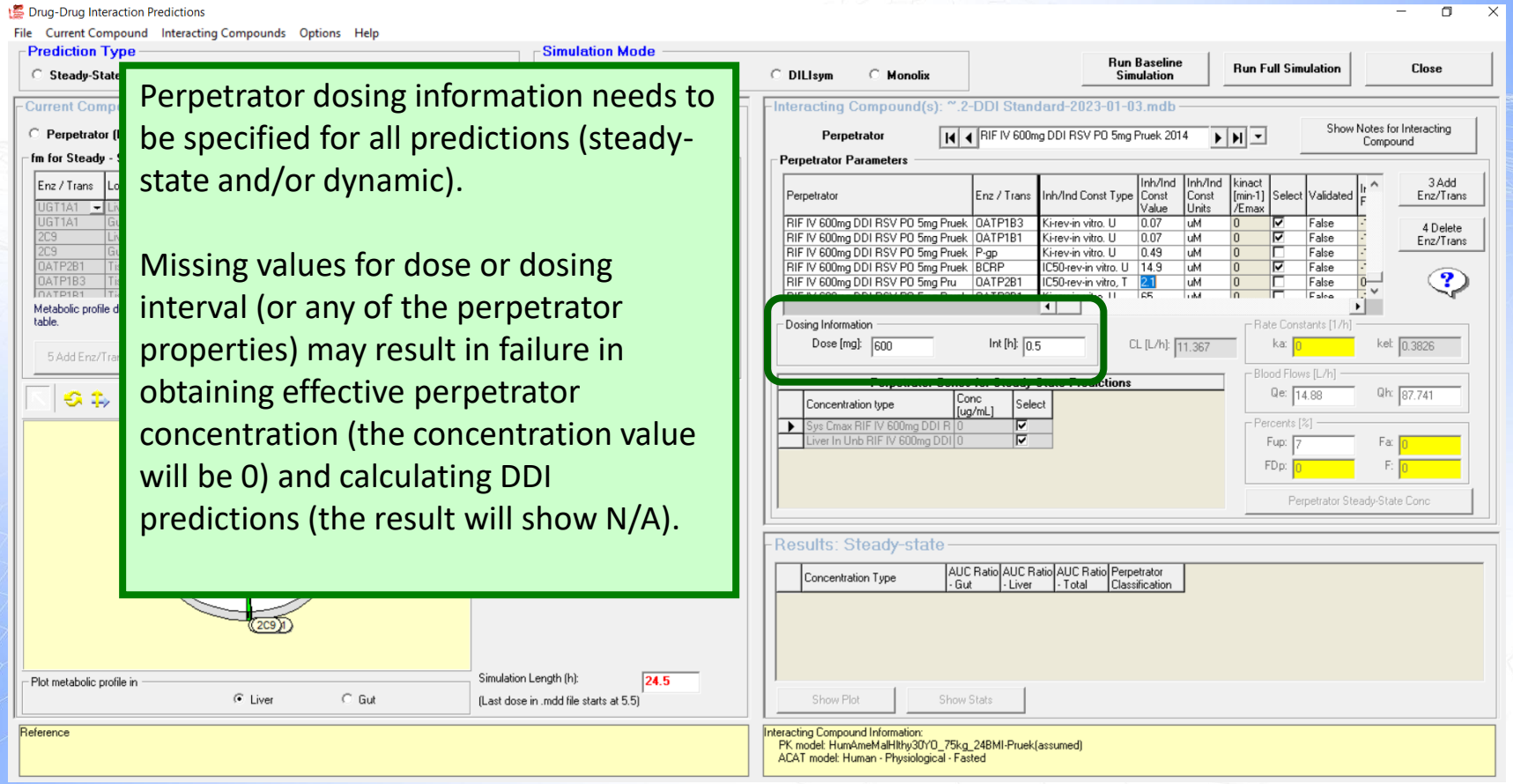
Inhibition/Induction constants for all proteins (enzymes or transporters) affected by a given compound and its metabolites need to be specified for all predictions (steady-state and/or dynamic simulations).

Multiple constants for the same compound-protein pair may be saved in the database.

Only one competitive inhibition, one time-dependent inhibition and one induction constant for each compound-protein pair may be used in any given prediction – you specify the value to use in the prediction by selecting the check box in the ***Select*** column.



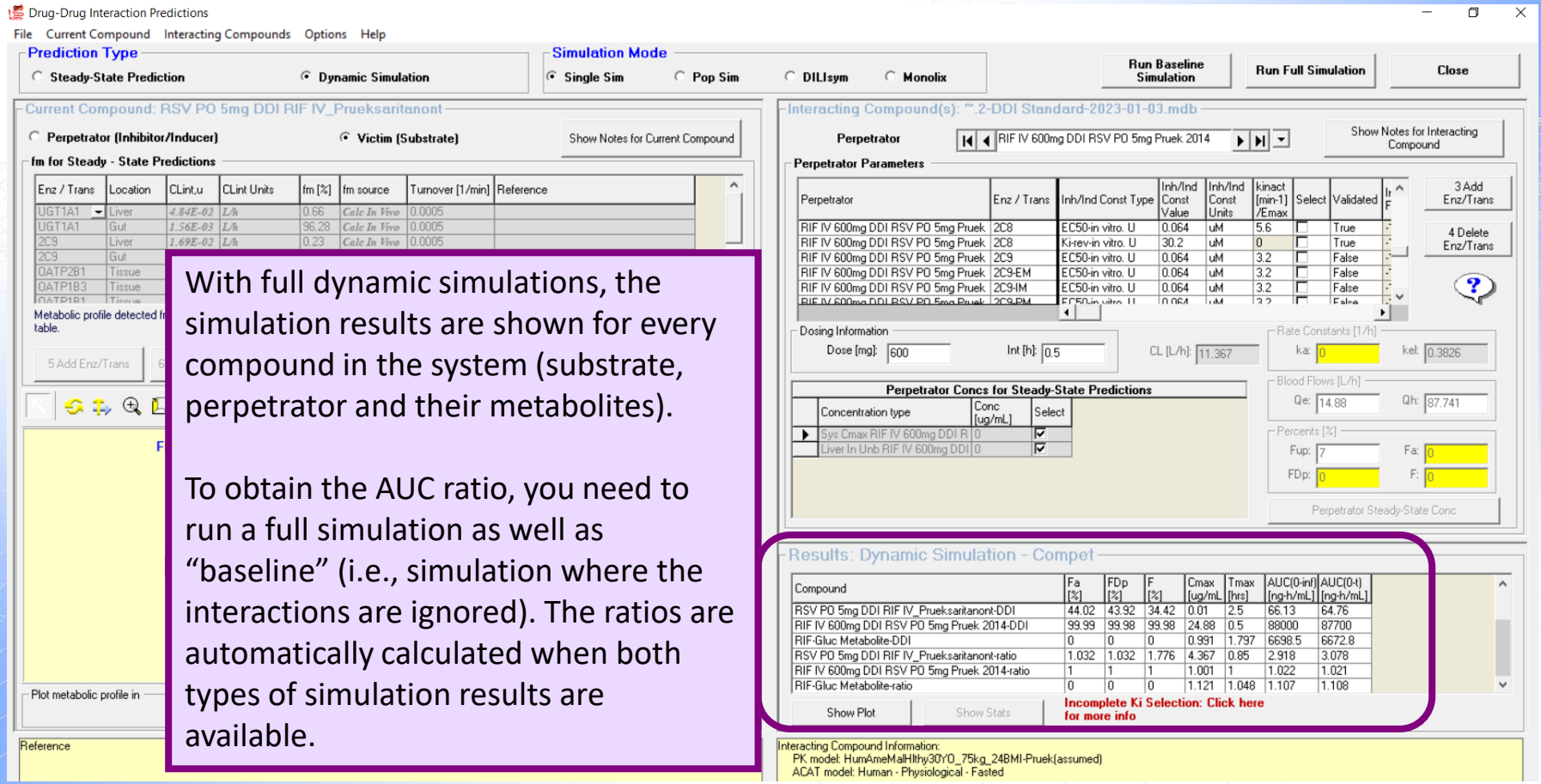
Perpetrator Settings



Perpetrator dosing information needs to be specified for all predictions (steady-state and/or dynamic).

Missing values for dose or dosing interval (or any of the perpetrator properties) may result in failure in obtaining effective perpetrator concentration (the concentration value will be 0) and calculating DDI predictions (the result will show N/A).

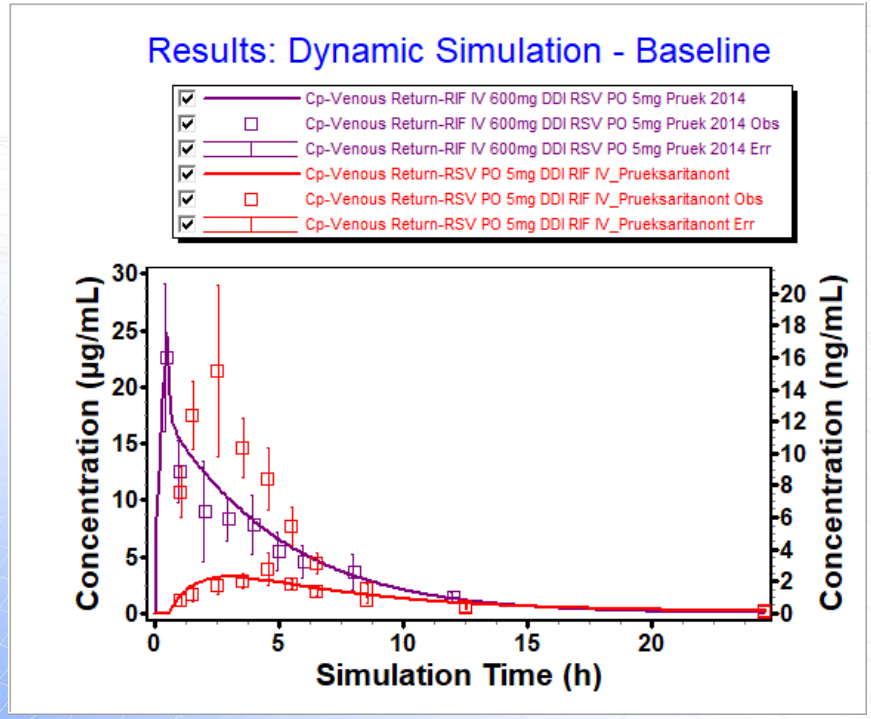
Prediction Results

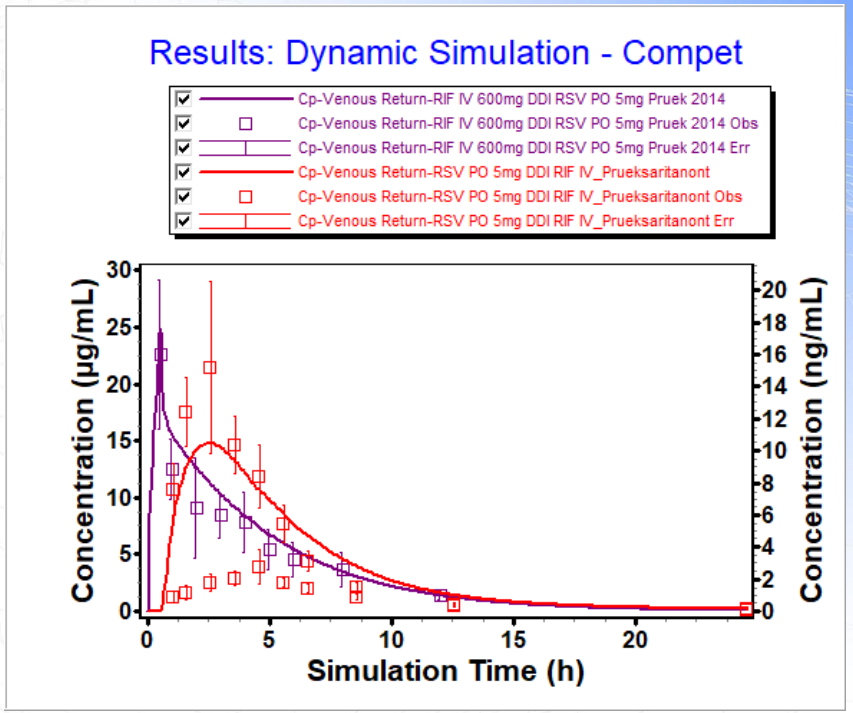


With full dynamic simulations, the simulation results are shown for every compound in the system (substrate, perpetrator and their metabolites).

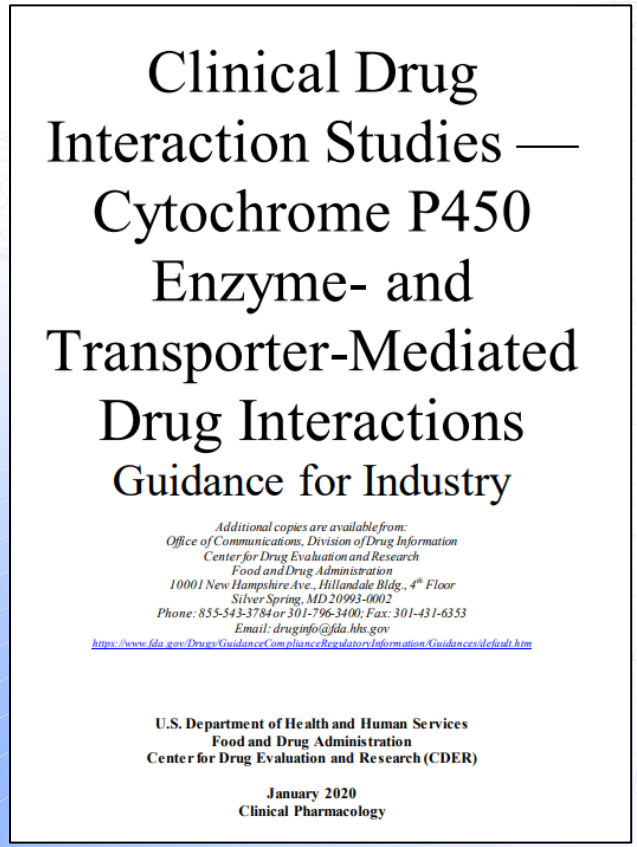
To obtain the AUC ratio, you need to run a full simulation as well as "baseline" (i.e., simulation where the interactions are ignored). The ratios are automatically calculated when both types of simulation results are available.

Prediction Results





FDA Guidance Document: DDI & PBPK



Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

January 2020
Clinical Pharmacology

In Silico DDI Studies: Physiologically based pharmacokinetic (PBPK) models can be used in lieu of some prospective DDI studies. For example, PBPK models have predicted the impact of weak and moderate inhibitors on the substrates of some CYP isoforms (e.g., CYP2D6, CYP3A) as well as the impact of weak and moderate inducers on CYP3A substrates. These predictions were made after prospective clinical trials showed a significant DDI between the investigational drug and strong index inhibitors or inducers. Before using a PBPK modeling approach to predict the effects of moderate or weak perpetrator drugs on the exposure of an investigational drug, the sponsor should verify the models using human pharmacokinetic data and information from DDI studies that used strong index perpetrators.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 June 2012
CPMP/EWP/560/95/Rev. 1 Corr. 2**
Committee for Human Medicinal Products (CHMP)

Guideline on the investigation of drug interactions

Discussion in the Efficacy Working Party (EWP)	June/October 1996 February 1997
Transmission to the CPMP	March 1997
Transmission to interested parties	March 1997
Deadline for comments	September 1997
Re-submission to the EWP	December 1997
Approval by the CPMP	December 1997
Date for coming into operation	June 1998
Draft Rev. 1 Agreed by the EWP	April 2010
Adoption Rev. 1 by CHMP for release for consultation	22 April 2010
End of consultation Rev. 1 (deadline for comments)	31 October 2010
Agreed by Pharmacokinetics Working Party	February 2012
Adopted by CHMP	21 June 2012
Date for coming into effect	1 January 2013

This guideline replaces guideline CPMP/EWP/560/95.

Keywords	***Interaction, guideline, metabolism, inhibition, induction, transport, enzyme, transport protein, transporter, absorption, food, distribution, PBPK, herbal, SmPC***

* The correction concerns section 5.3.4.1 (p 26) and the corresponding decision tree no. 6 (p 61) to read "if the observed Ki value is lower or equal to /.../"; Appendix VII, Table 5 to read "See section 5.4.2".* Decision tree 4.

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DRUG INTERACTION STUDIES

M12

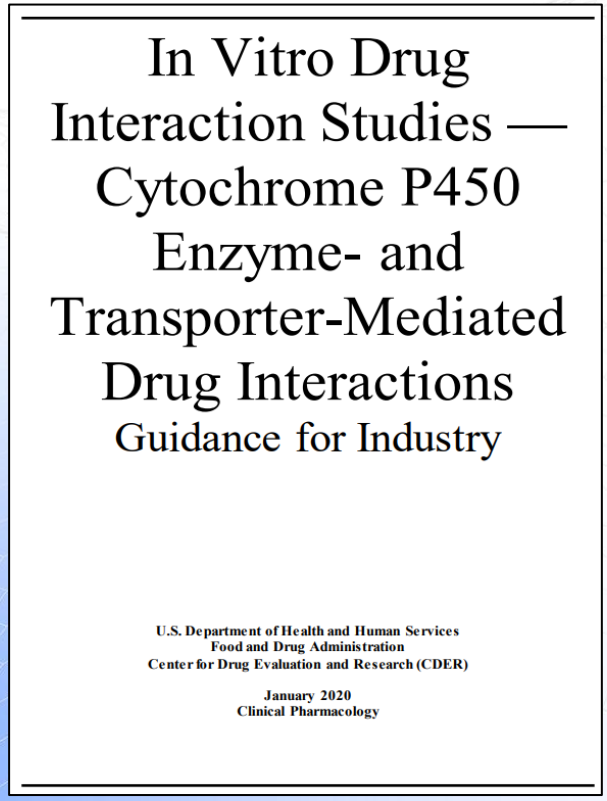
Draft version

Endorsed on 24 May 2022

Currently under public consultation

Utilization of PBPK models for DDIs studies is present in regulatory documents for different jurisdictions

FDA Guidance Document : Transporter mediated DDI



In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

January 2020
Clinical Pharmacology

The USFDA Guidance regulates that the sponsor should consider evaluating DDI of the NCE mediated by the following transporters:

- P-glycoprotein (P-gp) which also known as multidrug resistance 1 (MDR1);
- Breast cancer resistance protein (BCRP);
- Organic anion transporting polypeptide 1B1/1B3 (OATP1B1/1B3);
- Organic anion transporter 1/3 (OAT1/3);
- Organic cation transporter 2 (OCT2);
- Multidrug and toxin extrusion 1/2K (MATE1/2K).

Additionally, organic cation transporter 1 (OCT1), bile salt export protein (BSEP) and multidrug resistance-associated protein 2 (MRP2) which are responsible for hepatobiliary transport were recommended by EMA.

Regulatory Requirements of Transporter Evaluation

Table 1
The regulatory requirements of transporter evaluation in USFDA, NMPA, PMDA and EMA guidelines.

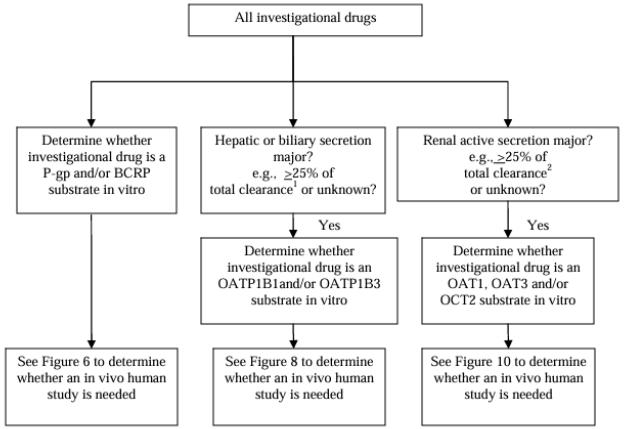
	Inhibition Study				Substrate Study			
	USFDA 2020	NMPA 2021	PMDA 2018	EMA 2013	USFDA 2020	NMPA 2021	PMDA 2018	EMA 2013
Efflux Transporter								
P-gp (MDR1)	yes	yes	yes	yes	yes	yes	yes	yes
BCRP	yes	yes	yes	yes	yes	yes	yes	yes
BSEP	no	no	no	prefer	no	no	no	consider
MRP2	no	no	no	no	no	no	no	consider
Uptake Transporter								
OATP1B1	yes, time-dependent	yes, time-dependent	yes, time-dependent	yes	the hepatic uptake or elimination is significant; or the uptake into the liver is clinically important; the other factors support the importance of active uptake into liver	the hepatic uptake or elimination is significant; or the uptake into the liver is clinically important; the other factors support the importance of active uptake into liver	hepatic metabolism or bile secretion is the major pathway for elimination	≧25% of the elimination is hepatic
OATP1B3	yes, time-dependent	yes, time-dependent	yes, time-dependent	yes				
OAT1	yes	yes	yes	yes	the active renal secretion is significant	the active renal secretion is significant	active secretion in the kidney is the major elimination pathway	≧25% of the elimination is through renal secretion or is/may be due to biliary/gut wall secretion
OAT3	yes	yes	yes	yes				
OCT2	yes	yes	yes	yes				
MATE1	yes, adjust pH	yes, adjust pH	yes, adjust pH	consider				
MATE2K	yes, adjust pH	yes, adjust pH	yes, adjust pH	consider				
OCT1	no	no	no	consider	no	no	no	no

Fu. S et al., *Medicine in Drug Discovery*. (2021)

Possible Model for Decision Making: Transporter-Based Drug-Drug Interaction Studies

Possible Model for Decision Making: Transporter-Based Drug-Drug Interaction Studies (Figure 5)

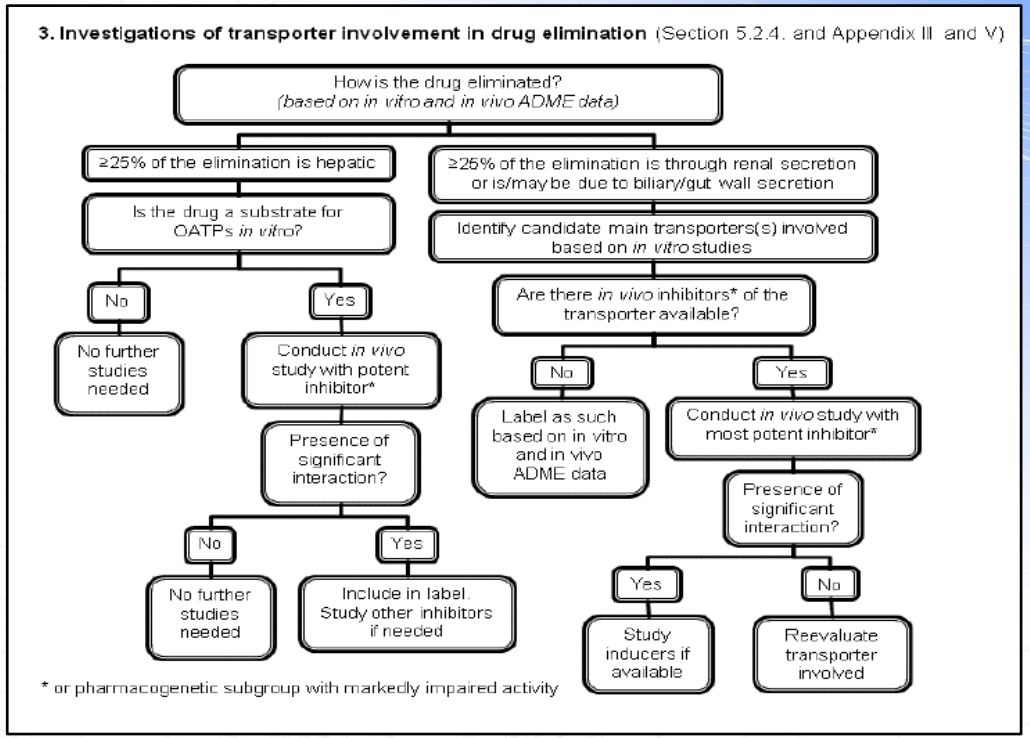
Figure 5. Evaluation of Investigational Drugs as Substrates for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 Transporters.



[1] Biliary secretion can be estimated from preclinical data, in vitro heptocyte uptake data or radiolabeled ADME data, and nonrenal clearance data.

[2] Percent (%) active renal secretion was estimated from $(CL_r – fu*GFR)/CL_{Total}$; fu is the unbound fraction in plasma.

US FDA

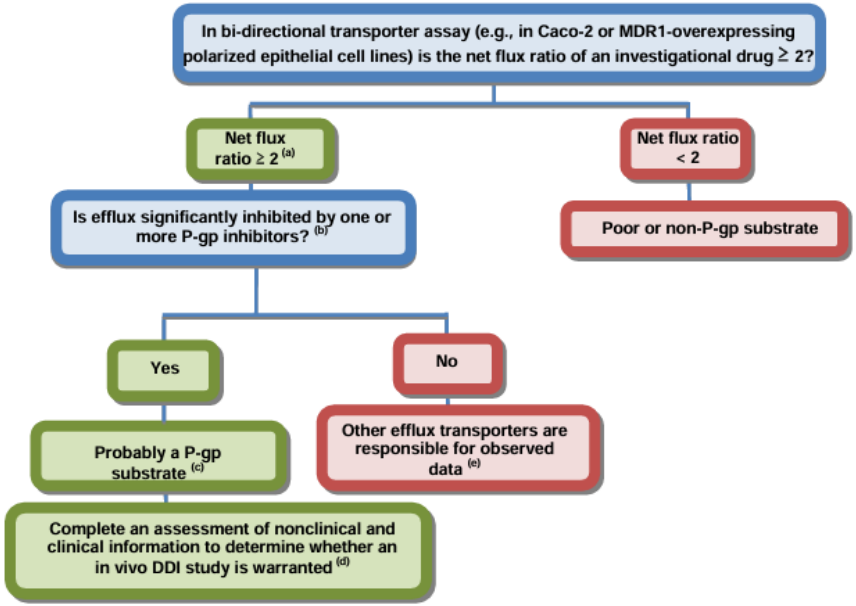


EMA, Guideline on the investigation of drug interactions

Decision Tree to Determine Whether An Investigational Drug is A Substrate for P-gp

P-gp and BCRP:

Figure 6. Decision tree to determine whether an investigational drug is a substrate for P-gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP substrate —(Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010).

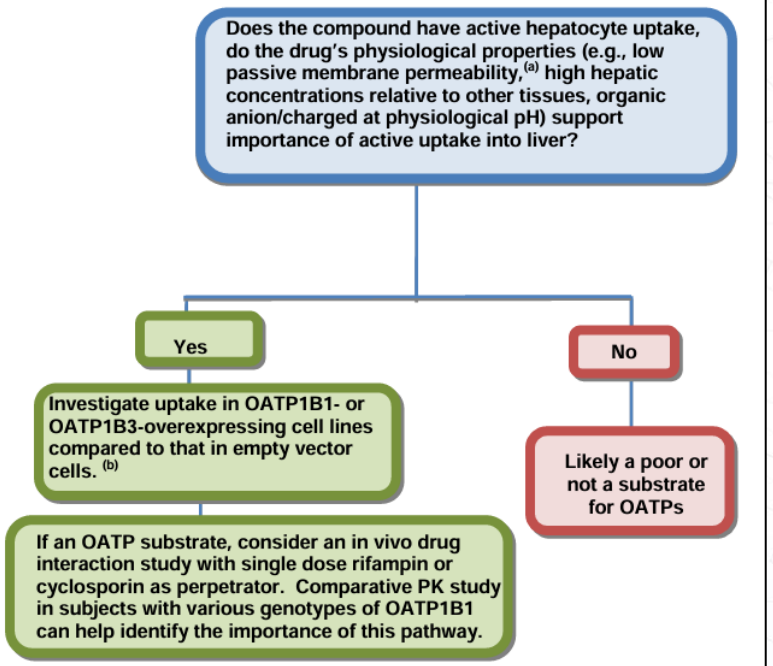


(a) An acceptable system produces net flux ratios of probe substrates similar to the literature values. A net flux ratio ≥ 2 for the investigational drug is a positive signal for further evaluation. A net flux ratio "cutoff" higher than 2 or a relative ratio to positive controls may be used to avoid false positives if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.
(b) Reduction of the flux ratio significantly (> 50%) or to unity.
(c) Additional data are needed to establish clinical relevance of the in vitro data. In particular, the relative contribution of the transporter-mediated pathway to the overall clearance of the drug is the primary determinant of whether an inhibitor will have a major effect on the disposition of the investigational new drug.
(d) Selection of inhibitors could be based on likelihood of co-administration and/or its inhibition potency on P-gp. Strong P-gp inhibitors (e.g., itraconazole, verapmil) provide the most sensitive assessment and should generally be tested first. If the drug is also a substrate for CYP3A, then inhibitors for both CYP3A and P-gp should be selected (Table 14).
(e) Based on existing knowledge of the compound class, further studies may be warranted to determine which efflux transporters are involved. Determining whether the drug is a BCRP substrate may be explored. A similar decision model may be used for a BCRP substrate; however, clinical studies would differ.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

Decision Tree to Determine Whether An Investigational Drug is A Substrate for OATP1B1 or OATP1B3

Figure 8. Decision tree to determine whether an investigational drug is a substrate for OATP1B1 or OATP1B3 and when an in vivo clinical study is needed— (Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010)



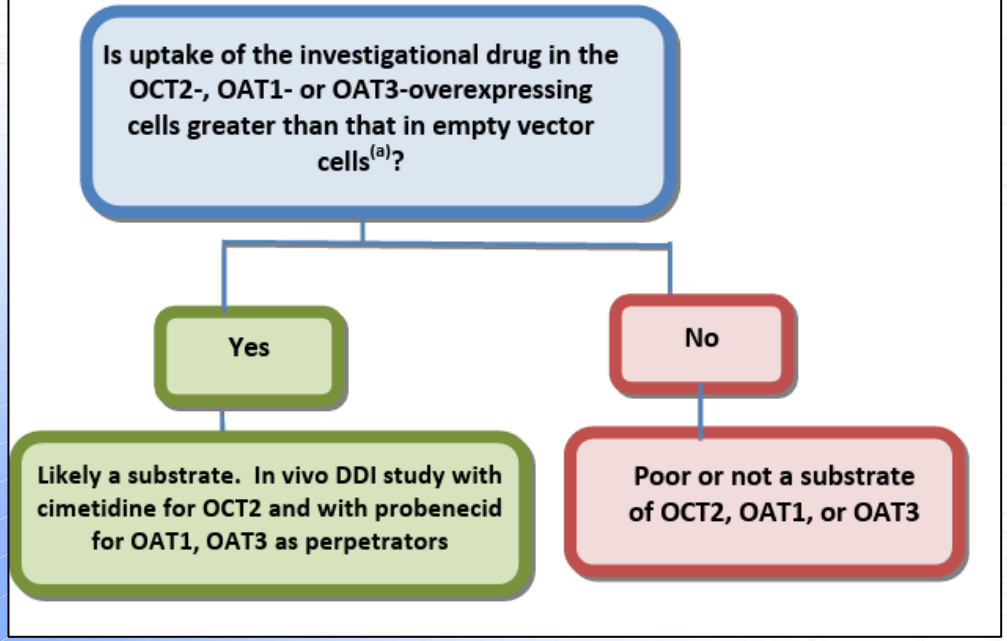
(a) Low permeability needs to be defined by each lab based on standards, such as atenolol (a biopharmaceutics classification system (BCS) reference drug). A general guide would be that 10^{-6} cm/sec (10 nm/sec) or lower is classified as "low" permeability.

(b) The following criteria suggest the investigational drug is a substrate of OATP1B1 or OATP1B3: Uptake in OATP1B1- or OATP1B3-transfected cells greater than 2-fold of that in empty vector transfected cells and is inhibitable (e.g, >50% reduction to unity) by a known inhibitor (e.g., rifampin) at a concentration at least 10 times of its K_i. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a ≥ 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

Decision Tree to Determine Whether An Investigational Drug is A Substrate for OCT2, OAT1 or OAT3

Figure 10. Decision tree to determine whether an investigational drug is a substrate for OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed —(Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010)



[(a)] The ratio of the investigational drug uptake in the cells expressing the transporter versus the control (or empty vector) cells should be greater than 2. It is important that uptake into the transfected cells be significantly greater than background in a control cell line and be inhibited by a known inhibitor of the transporter. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a ≥ 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

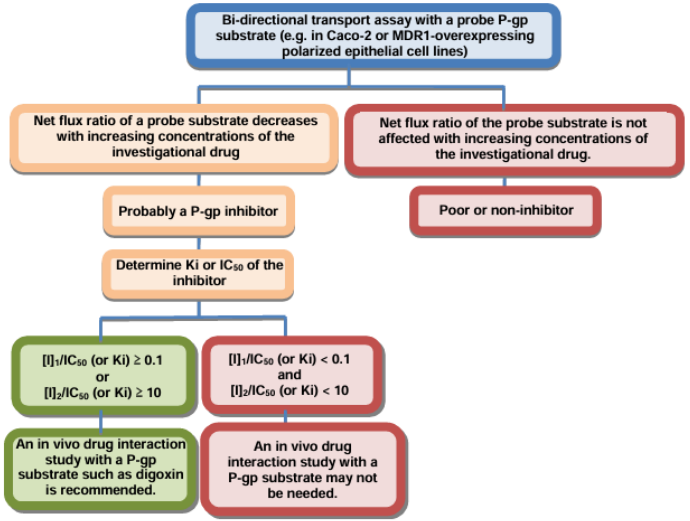
Regulatory Requirements of Transporter Evaluation for Inhibition Studies

	Inhibition Study			
	USFDA 2020	NMPA 2021	PMDA 2018	EMA 2013
Efflux Transporter				
P-gp (MDR1)	yes	yes	yes	yes
BCRP	yes	yes	yes	yes
BSEP	no	no	no	prefer
MRP2	no	no	no	no
Uptake Transporter				
OATP1B1	yes, time-dependent	yes, time-dependent	yes, time-dependent	yes
OATP1B3	yes, time-dependent	yes, time-dependent	yes, time-dependent	yes
OAT1	yes	yes	yes	yes
OAT3	yes	yes	yes	yes
OCT2	yes	yes	yes	yes
MATE1	yes, adjust pH	yes, adjust pH	yes, adjust pH	consider
MATE2K	yes, adjust pH	yes, adjust pH	yes, adjust pH	consider
OCT1	no	no	no	consider

Fu. S et al., *Medicine in Drug Discovery*. (2021)

Decision Tree to Determine Whether An Investigational Drug is An Inhibitor of P-gp

Figure 7. Decision tree to determine whether an investigational drug is an inhibitor of P-gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP inhibitor) — (Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010)

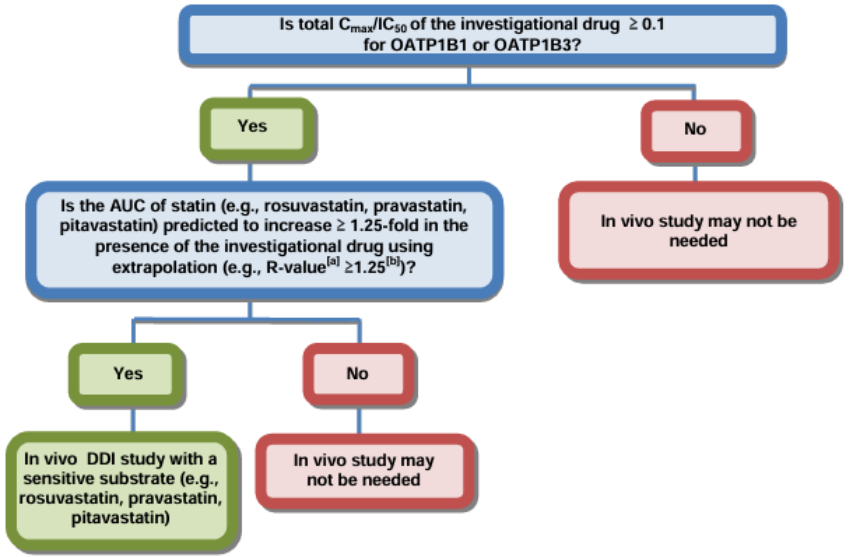


$[I]_1$ represents the mean steady-state total (free and bound) C_{max} following administration of the highest proposed clinical dose. $[I]_2$= Dose of inhibitor (in mol)/250 mL (if IC_{50} is in a molar unit). For IC_{50} determination, a unidirectional assay (e.g., B to A) based on the probe substrate can also be considered.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

Decision Tree to Determine Whether An Investigational Drug is An Inhibitor of OATP1B1 or OATP1B3

Figure 9. Decision tree to determine whether an investigational drug is an inhibitor of OATP1B1 or OATP1B3 and when an in vivo clinical study is needed —(Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010)



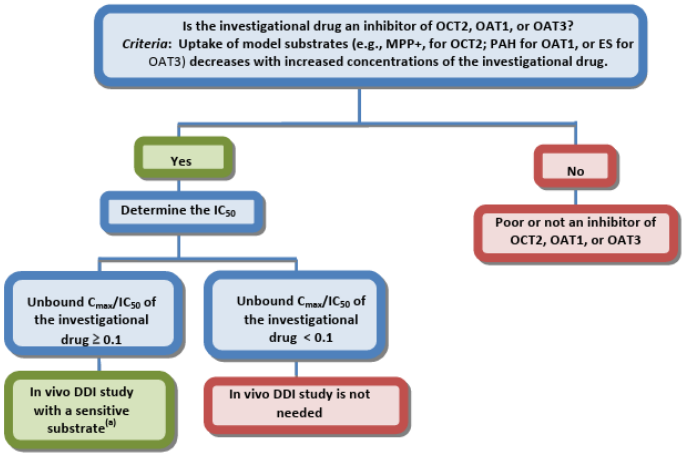
[a] R-value = 1+ (fu x $I_{in,max}/IC_{50}$), where, $I_{in,max}$ is the estimated maximum inhibitor concentration at the inlet to the liver and is equal to: C_{max} + (k_a x Dose x F_a F_g/Qh). C_{max} is the maximum systemic plasma concentration of inhibitor; Dose is the inhibitor dose; F_aF_g is the fraction of the dose of inhibitor which is absorbed; k_a is the absorption rate constant of the inhibitor and Qh is the estimated hepatic blood flow (e.g., 1500 mL/min). If Fa Fg values and ka values are unknown, use 1 and 0.1 min^{-1} (Ito et al. *Pharmacol Rev.* 50 (3): 387-412, 1998) for FaFg and ka, respectively because the use of theoretically maximum value can avoid false-negative prediction. For drugs whose fu values are less than 0.01 or fu cannot be accurately determined due to high protein-binding, then assume fu = 0.01, to err on the conservative side to avoid false negative predictions.

[b] These are the suggested values according to the upper limit of equivalence range. We are open to discussion based on sponsors' interpretation.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

Decision Tree to Determine Whether An Investigational Drug is An Inhibitor of OCT2, OAT1 or OAT3

Figure 11. Decision tree to determine whether an investigational drug is an inhibitor of OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed — (Modified From Figures in Giacomini KM, *et al*, Nat. Rev Drug Discov. 9: 215-236, 2010)



MPP^+, 1-methyl-4-phenylpyridinium; PAH, *para*-aminohippuric acid; ES, estrone-3-sulfate.

[a] For the investigational drug that is an OCT2 inhibitor, metformin may be used as the substrate for the clinical drug interaction study.

For investigational drugs that are OAT1 or OAT3 inhibitors, multiple OAT1 or OAT3 substrates could be used in clinical DDI studies, including zidovudine, acyclovir, ciprofloxacin, tenofovir, or methotrexate.

https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-possible-models-decision-making

Determining if the Investigational Drug is an Inducer of a Transporter

- Transporters such as P-gp are induced through mechanisms similar to those for CYP enzymes (ex: by activation of specific nuclear receptors).
- Because of these similarities, information from CYP3A inductions studies can inform P-gp induction studies.
- *In vitro* methods to evaluate the induction of P-gp and other transporters are not well established.

Analysis and Interpretation of Transporter Inhibition Assay

The data analysis and interpretation of transporter inhibition assay recommended by USFDA, NMPA, PMDA and EMA guidelines.

Transporters	USFDA 2020	NMPA 2021	PMDA 2018	EMA 2013
P-gp (MDR1), BCRP	For NCE administered orally, I_{gut}/IC_{50} or $K_i \geq 10$; Where, I_{gut} = dose of NCE/250 mL For NCE administered by parental route, I_1/IC_{50} or $K_i \geq 10$ Where, I_1 = C_{max} of NCE	For NCE administered orally, I_{gut}/IC_{50} or $K_i \geq 10$; Where, I_{gut} = dose of NCE/250 mL For NCE administered by parental route, I_1/IC_{50} or $K_i \geq 10$ Where, I_1 = C_{max} of NCE	$I_{gut}/IC_{50} \geq 10$ Where, I_{gut} = maximum single dose of NCE/250 mL	$Ki \leq 0.1 \times I_{gut}$ Where, I_{gut} = dose of NCE/ 250 mL, or if low solubility, I_{gut} = the maximum possible concentration at the pH range of the GI tract
OATP1B1, OATP1B3	$1+(f_{u,p} \times I_{max\ inlet})/IC_{50} \geq 1.1$ Where, $f_{u,p}$ is the unbound fraction in plasma. Imax inlet is the estimated maximum plasma NCE concentration at the inlet to the liver, which is calculated as: $I_{max\ inlet} = I_{max}+(F_a \times F_g \times k_a \times dose)/Q_h/R_BF_a$ is the fraction absorbed. F_g is the intestinal availability. k_a is the absorption rate constant. Q_h is the hepatic blood flow rate. R_B is the blood-to-plasma concentration ratio. $F_a = 1$, $F_g = 1$ and $k_a = 0.1$/min can be used as a worst-case estimate. The unbound fraction ($f_{u,p}$) should be set to 1% if experimentally determined to be less than 1%.	$1+(f_{u,p} \times I_{max\ inlet})/IC_{50} \geq 1.1$ Where, $f_{u,p}$ is the unbound fraction in plasma. Imax inlet is the estimated maximum plasma NCE concentration at the inlet to the liver, which is calculated as: $I_{max\ inlet} = I_{max}+(F_a \times F_g \times ka_\times dose)/Q_h/R_BF_a$ is the fraction absorbed. F_g is the intestinal availability. k_a is the absorption rate constant. Q_h is the hepatic blood flow rate. R_B is the blood-to-plasma concentration ratio. $F_a = 1$, $F_g = 1$ and $k_a = 0.1$/min can be used as a worst-case estimate. The unbound fraction ($f_{u,p}$) should be set to 1% if experimentally determined to be less than 1%.	$1+(f_{u,b} \times I_{max\ inlet})/K_i \geq 1.1$ Where, Imax inlet = $C_{max}+(k_a \times dose \times F_aF_g/Q_h)$. C_{max} = the maximum blood concentration of the inhibitor, dose is the dose of the inhibitor, F_aF_g is the intestinal availability of the inhibitor, k_a is the absorption rate constant of the inhibitor, and Q_h is the hepatic blood flow rate (97 L/hr/70 kg). If the F_aF_g and k_a values are unknown, 1 and 0.1 min^{-1}can be used as the values for the F_aF_g and k_a, respectively. $f_{u,b}$ is blood unbound fraction of drugs.	$K_i \leq 25 \times I_{max\ u\ inlet}$ Where, $I_{max\ u\ inlet}$ = the unbound hepatic inlet concentration
OAT1, OAT3, OCT2, MATE1, MATE2K	$I_{max\ u}/IC_{50} \geq 0.1$ Where, $I_{max\ u}$ is maximal unbound plasma concentration of NCE at steady state.	$I_{max\ u}/IC_{50} \geq 0.1$ Where, $I_{max\ u}$ is maximal unbound plasma concentration of NCE at steady state.	For OAT1, OAT3, OCT2, $1 + C_{max\ u}/K_i \geq 1.1$; For MATE1, MATE2K, $1 + C_{max\ u}/K_i \geq 1.02$ Where, $C_{max\ u}$ is the unbound maximum blood concentration of NCE.	$K_i \leq 50 \times C_{max\ u}$ Where, $C_{max\ u}$ is the unbound maximum blood concentration of NCE.

Fu. S et al., *Medicine in Drug Discovery*. (2021)

Regulatory Guidance Assessing tDDIs
PBPK Modeling Considerations

Parameters	USFDA (2020)	PMDA (2018)	EMA (2013)
PBPK modeling considerations	• ADME processes-mediated by transporters • Verify models for transporter substrates when evaluating inhibitory effect • Limitations of establishing models for tDDI's, enzyme transporter interplay	• PBPK models to guide clinical studies or designs • Can determine necessity *of* in vivo interaction studies • To support interaction study in case of dose, regimen or formulation change	• Can be used to inform the design of in vivo DDI studies and to support labeling • Subpopulation impact (PBPK prediction of relative contribution of enzymes to clearance, inhibition of enzymes, well validated)

Vijaywargi G., *Biopharm Drug Dispos*. 2023

Literature – tDDI and PBPK Modeling

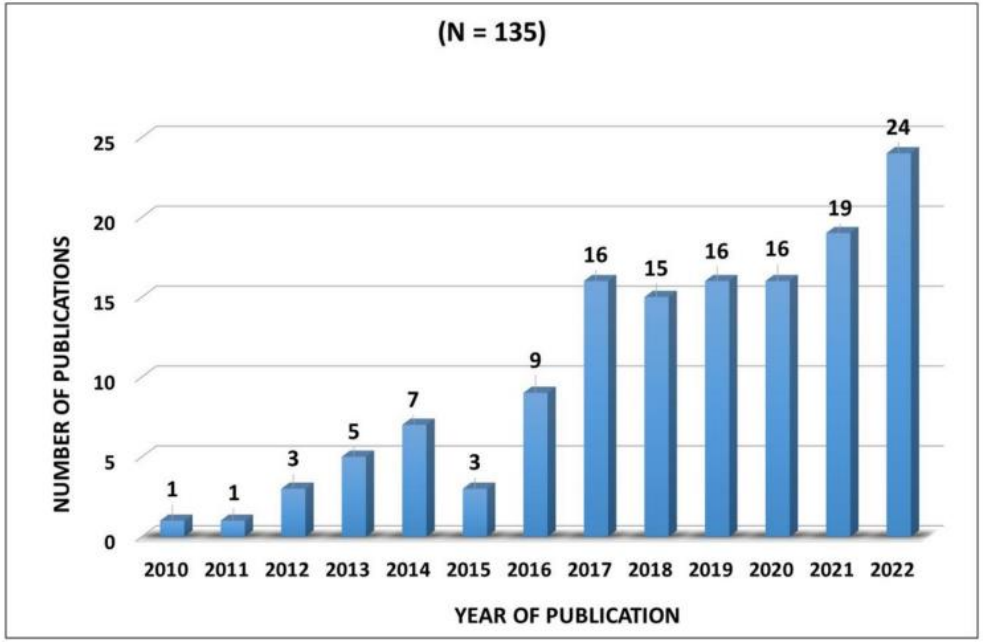


FIGURE 1 Bar graph showing the steady increase in the number of publications in last decade (2010–September 2022) where transporter-mediated disposition of drug molecules has been captured using PBPK modeling

Vijaywargi G., *Biopharm Drug Dispos*. 2023

Transporter based PBPK Models in Healthy

TABLE 1 Literature survey of available transporters-based PBPK models of drug molecules in normal, diseased, and special populations

Transporter	Molecule	Mechanism	Model objective	Minimal or full PBPK	Data input	Data source	Acceptance criterion[a]	Outcome[b]	Software used	Reference
Healthy population										
OAT3, MRP4	Furosemide Probenecid	Substrate Inhibitor	DDI	Full PBPK	Uptake parameters Inhibition parameters	In vitro, PE, OP, SA	3	1	PK-Sim	Britz et al. (2020)
P-gp	Rivaroxaban	Substrate	DDI	Full PBPK	Metabolism by CYPs, non-CYPs, GFR and renal secretion by P-gp	In vitro, PE, SA	3	2	PK-Sim and MoBi	Willmann et al. (2021)
OATP1B1 BCRP	Simvastatin acid Simvastatin lactone	Substrate	DGI	Full PBPK	Literature reported uptake parameters	In vivo, PE, OP	3	1	PK-Sim	Wojtyniak et al. (2021)
OATP1B1, 1B3 and BCRP	Rosuvastatin	Substrate	tDDI	Full PBPK	Literature reported uptake parameters	In vivo, PE, OP	3	1	PK-Sim	Hanke et al. (2021)
OATP1B1, 1B3, MATEs and OCT2	Dasatinib	Inhibitor	tDDI	Full PBPK	Literature reported inhibition parameters	In vitro, PE, OP, BC	3	1	Simcyp	Chang et al. (2022)
P-gp	Edoxaban	Substrate	P-gp impact on absorption	Full PBPK	P-gp clearance in each GI segment, biliary and metabolic CL	In vitro, in vivo, PE, OP, BC	2	1	Gastroplus	Kato et al. (2021)
OATP1B1, 1B3	Vemurafenib	Inhibitor	tDDI	Minimal PBPK	Experimental inhibition parameters	Experimental in vitro	7	1	Simcyp	Kayesh et al. (2021)
PEPT1	Cefadroxil	Substrate	PEPT1 impact on absorption	Minimal PBPK	Experimental transporter kinetics in mouse (extrapolated to humans)	Experimental in vitro, OP	5	1	Gastroplus	Tan et al. (2021)

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Transporter based PBPK Models in Diseased and Geriatric Population

Transporter	Molecule	Mechanism	Model objective	Minimal or full PBPK	Data input	Data source	Acceptance criterion[a]	Outcome[b]	Software used	Reference
Diseased population (hepatic[A], renal impaired[B] and cancer[C])										
[A]OATP1B1	Pemafibrate	Substrate	Clinical PK	Full PBPK	Experimental metabolic and uptake clearances	Experimental in vitro, PE, OP	5	1	Simcyp	Ogawa et al. (2020)
[B]OCT2	Pramipexole	Substrate	Clinical PK	Full PBPK	RAF value for OCT2 in PD patients and literature reported disposition parameters	In vitro, in vivo, PE, OP	5	1	Gastroplus	You et al. (2020)

Transporter	Molecule	Mechanism	Model objective	Minimal or full PBPK	Data input	Data source	Acceptance criterion[a]	Outcome[b]	Software used	Reference
Geriatric population										
Specific transporter is not characterized	Bilastine	Substrate of efflux and influx transporters	Clinical PK	Full PBPK and Pop-PK	Transporter kinetics estimated using SA and attributed to P-gp and BCRP	In vivo, SA, PE	3	1	Gastroplus and NLME	Kim et al. (2021)

Vijaywargi G., *Biopharm Drug Dispos*. 2023

Transporter Mediated DDI in Regulatory Submissions

Table 2 Examples of DDI PBPK analyses and their impact on drug development and regulatory decision

Drug	Key theme (impact level) and question(s)	Victim/perpetrator?	Brief description	Internal impact	Qualification dataset	FDA/EMA response
Trametinib (marketed) Chen *et al.*, 2015[61]	DDI (high) Requested to provide clinical studies to investigate the inhibition of intestinal BCRP. *In vitro* BCRP inhibition data flagged the potential risk of *in vivo* DDI according to the EMA regulatory guidelines.	Perpetrator: Weak BCRP inhibitor	*In vitro* Trametinib is a weak BCRP inhibitor, however based upon the EMA DDI guidance criteria the *in vivo* risk in the gut could not be excluded using *in vitro* data alone. Predicted intestinal concentrations were simulated using GastroPlus. Complete inhibition was predicted for the first 40 minutes post dose and partial inhibition was predicted up to 1.6 hours post dose and restricted to the duodenum and jejunum. Recommendation was to limit the co-administration of sensitive BCRP substrates to 2 hours post trametnib administration	Previously constructed GastroPlus Model of trametinib was developed for other applications, therefore minimal work was required to construct the model in response to the agency. Absorption was simulated and the outputs of the model (predicted concentrations vs. time) along the intestinal track were used as input in the DDI prediction guidelines, internal static modeling as well as cross referencing data in the Washington database to inform concomitant medications at risk. No clinical BCRP DDI study was conducted	*In vitro* BCRP inhibition data. Sponsor was requested to further discuss the interaction potential between trametinib and drugs mainly absorbed in the duodenum and jejunum. Outcome: Using the University of Washington database a list of BCRP substrates absorbed within 1-2 hours after oral administration was constructed. This list was further refined to exclude those substrates in which the DDI mechanism was known, leaving behind a list of substrates that may potentially be affected by BCRP inhibition.	FDA: Not submitted by the sponsor. EMA: Accepted.

Shebley *Clin Pharm Ther* 2018

Table 3 Examples of transporter-mediated DDI PBPK analyses and their impact on drug development and regulatory decision

Example number	Drug	Key theme Transporter (location function) Inhibitor – inh Substrate - sub	Victim/perpetrator/ and question(s)?	Brief description	Impact[a]	Qualification dataset	FDA/EMA response
4	Axitinib (marketed)	Intestinal transporter: P-gp (apical efflux) inhibitor	Does P-gp inhibition *in vitro* translate to clinical DDI liability unbound C_{max} of 0.0008 μM,	ACAT model using Gastroplus was built to simulate axitinib concentrations in segments of GI tract	High Impact: Agreement of HA that no formal DDI trial with P-gp substrate is needed		FDA: Accepted EMA: Not submitted

Taskar *Clin Pharm* Ther 2019

Strategies for Validating tDDI Models

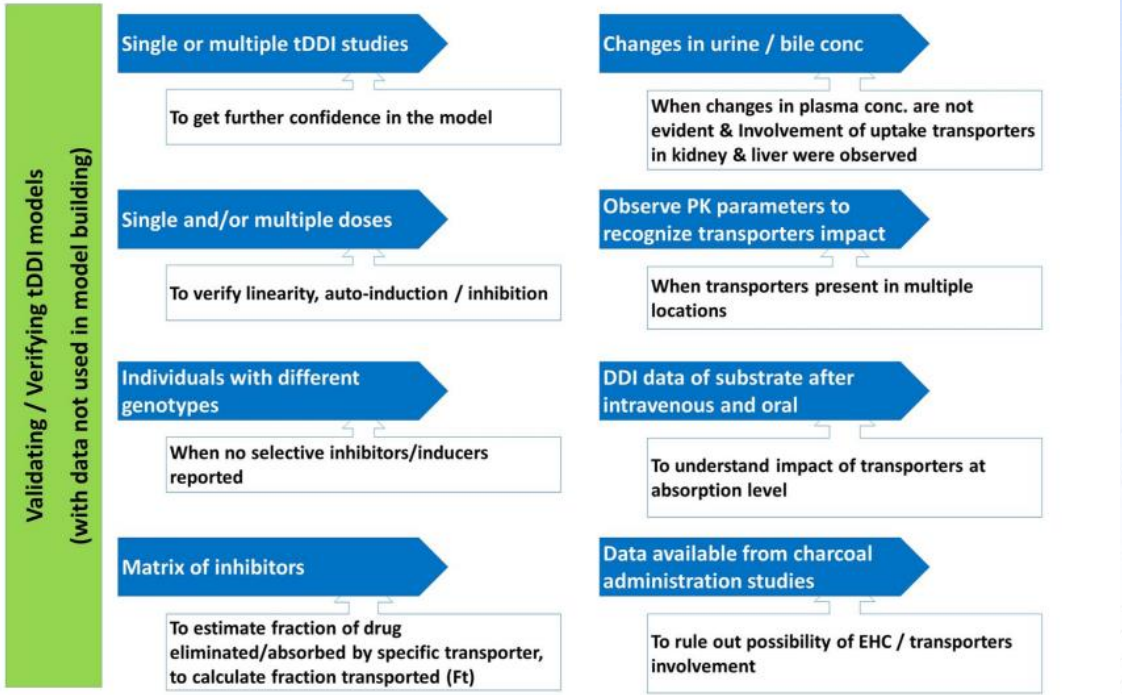


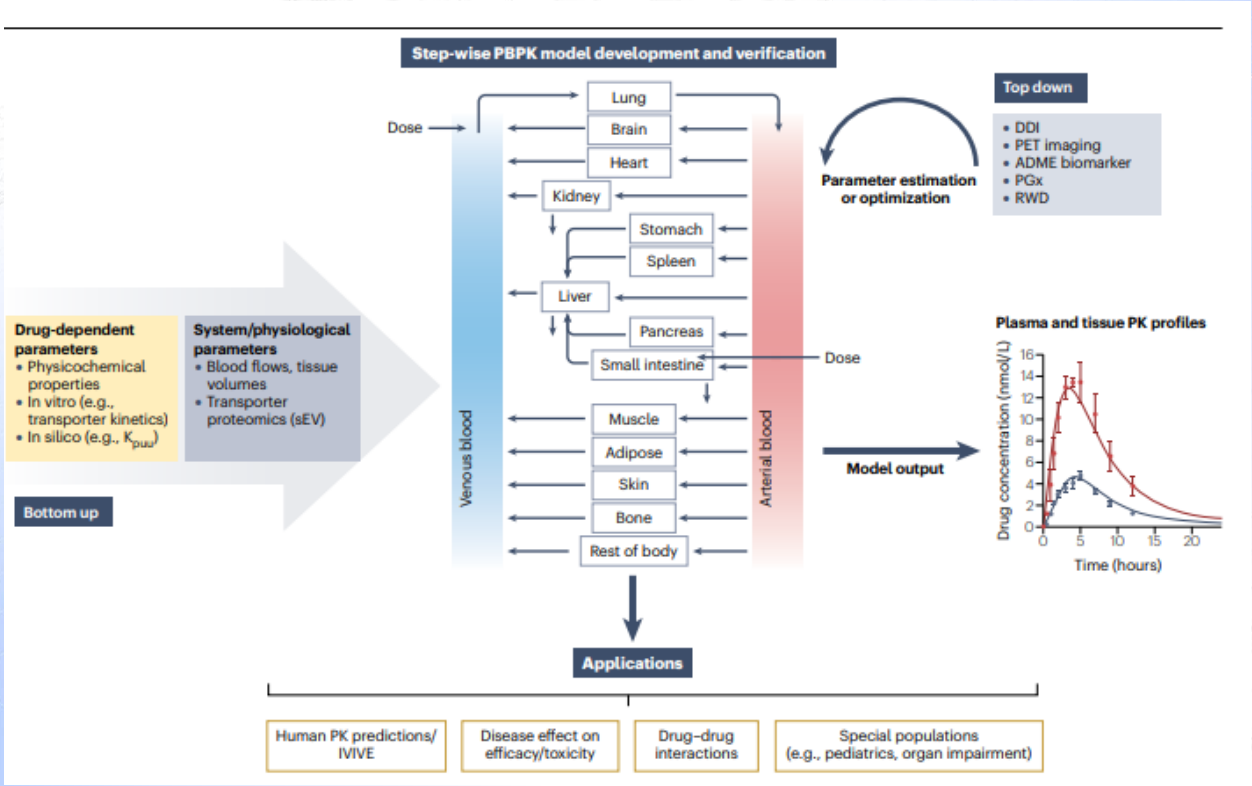
FIGURE 5 Various strategies for validating tDDI models with external data. tDDI, transporter-mediated drug–drug interaction.

Vijaywargi G., *Biopharm Drug Dispos*. 2023

Challenges – PBPK Modeling Involving Drug Transporters

- There has been considerable effort in the area of transporters over the last decade to understand and build robust IVIVE for transporters
- Some information is still lacking like
  - in vitro data (ex: K_m, J_{max}, CL_{PD}),
  - Protein abundance/ expression of the transporters in different tissues
  - Localization of transporters
  - Transporter Induction/inhibition parameters
  - Time-dependent inhibition of transporters

Development, Validation, and Application of PBPK Models of Transporter- Mediated Processes



DDI Qualification Approach

- Literature collection collated in a spreadsheet
- Model building and verification of single doses and multiple doses
- Verification for all mechanisms of DDI
- PowerPoint
- MS-Word Reports (context of use) that can be submitted along with PBPK reports for investigational drugs

In Silico DDI Predictive Performance: Guest Criteria

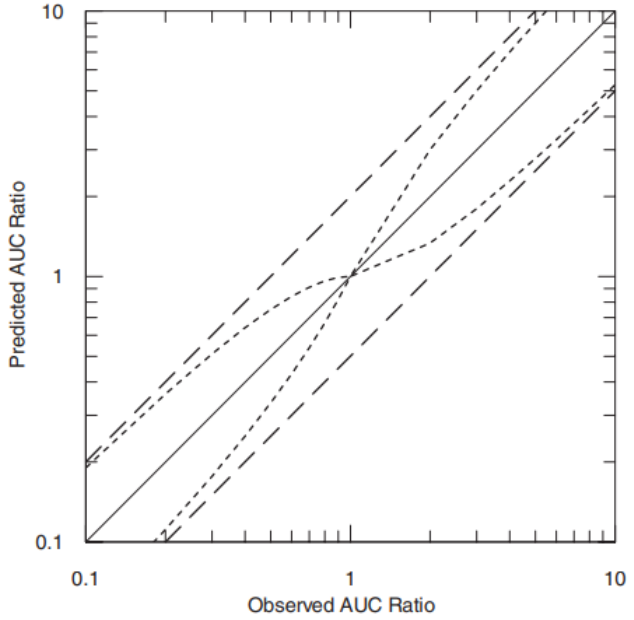


FIG. 1. Schematic graph displaying the limits of the different predictive measures; the traditional two-fold predictive measure (dashed lines) and the proposed new predictive measure (dotted lines). Observed AUC ratios include both induction and inhibition DDIs.

Guest EJ, Aarons L, Houston JB, Rostami-Hodjegan A, Galetin A. Critique of the two-fold measure of prediction success for ratios: application for the assessment of drug-drug interactions. Drug Metab Dispos. 2011 Feb;39(2):170-3

Upper limit: $R_{obs} * Limit$

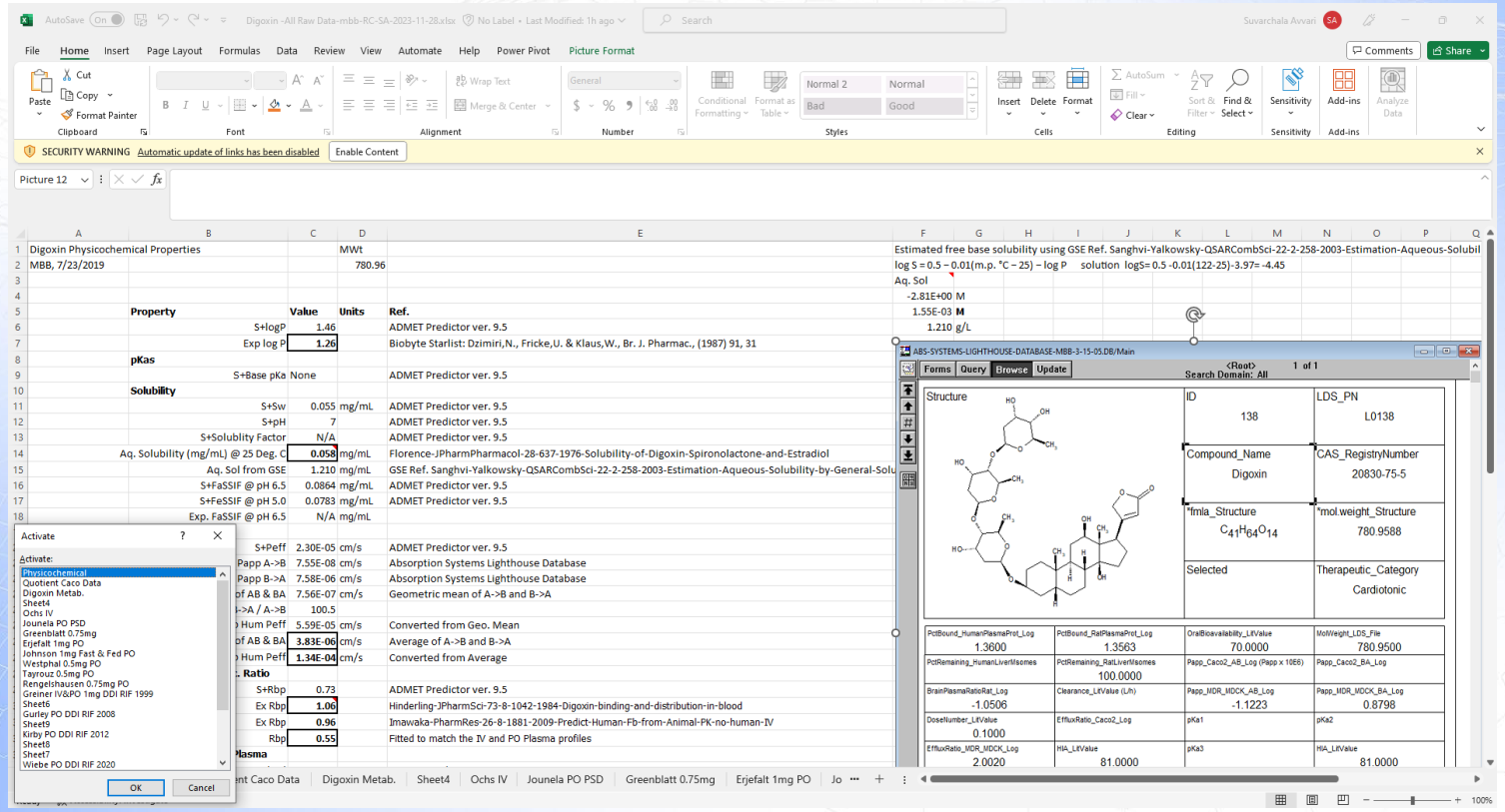
Lower limit: $R_{obs}/Limit$

$$Limit = \frac{1 + 2(R_{obs} - 1)}{R_{obs}}$$

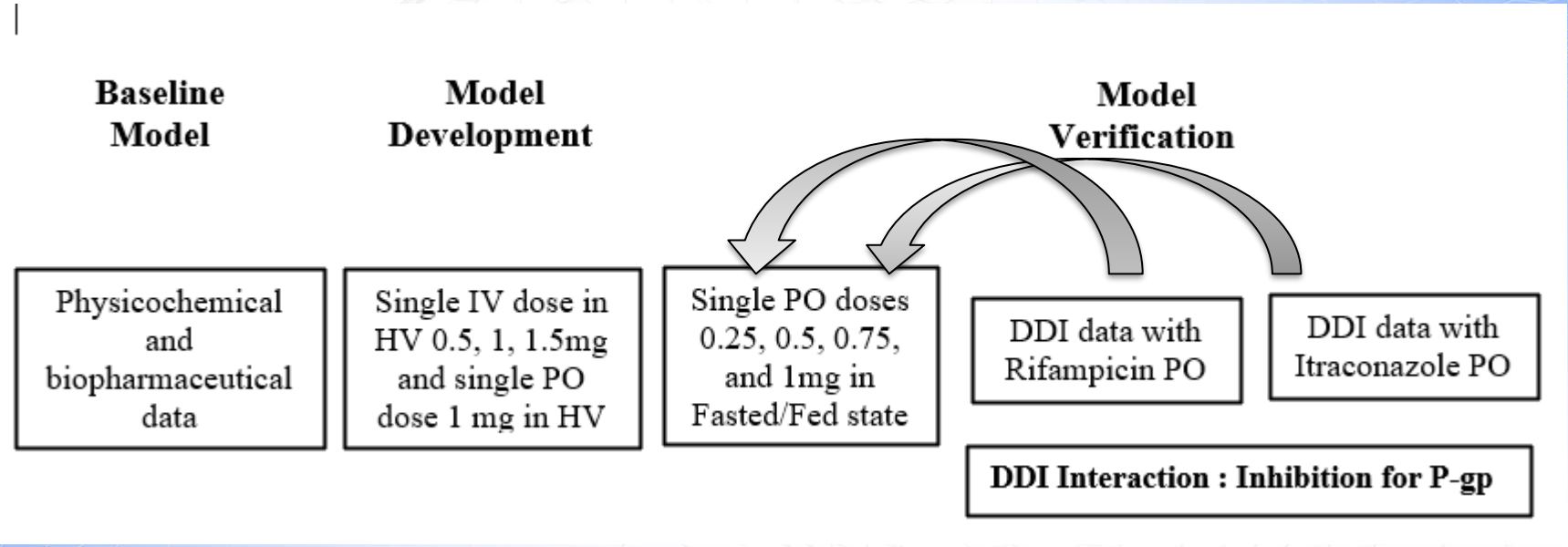
Predictive performance (i.e., fold of deviation) is related to the magnitude of DDI interactions (i.e., if the ratio of observed post- and pre- DDI values are greater, the acceptable limits for *in silico* DDI predictive performance are wider.

Development of a Physiologically Based Pharmacokinetic (PBPK) Model for the P-gp Substrate Digoxin, and Model Validation of Known Drug-Drug Interactions (DDIs) with Rifampicin and Itraconazole

Literature collection



Overview of Modeling Strategy of Digoxin



Key Physicochemical and Biopharmaceutical Parameters for Digoxin Used in GastroPlus Simulations

Parameter	Value	Reference
logP	1.26	[PUBCHEM]
Diffusion coefficient	0.44x10-5 cm2/s	ADMET Predictor
pKa	NA (None in Physiological Range)	
Reference solubility	0.058 mg/mL @ pH = 7.0	[Florence et al. 1976]
Dissolution Model	Johnson with Particle size of 5 µM	GastroPlus default (Lu et al. 1993)
Precipitate radius	1 µm	GastroPlus default
Drug particle density	1.2 g/mL	GastroPlus default
Mean precipitation time	900 s	GastroPlus default
Human Jejunal P_{eff} ($\times 10^{-4}$)	1.765 cm/sec	Assumed to be 10 times of rat permeability 0.4 $\times 10^{-4}$ cm/s [Varma et al. 2005]
Blood: plasma concentration ratio (Rbp)	0.55	ADMET Predictor
Plasma protein binding (Fup%)	75 %	US FDA
Adjusted plasma fraction unbound %	69.249	GastroPlus algorithm

Parameter	Value	Reference
OATP4C1 **(kidney)**		
K_m (µM)	7.8	[Mikkaichi et al. 2004]
V_{max} (mg/s/mg trans protein)	0.1	Optimized value
Na^+/K^+-ATPase **(muscle)**		
K_m (mg/L)	6.2	Assumed
V_{max} (mg/s/mg trans protein)	0.03	Optimized value
P-gp **(PBPK)**		
K_m (µM)	177	(Troutman et al. 2003)
V_{max} (mg/s/mg trans protein)	0.018	Optimized value
P-gp (**gut-apical**)		
K_m (µM)	177	(Troutman et al. 2003)
V_{max} (mg/s/mg trans protein)	0.15	Optimized value
Liver Apical PStc	0.5 (mL/s)	Optimized value (MDR3 P-gp transporter in liver)
SpecPStc	0.35 (mL/s/mL)	Optimized value
Renal Clearance Estimation method	f_{up}* GFR	

[a] Predicted using ADMET Predictor® v10.0

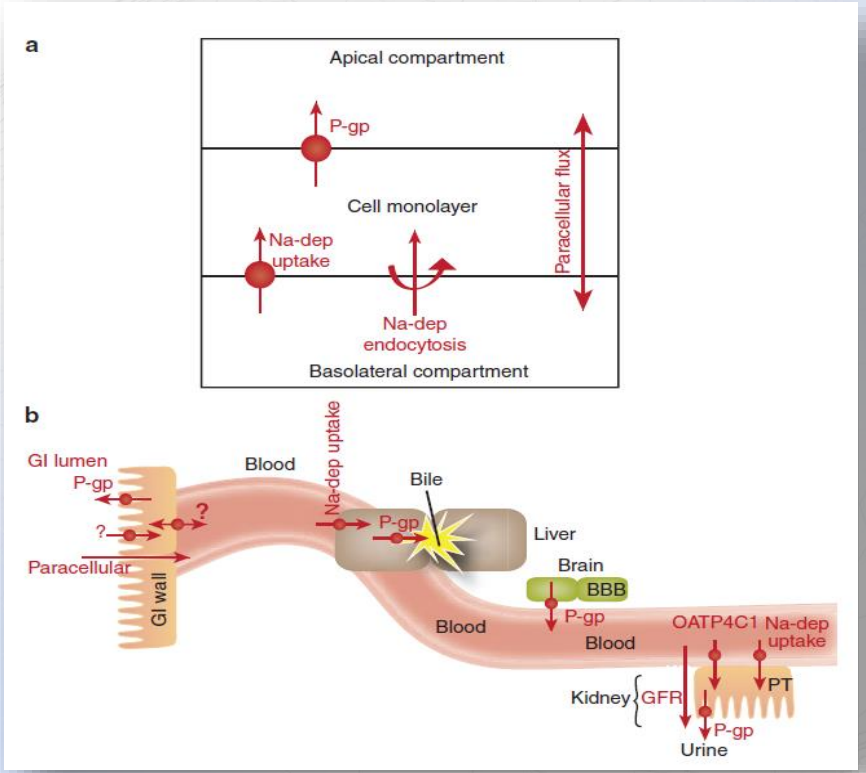
Digoxin Characteristic Properties

- P-gp substrate that reaches C_{max} 1-3 hrs after oral administration
- Mainly excreted unchanged in human urine (only16 – 25 % of a dose is metabolized)
- The fact that renal CL of Digoxin is greater than creatinine CL indicates that it is excreted by tubular secretion as well as by glomerular filtration
- Studies indicate OATP uptake of Digoxin in rat small intestine

Distribution Characteristics

- Na+/K+ - ATPase (sodium pump) acts as receptor for Digoxin
- Skeletal muscle pool of sodium pumps constitutes the main determinant of the V_{ss} of Digoxin
- Receptor binding is relatively slow
- Transcapillary permeation of Digoxin is rapid relative to tissue binding
- So, tissue binding is the rate-limiting step in Digoxin distribution kinetics

Schematic View of Transporters Involved in Absorption and Disposition of Digoxin in Gut, Liver, Muscle and Kidney



Lee CA. CPT- 96(3)-298-301

Digoxin and MDR3 P-gp Transporter

MDR3 P-glycoprotein, a Phosphatidylcholine Translocase, Transports Several Cytotoxic Drugs and Directly Interacts with Drugs as Judged by Interference with Nucleotide Trapping*

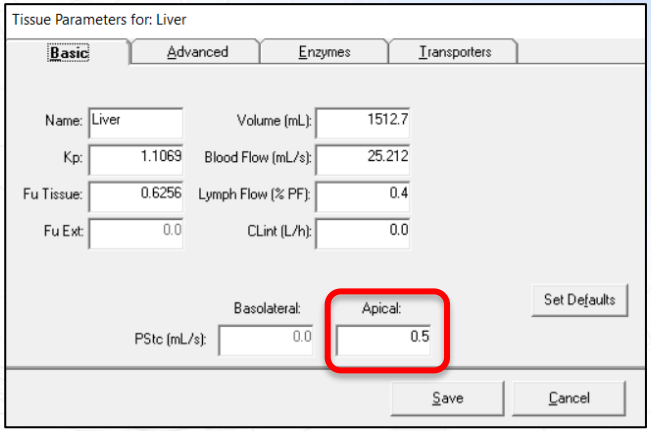
Received for publication, November 8, 1999, and in revised form, April 20, 2000
Published, JBC Papers in Press, May 1, 2000, DOI 10.1074/jbc.M909002199

The human *MDR3* gene is a member of the multidrug resistance (MDR) gene family. The MDR3 P-glycoprotein is a transmembrane protein that translocates phosphatidylcholine. The MDR1 P-glycoprotein related transports cytotoxic drugs. Its overexpression can make cells resistant to a variety of drugs. Attempts to show that MDR3 P-glycoprotein can cause MDR have been unsuccessful thus far. Here, we report an increased directional transport of several MDR1 P-glycoprotein substrates, such as digoxin, paclitaxel, and vinblastine, through polarized monolayers of *MDR3*-transfected cells. Transport of other good MDR1 P-glycoprotein substrates, including cyclosporin A and dexamethasone, was not detectably increased. MDR3 P-glycoprotein-dependent transport of a short-chain phosphatidylcholine analog and drugs was inhibited by several MDR reversal agents and other drugs, indicating an interaction between these compounds and MDR3 P-gp. Insect cell

Asterisk indicates important transporters in the organ as identified in the organ diagram.

Organ	Source	Relative Expression
Brain	Nishimura	0.0000887
Kidney	Nishimura	0.000425
Liver*	Nishimura	0.150
Placenta	Nishimura	0.000122
Small Intestine	Nishimura	0.000614
Kidney	Mean across all PMT Samples	BLQ
Liver*	Mean across all PMT Samples	5.061

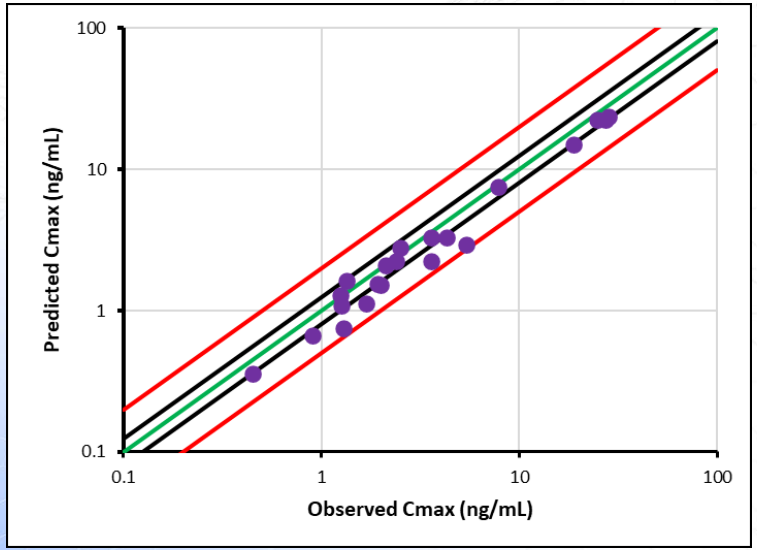
Note that relative expression values should only be compared between entries of the same source.

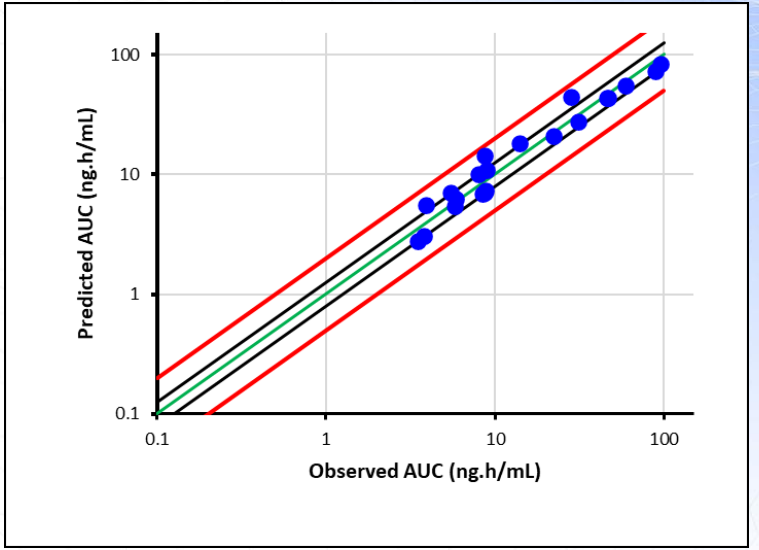


Smith J- J Biolo Chemistry-275(31)-23530-2000

Model Validation of Digoxin

Observed vs Predicted Values for C_{max} and AUC of Digoxin

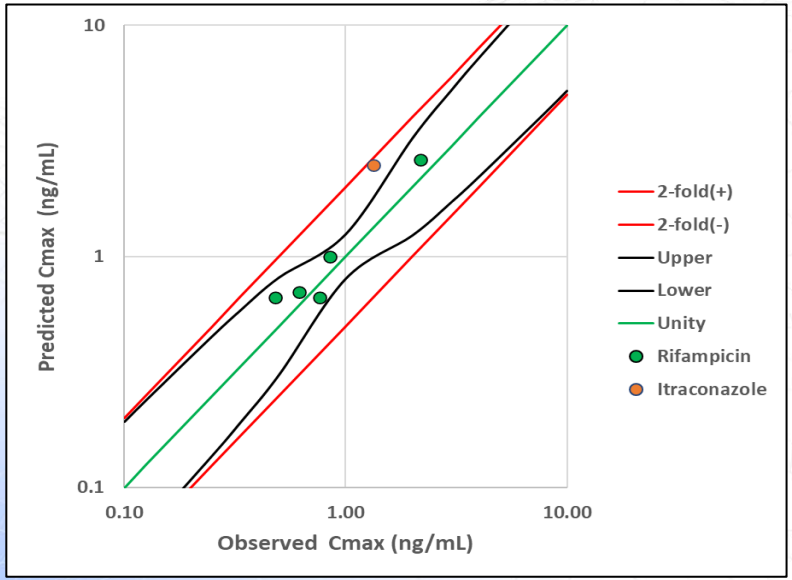


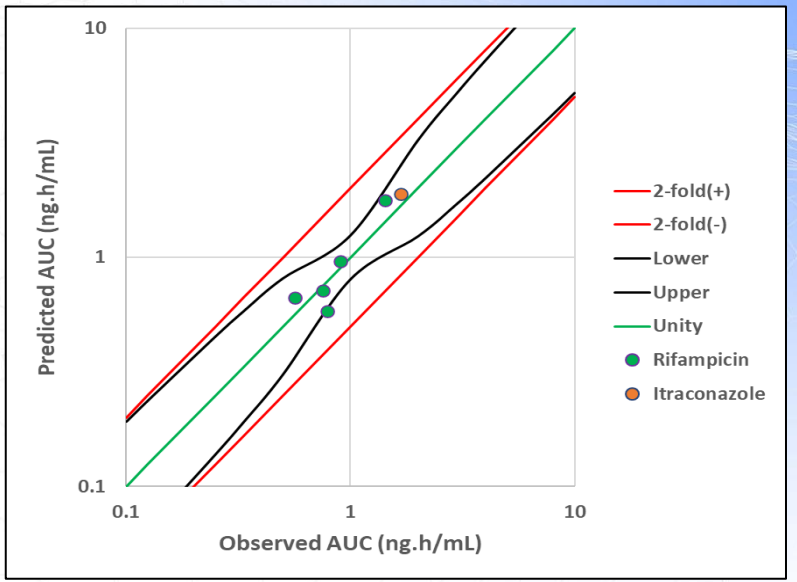


Purple Circles and Blue Circles represent C_{max} and $AUC_{0\text{-}inf}$, respectively.

Red lines (—) represent 2-fold prediction error, Black lines (—) represent the 1.25-fold prediction error.

DDI Accuracy



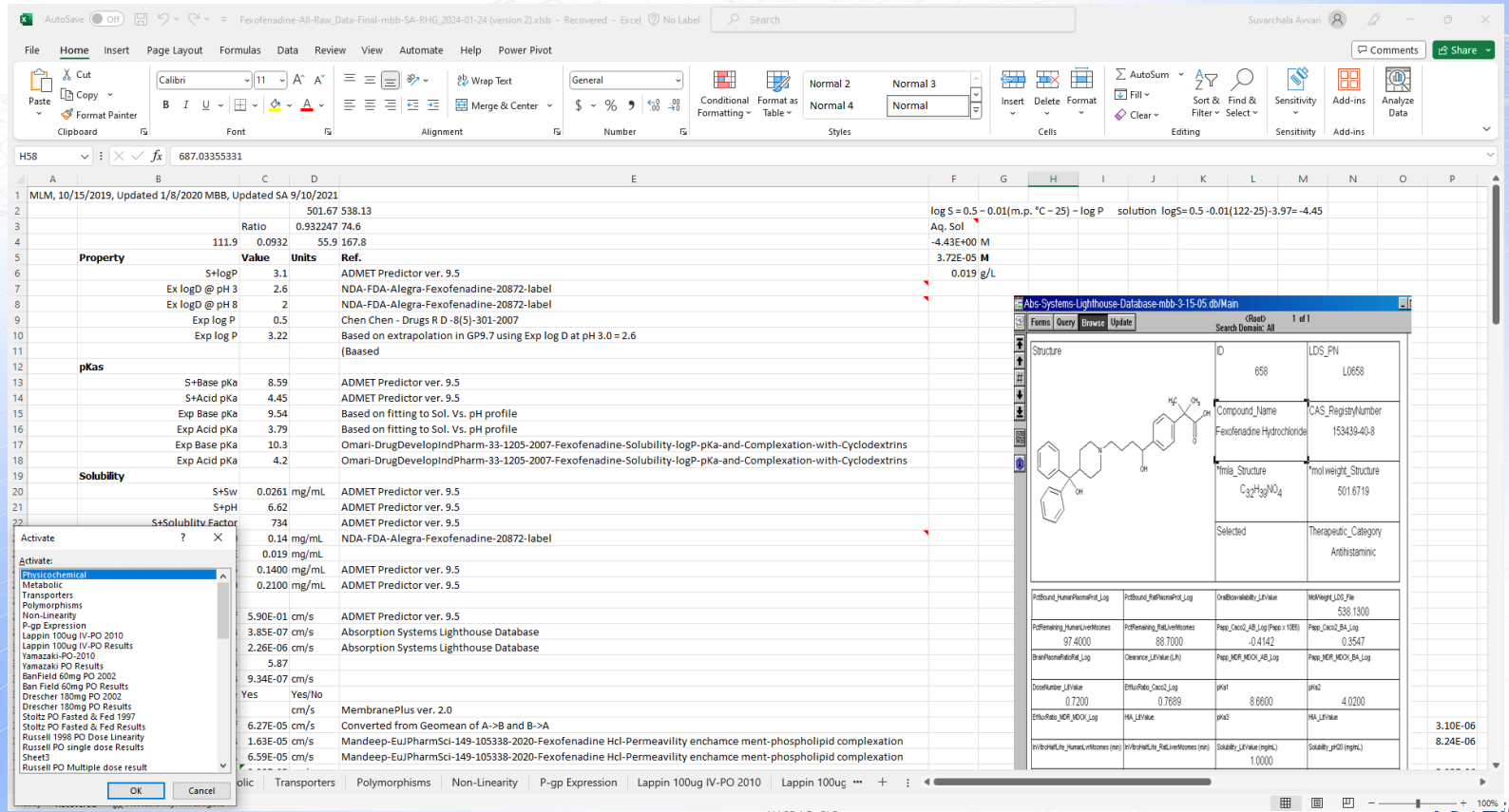


Observed vs Predicted AUC_{0-t} and C_{max} Ratios for DDI Between Digoxin, Rifampicin, and Itraconazole

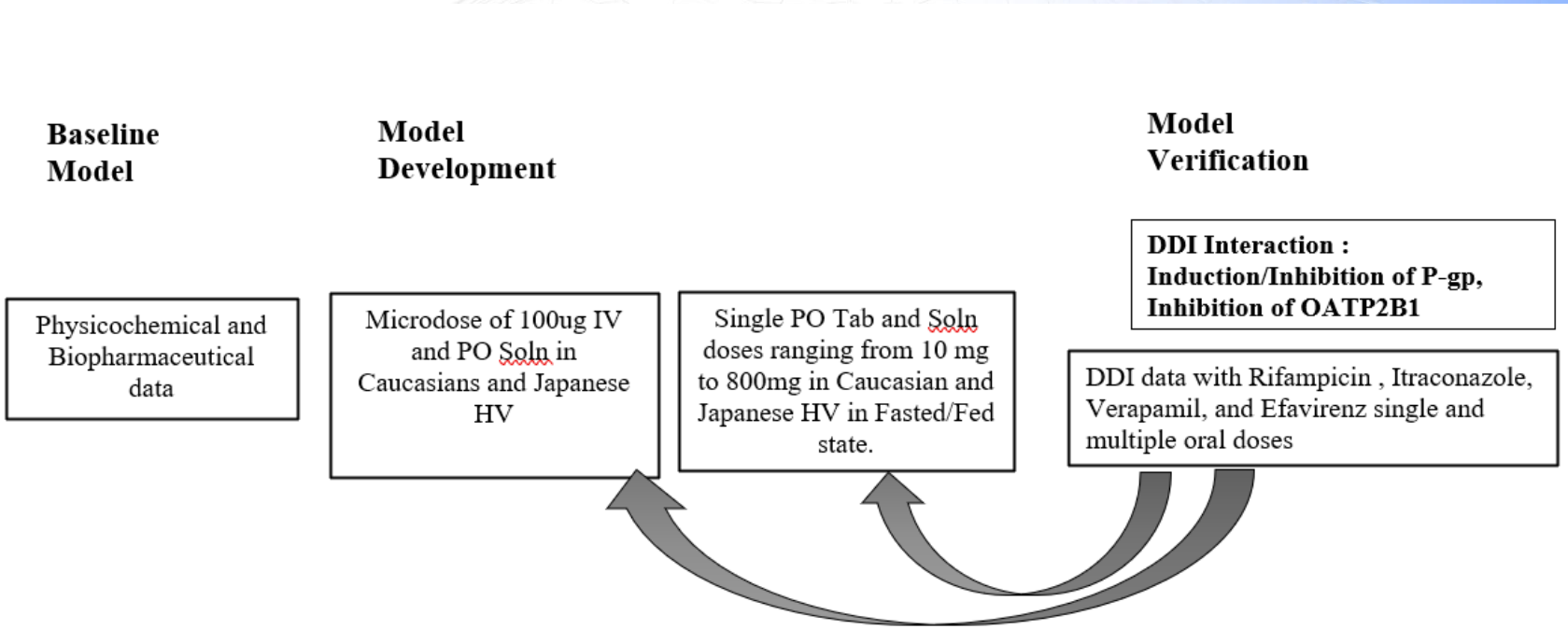
Green (circles) represent the AUC and Cmax for DDI with Rifampicin, and Orange (Circles) represent the AUC and Cmax for DDI with Itraconazole. Red lines (—) represent 2-fold prediction error, and black lines (—) represent fold prediction error per Guest's criteria (Guest *et al.* 2011).

Development of a Physiologically Based Pharmacokinetic (PBPK) Model for the P-gp, OATP2B1, OAT3 Substrate Fexofenadine and Model Validation of Known Drug-Drug Interactions (DDIs) with Rifampicin, Itraconazole, Verapamil, and Efavirenz

Literature Collection



Overview of Modeling Strategy of Fexofenadine



Key Physicochemical and Biopharmaceutical Parameters for Fexofenadine Used in GastroPlus Simulations

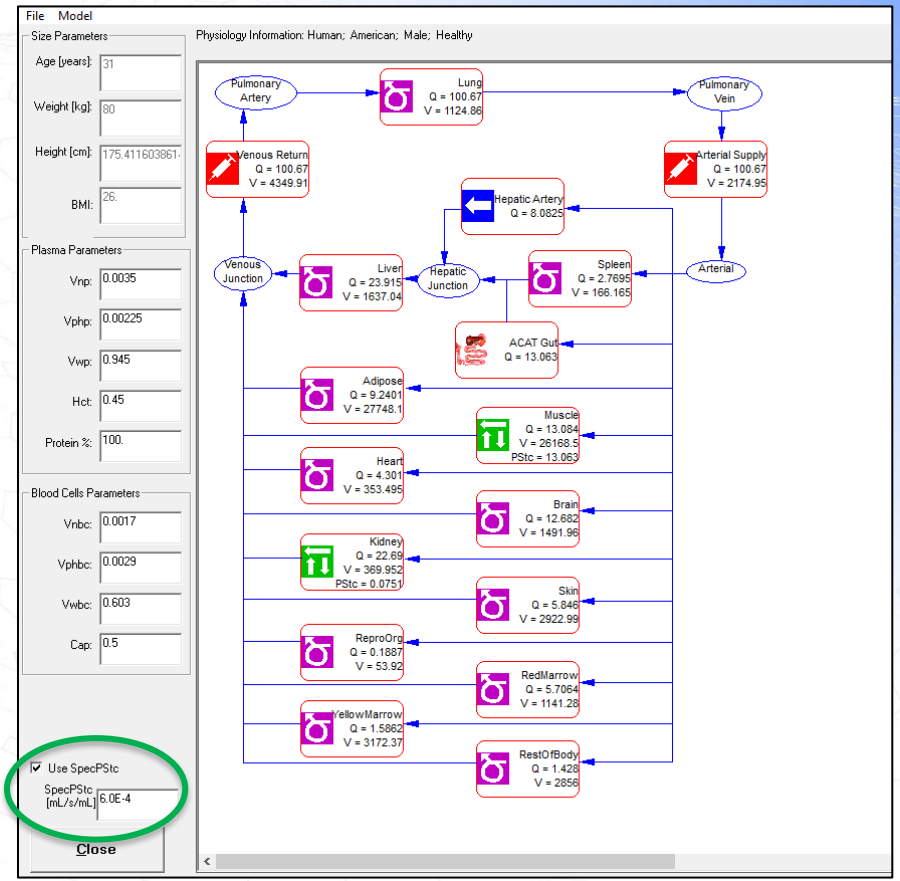
Parameter	Value	Reference
logP	0.5	(Chen chen et al. 2007)
Diffusion coefficient	0.53x10^{-5} cm^2/s	ADMET Predictor[a]
pKa	9.462 (base) 3.931 (acid)	Based on fitting to Sol. vs. pH profile
Reference solubility	0.14 mg/mL @ pH = 6.0	(NDA-FDA-Alegra-Fexofenadine-20872-label)
Solubility Factor	59.31 (base) 14.76 (acid)	Based on fitting to Sol. vs. pH profile
FaSSIF solubility	0.14 mg/mL	ADMET Predictor[a]
FeSSIF solubility	0.21 mg/mL	ADMET Predictor[a]
Bile salt solubilization ratio	1802.1	GastroPlus algorithm
Human effective permeability (P_{eff}) (derived from Caco-2 assay)	0.626 x 10^{-4} cm/s	(Absorption Systems Lighthouse Database)
Particle radius	25 mm	GastroPlus default
Precipitate radius	1 mm	GastroPlus default
Drug particle density	1.2 g/mL	GastroPlus default
Mean precipitation time	20000 s	Fitted

Parameter	Value	Reference
Blood:plasma concentration ratio (R_{bp})	0.74	(Takano et al.,2016)
Plasma protein binding (F_{up})	31 % 22% (R-Fexo) 40% (S-Fexo)	(NDA-FDA-Alegra-Fexofenadine-20872-label) (Kusuhara et al. 2013)
Spec PStc	6.0 x 10^{-4} mL/s/mL tissue	Fitted
Transporters		
P-gp K_m	25.9 µM 20 µM	(Takano et al. 2016) Fitted
P-gp V_{max}	0.05 mg/s (Gut) 0.02 mg/s/mg-trans (PBPK)	(Fitted)
OATP2B1 K_m	428 µM	(Shirasaka et al. 2014)
OATP2B1 V_{max}	4.2 nmol/min/mg protein 0.06 mg/s	(Fitted)
OAT3 K_m	70.2 µM	(Tahara et al. 2006)
OAT3 V_{max}	0.12 nmol/min/mg protein 0.012 mg/s/mg-trans	(Tahara et al. 2006)

[a] Predicted using ADMET Predictor® v10.0

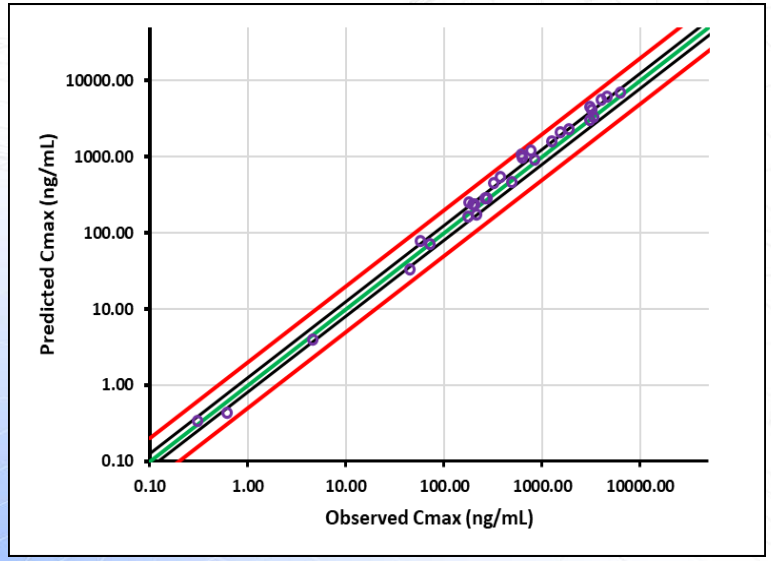
GastroPlus PBPK Model : Fexofenadine

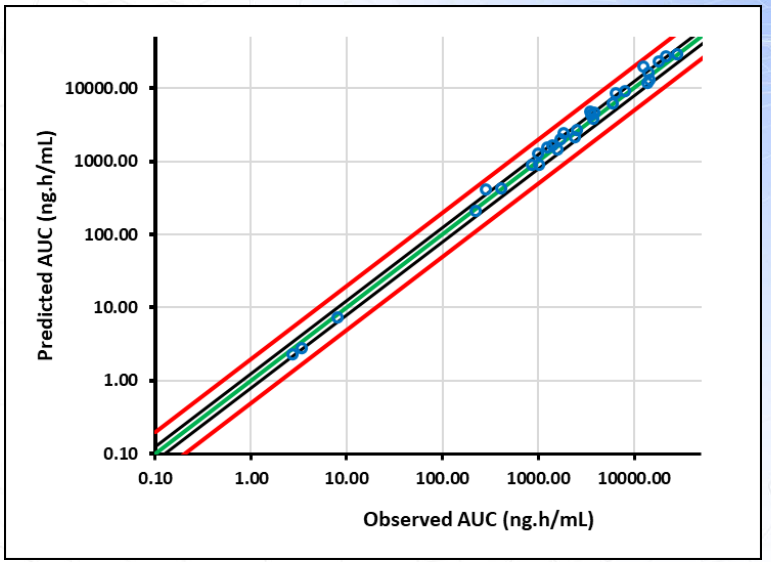
- Used the log P = 0.5
- Changed the kidney and muscle model to Permeability-limited with SpecPstc =6E-4 mL/s/mL
- PBPK Vmax values (P-gp & OATP2B1) and SpecPStc were optimized against IV dose
- Gut transporter Vmax values were fitted against the PO dose



Model Validation of Fexofenadine

Observed vs Predicted Values for C_{max} and AUC of Fexofenadine

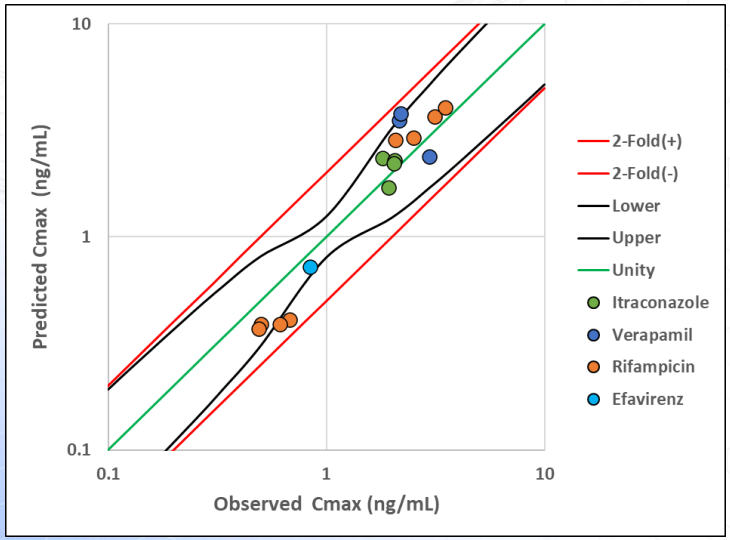


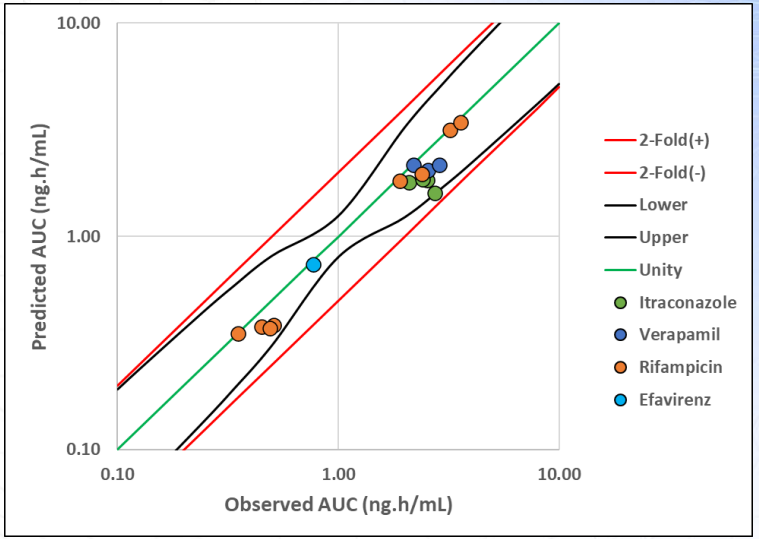


Purple Circles and Blue Circles represent C_{max} and $AUC_{0\text{-}inf}$, respectively.

Red lines (—) represent 2-fold prediction error, Black lines (—) represent the 1.25-fold prediction error.

DDI Accuracy



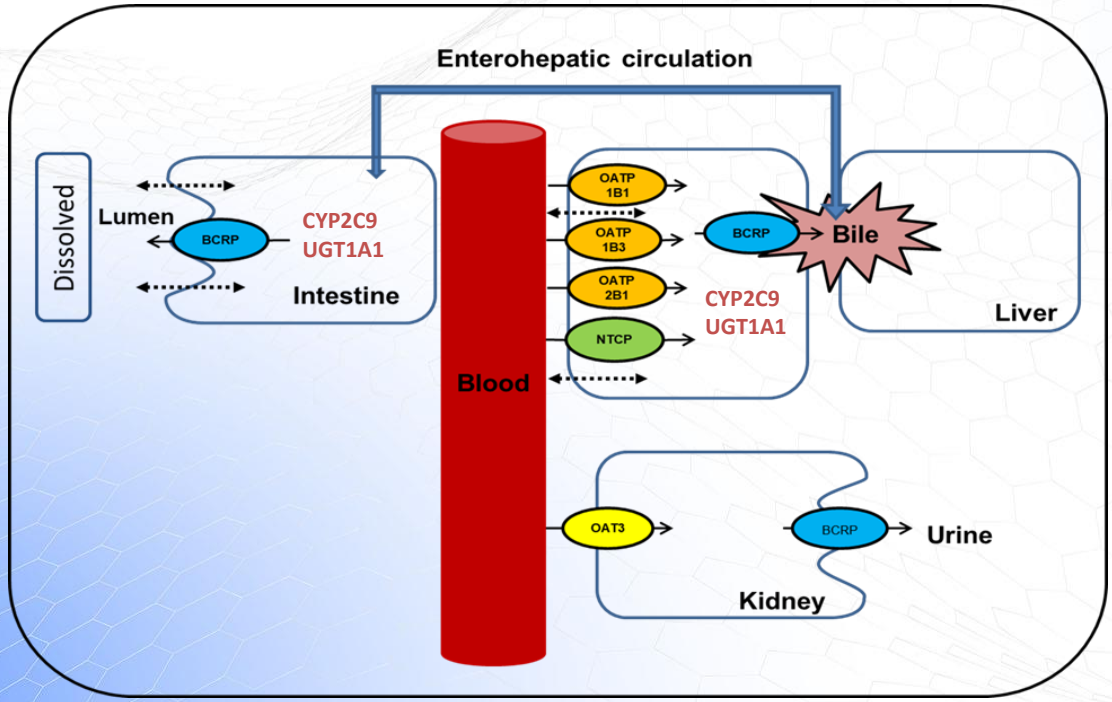


Observed vs Predicted AUC_{0-t} and C_{max} Ratios for DDI Between Fexofenadine, Itraconazole, Verapamil, Rifampicin, and Efavirenz.

Green (circles), Blue (Circles), and Orange (Circles) represent the AUC and Cmax respectively. Red lines (—) represent 2-fold prediction error, and black lines (—) represent fold prediction error per Guest's criteria (Guest *et al.* 2011).

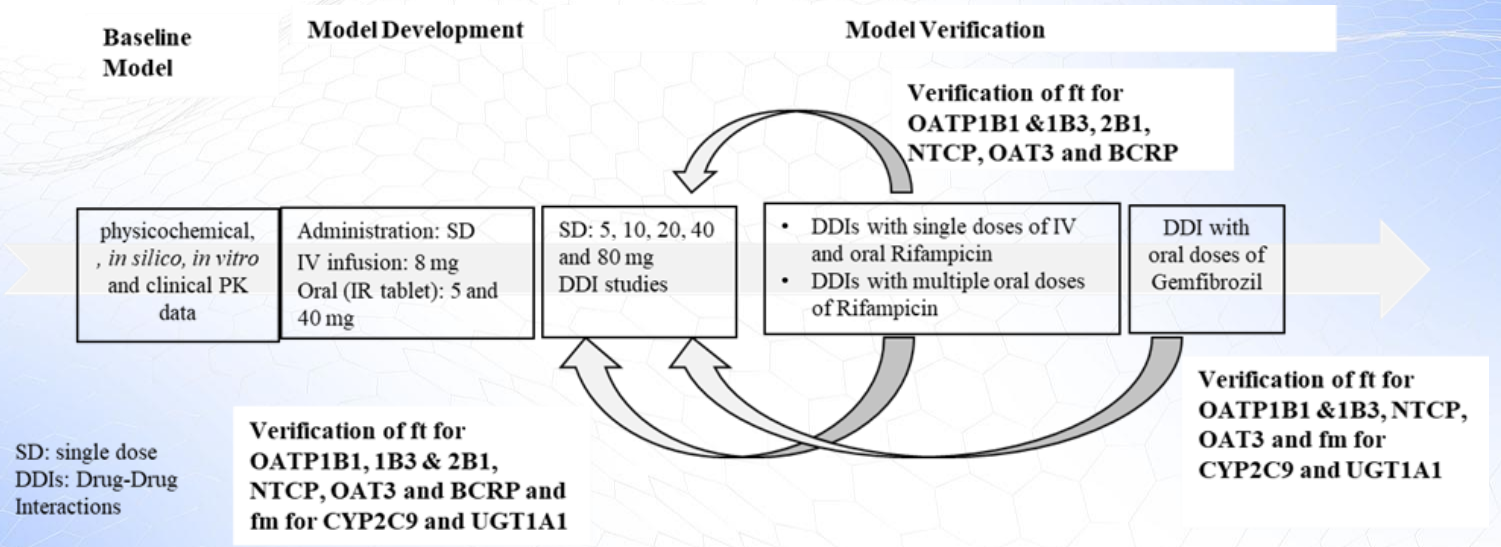
Development of a Physiologically Based Pharmacokinetic (PBPK) Model for the BCRP, OATP1B1, and OATP1B3 Substrate Rosuvastatin, and Model Validation of Known Drug-Drug Interactions (DDIs) with Rifampicin and Gemfibrozil

Schematic View of Enzymes and Transporters Involved in Absorption and Disposition of Rosuvastatin in Gut, Liver and Kidney of Human Body



NTCP	sodium-taurocholate co-transporting polypeptide
OAT3	organic anion-transporter
BCRP	breast cancer resistance protein
OATP1B1	organic anion-transporting polypeptide 1B1
OATP1B3	organic anion-transporting polypeptide 1B3
OATP2B1	organic anion-transporting polypeptide 2B1

Overview of Modeling Strategy of Rosuvastatin



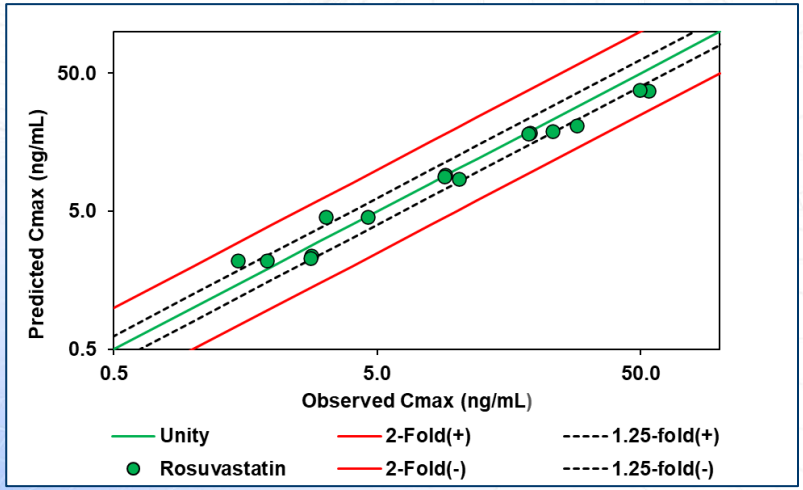
Key Physicochemical and Biopharmaceutical Parameters for Rosuvastatin Used in GastroPlus Simulations

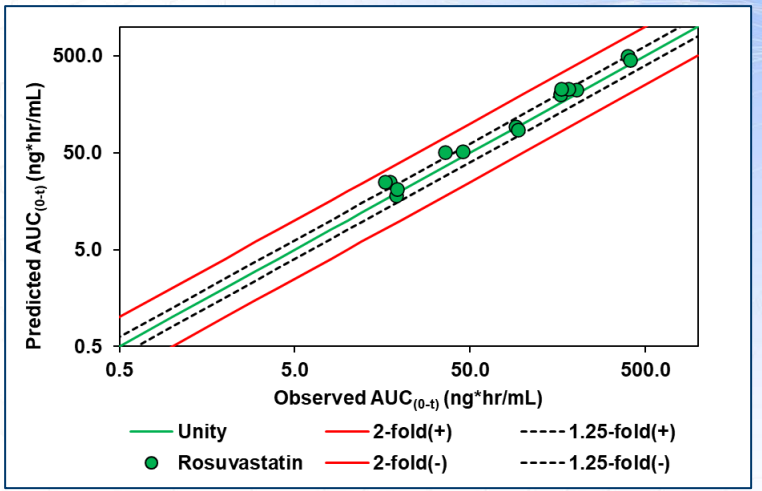
Parameter	Value	Reference
logD	-0.33 @ pH=7.4	(Jones et al. 2012)
Diffusion coefficient	0.57x10^{-5} cm^2/s	ADMET Predictor[a]
pKa	4.329 (acid), 2.26 (base)	(Jamei et al. 2014), ADMET Predictor[a]
Reference solubility	0.5 mg/mL @ pH = 1.2	(FDA 2003)
Human effective permeability (P_{eff})	1.02 x10^{-4} cm/s	(Human jejunal P_{eff} value is estimated from geo mean of $P_{app(A-B)}$ and $P_{app(B-A)}$ data in Caco-2) using built-in ABSCa conversion. (Li et al. 2012)
Particle radius	25 mm	GastroPlus default
Precipitate radius	1 mm	GastroPlus default
Drug particle density	1.20 g/mL	GastroPlus default
Mean precipitation time	900 s	GastroPlus default
Blood: plasma concentration ratio (R_{bp})	0.625	(Jamei et al. 2014)
Plasma protein binding (F_{up})	10.7 %	(Jamei et al. 2014)
Adjusted F_{up}	10.697 %	GastroPlus algorithm[b]
Metabolism		
CYP2C9 $K_{m,u}$ (mM)	23.03	ADMET Predictor[a]
CYP2C9 V_{max} (nmol/min/mg protein)	0.0001	Optimized value
UGT1A1 $K_{m,u}$ (mM)	16	(Schirris et al. 2015)
UGT1A1V_{max} (nmol/min/mg protein)	0.0002	Optimized value

Parameter	Value	Reference
Transporters		
Influx: Basolateral side		
OATP1B1 (liver)		
$K_{m,u}$ (mM)	4	(Ho et al. 2006)
V_{max} (mg/s/mg-trans)	0.069	Optimized value
OATP1B3 (liver)		
$K_{m,u}$ (mM)	9.8	(Ho et al. 2006)
V_{max} (mg/s/mg-trans)	0.086	Optimized value
NTCP (liver)		
$K_{m,u}$ (mM)	65	(Ho et al. 2006)
V_{max} (mg/s/mg-trans)	0.261	Optimized value
OATP2B1 (liver)		
$K_{m,u}$ (mM)	2.4	(Ho et al. 2006)
V_{max} (mg/s/mg-trans)	0.0054	Optimized value
OAT3 (kidney)		
$K_{m,u}$ (mM)	7.4	(Windass et al. 2007)
V_{max} (mg/s/mg-trans)	0.08	Optimized value
Efflux: Apical side		
BCRP (liver, kidney)		
$K_{m,u}$ (mM)	307	(Huang et al. 2006)
V_{max} (mg/s/mg-trans)	0.012	Optimized value
BCRP (gut)		C
$K_{m,u}$ (mM)	307	(Huang et al. 2006)
BCRP V_{max} (mg/s)	0.11	Optimized value
CL_{PD} (mL/min/million cells)	0.0264	Optimized value

Model Validation of Rosuvastatin

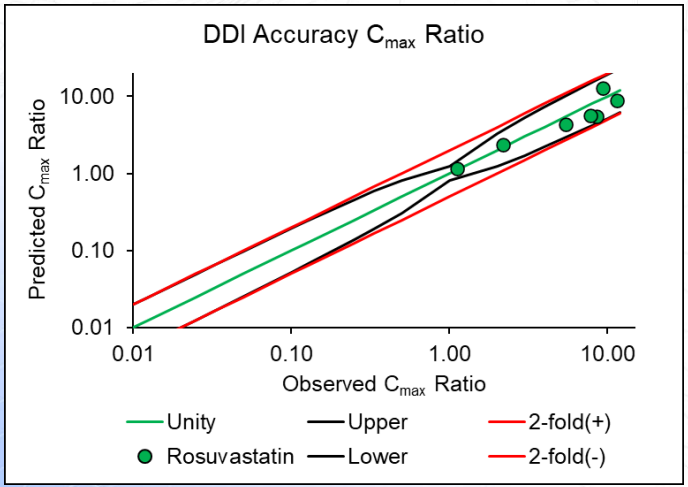
Goodness-of-Fit Plots Showing Observed vs Predicted Values for C_{max} and AUC of Rosuvastatin

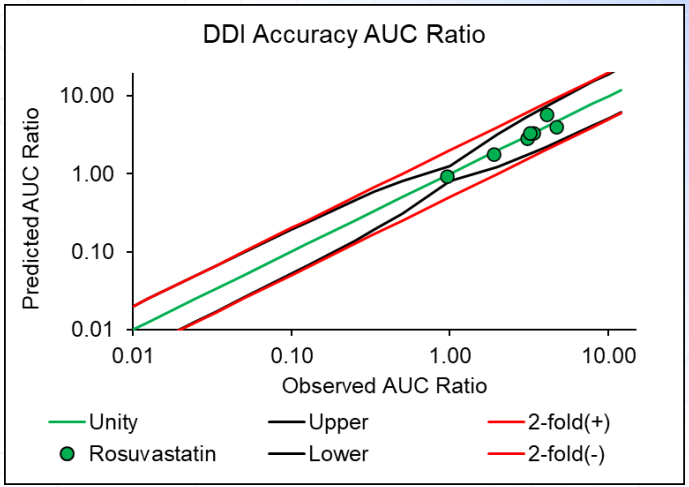




Model Validation of Rosuvastatin: DDI Accuracy

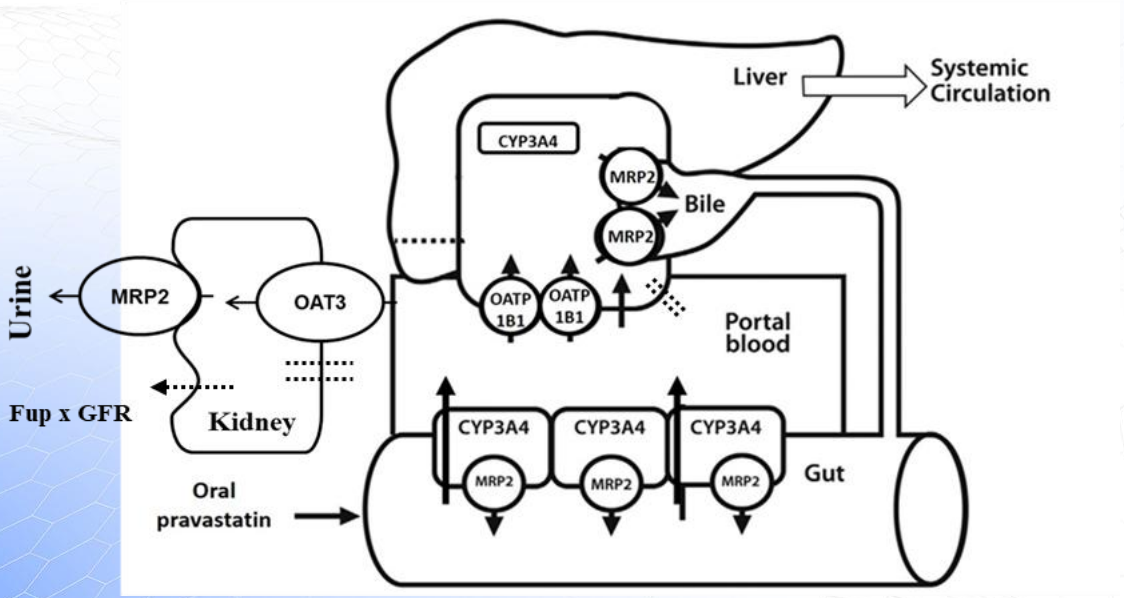
Observed vs Predicted DDI Ratios for C_{max} and AUC of Rosuvastatin with Perpetrators (Rifampicin and Gemfibrozil)





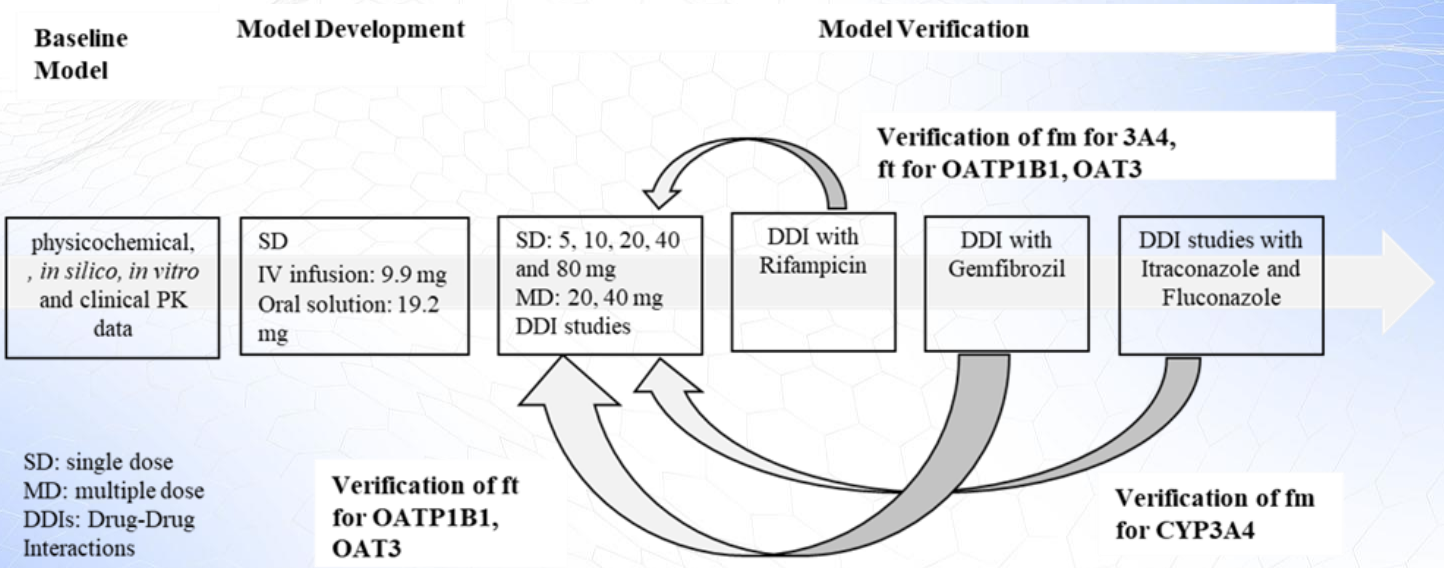
Development of a Physiologically Based Pharmacokinetic (PBPK) Model for the OATP1B Substrate Pravastatin, and Model Validation of Known Drug-Drug Interactions (DDIs) with Rifampicin, Gemfibrozil, Fluconazole, and Itraconazole

Schematic View of Enzymes and Transporters Involved in Absorption and Disposition of Pravastatin in Gut, Liver and Kidney of Human Body



OATP1B1	organic anion-transporting polypeptide 1B1
OATP1B3	organic anion-transporting polypeptide 1B3
OAT3	organic anion-transporter
MRP2	multi-drug resistance protein 2

Overview of Modeling Strategy of Pravastatin



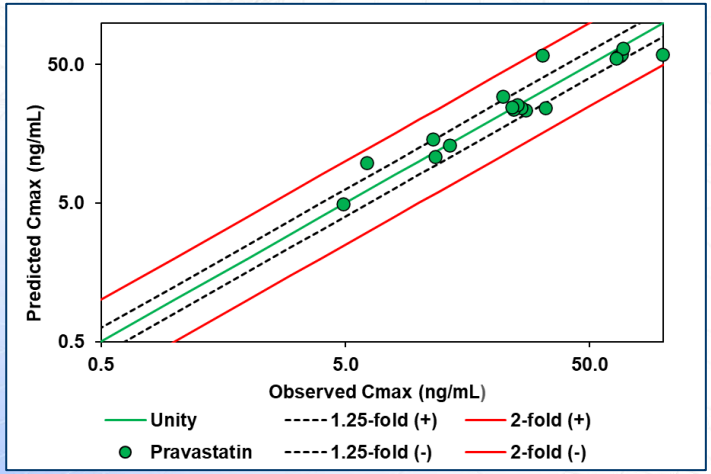
Key Physicochemical and Biopharmaceutical Parameters for Pravastatin Used in GastroPlus Simulations

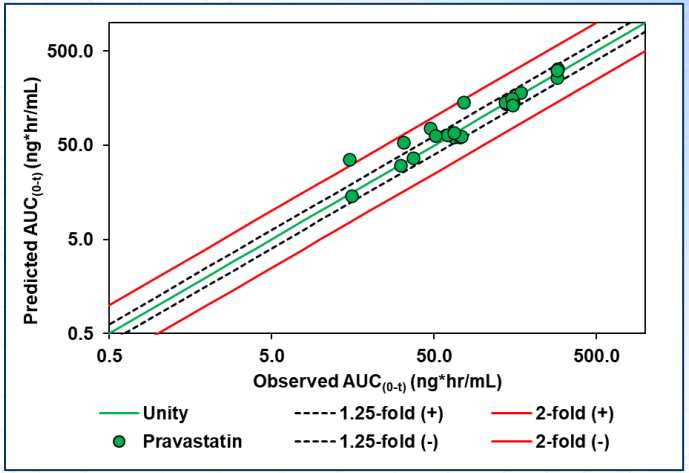
Parameter	Value	Reference
Molecular weight	424.5	ADMET predictor
LogD at pH 7	0.59	(FDA)
LogP	1.8[a]	Optimized to describe IV C_p-time profile and to better capture observed V_{ss}
Ionization constant (pKa)	4.92 (acid)	ADMET predictor
Reference solubility (mg/mL)	479.6 @ pH 6.8	(Ruiz-Picazo et al. 2019)
Papp (10^{-5} cm/s, Caco-2)	0.3	(Varma et al. 2012)
Peff (10^{-4} cm/s)	1.18	ABSCa conversion[b]
Mean precipitation time (s)	900	GastroPlus default value
Blood to Plasma concentration ratio	0.56	(Watanabe et al. 2009)
Plasma fraction unbound (%)	50	(FDA)
Adjusted plasma fraction unbound (%)	49.96	GastroPlus algorithm[c]
Metabolism		
CYP3A4 (gut and liver)		
Km (µM)	3480	(Jacobsen et al. 1999)
V_{max} (nmol/min/mg-enz)	75	Optimized

Parameter	Value	Reference
Transporters		
Influx: Basolateral side		
OATP1B1 (liver)		
Km (µM)	27	(Izumi et al. 2015)
V_{max} (mg/s/mg-trans)	0.023	Optimized
OAT3 (kidney)		
Km (µM)	27.7	(Nakagomi-Hagihara et al. 2007a)
V_{max} (mg/s/mg-trans)	0.1	Optimized
Efflux: Apical side		
MRP2 (liver, kidney)		
Km (µM)	7.2	(Ellis et al. 2013)
V_{max} (mg/s/mg-trans)	0.1	Optimized
MRP2 (gut)		
Km (µM)	7.2	(Ellis et al. 2013)
V_{max} (mg/s/)	0.002	Optimized
CL_{PD} (µL/min/million cells)	0.5	(Varma et al. 2012)

Model Validation of Pravastatin

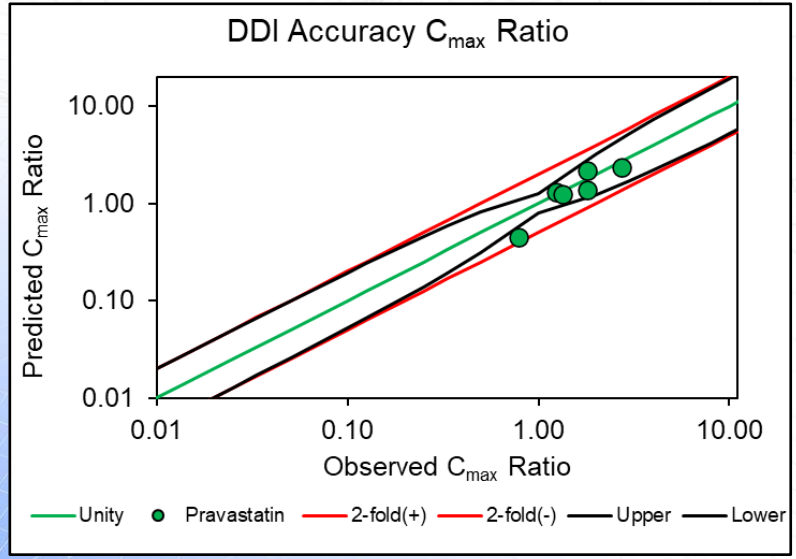
Goodness-of-Fit Plots Showing Observed vs Predicted Values for C_{max} and AUC of Pravastatin

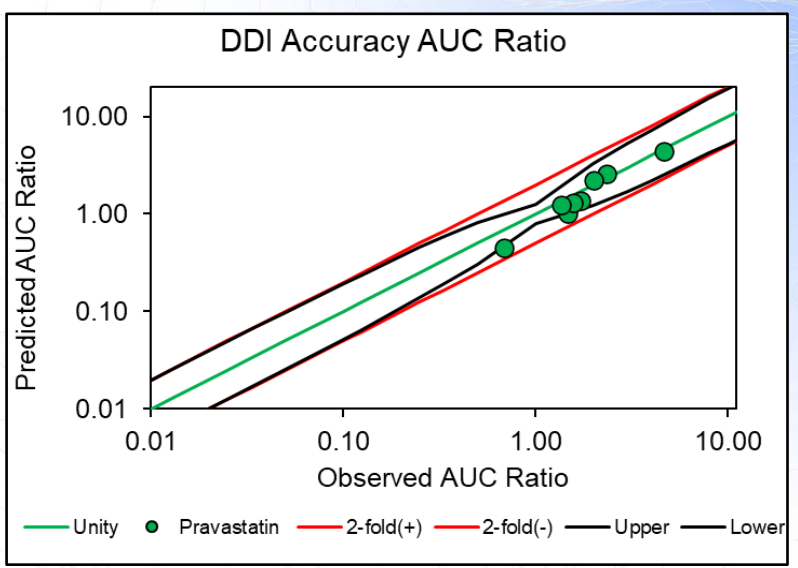




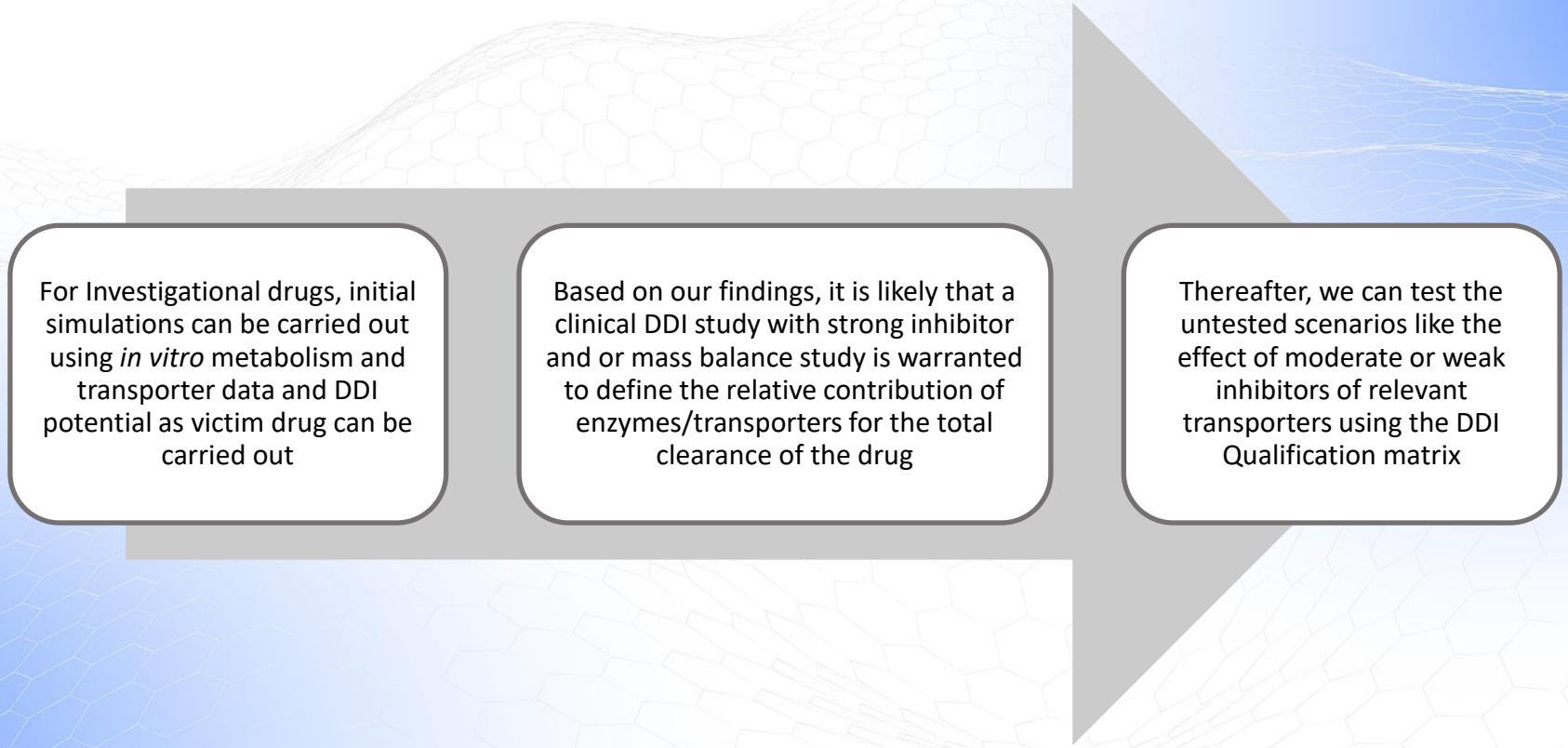
Model Validation of Pravastatin: DDI Accuracy

Observed vs Predicted DDI Ratios for C_{max} and AUC of Pravastatin with Perpetrators (Rifampicin, Gemfibrozil, and Its Metabolite, Itraconazole, and Fluconazole)





Investigational Drugs as Victim Drug-Findings/Guidance



Investigational Drugs as Transporter Inhibitors- Findings/Guidance

For Investigational drugs, using the *in vitro* Ki values, if the R values calculated are higher than cut-off values, then relevant substrates (our compounds in the DDI matrix can be used) can be used to test the effect of IND on these substrates

Depending on the predicted magnitude of interaction, whether significant or not.

In the former case, a clinical DDI study is still required whereas in the later a sensitivity analysis will suffice

Additional Literature on GastroPlus DDI Applications

- Perrier Jeremy, Gualano V, Helmer E, Namour F, Lukacova V, Taneja A. Drug-drug interaction prediction of Ziritaxestat using a physiologically based enzyme and transporter pharmacokinetic network interaction model. 2023 Sep; Clin Transl Sci. 16:2222-2235.

- Deb S, Hopefl R. Simulation of drug-drug interactions between **breast cancer chemotherapeutic agents and antiemetic drugs**. Daru. 2023 May 24. doi: 10.1007/s40199-023-00463-1.

- Deb S, Reeves AA. Simulation of **Remdesivir** Pharmacokinetics and Its Drug Interactions. J Pharm Pharm Sci. 2021;24:277-291.

- Yamada M, Ishizuka T, Inoue S, Rozehnal V, Fischer T, Sugiyama D. Drug-drug risk assessment of Esaxerenone as a perpetrator by In vitro studies and static and physiologically based pharmacokinetic models. Drug Metab Dispos. 2020;48:769-777.

Additional Literature on GastroPlus DDI Applications

- Sohlenius-Sternbeck AK, Meyerson G, Hagbjörk AL, Juric S, Terelius Y. A **strategy for early-risk predictions** of clinical drug-drug interactions involving the GastroPlus™ DDI module for time-dependent CYP inhibitors. Xenobiotica. 2018 Apr;48(4):348-356

- Dodd S, Kollipara S, Sanchez-Felix M, Kim H, Meng Q, Beato S, Heimbach T. Prediction of **ARA/PPI Drug-Drug Interactions** at the Drug Discovery and Development Interface. J Pharm Sci. 2019 Jan;108(1):87-101.

Important Resources

- In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry | FDA
- Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry | FDA
- Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers | FDA
- European Medical Agency (EMA)-Guideline on the investigation of drug interactions
- Japanese Pharmaceuticals and Medical Devices Agencies (PMDA) 2019-Development of a new Japanese guideline on drug interaction for drug development and appropriate provision of information
- Question & Answer document (live document, EMA website)- Section 2. Drug interactions
- ICH Guideline M12 on drug interaction studies (draft)

Additional Information

DDI Inhibition Parameters

Supplementary Table 1 DDI Inhibition and Induction Input Parameters for Rifampicin

Enzyme	Interaction Parameter Value (Inhibition/Induction)	Reference
CYP3A4	Inhibition: Competitive, K_i-rev-*in vitro*, u = 18.5 µM	(Kajosaari et al. 2005)
MRP2	Inhibition: Competitive, K_i-rev-*in vitro*, u = 0.87 µM	(Yoshikado et al. 2016)
OATP1B1	Inhibition: Competitive, K_i-rev-*in vitro*, u = 0.07 µM	(Morse et al. 2019)
OATP1B3	Inhibition: Competitive, K_i-rev-*in vitro*, u = 0.07 µM	(Morse et al. 2019)
OATP2B1	Inhibition: Competitive, IC50-*in vitro*, u = 65 µM	(Karlgren et al. 2012)
BCRP	Inhibition: Competitive, IC50-*in vitro*, u = 14.9 µM	(Costales et al. 2021)
NTCP	Inhibition: Competitive, IC50-*in vitro*, u = 127 µM	(Zhang et al. 2019)
OAT3	Inhibition: Competitive, IC50-*in vitro*, u = 33 µM	(Parvez et al. 2016)
CYP3A4	Induction: EC_{50} (unbound) = 0.064 µM E_{max} = 15	(Asaumi et al. 2018) Fitted value#
UGT1A3	Induction: EC_{50} (unbound) = 0.064 µM E_{max} = 4.4	(Asaumi et al. 2018) Fitted value#
UGT1A1	Induction: EC_{50} (unbound) = 0.064 µM E_{max} = 4.4	(Asaumi et al. 2018) Fitted value#
CYP2C9	Induction: EC_{50} (unbound) = 0.064 µM E_{max} = 3.2	(Asaumi et al. 2018) (Buckley et al. 2013)

"Rev" represents reversible inhibition and "u" stands for unbound.

#fitted values for UGT1A3 and CYP3A4 were previously validated against clinical rifampicin PK studies and DDI studies with rifampicin and CYP3A4 substrates (midazolam, triazolam, alfentanil); the same E_{max} value as fitted for UGT1A3 is assumed also for UGT1A1.

DDI Inhibition Parameters Cont.,

Supplementary Table 2 DDI Inhibition Parameters for Gemfibrozil and Gemfibrozil-Glucuronide

Perpetrator	Enzyme	Interaction Parameter Value (Inhibition/Induction)	Reference
Gemfibrozil	OATP1B1	Inhibition: Competitive, IC50-*in vitro*, u = 7.4 µM	(Säll 2013)
Gemfibrozil	OATP1B3	Inhibition: Competitive, IC50-*in vitro*, u = 10 µM	(Yoshida et al. 2012)
Gemfibrozil	OAT3	Inhibition: Competitive, K_i-rev-*in vitro*, u = 3.4 µM	(Nakagomi-Hagihara et al. 2007)
Gemfibrozil	NTCP	Inhibition: Competitive, IC50-*in vitro*, u = 23 µM	(Ho et al. 2006)
Gemfibrozil	CYP2C9	Inhibition: Competitive, K_i-rev-*in vitro*, u = 4 µM	(Wang et al. 2002)
Gemfibrozil	UGT1A1	Inhibition: Competitive, IC50-*in vitro*, u = 110 µM	(Gan et al. 2010)
Gemfibrozil-glucuronide	OATP1B1	Inhibition: Competitive, IC50-*in vitro*, u = 4.3 µM	(Säll 2013)
Gemfibrozil-glucuronide	OATP1B3	Inhibition: Competitive, IC50-*in vitro*, u = 74 µM	(Yoshida et al. 2012)
Gemfibrozil-glucuronide	OAT3	Inhibition: Competitive, K_i-rev-*in vitro*, u = 9.9 µM	(Nakagomi-Hagihara et al. 2007)
Gemfibrozil-glucuronide	UGT1A1	Inhibition: Competitive, IC50-*in vitro*, u = 130 µM	(Gan et al. 2010)

"Rev" represents reversible inhibition and "u" stands for unbound and "T" represents total binding.

DDI Inhibition Parameters Cont.,

Supplementary Table 4 **DDI Inhibition Parameters of Itraconazole and Its Metabolites**

Perpetrator	Enzyme	Interaction Parameter Value (Inhibition/Induction)	Reference
Itraconazole (ITZ)	CYP3A4	Inhibition: Competitive Ki-rev-*in vitro*, u = 1.3 nM	(Isoherranen et al. 2004)
Hydroxy itraconazole (OH-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev-*in vitro*, u = 14.4 nM	(Isoherranen et al. 2004)
Keto itraconazole (Keto-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev-*in vitro*, u = 1.4 nM	(Isoherranen et al. 2004)
N-desalkyl itraconazole (ND-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev-*in vitro*, u = 0.38 nM	(Isoherranen et al. 2004)

"rev" represents reversible inhibition and "u" stands for unbound.

DDI Inhibition Parameters Cont.,

Supplementary Table 5 DDI Inhibition Parameters of Fluconazole

Perpetrator	Enzyme	Interaction Parameter Value (Inhibition/Induction)	Reference
Fluconazole	CYP3A4	Inhibition: Competitive Ki-rev-*in vitro*, T = 15 µM	(Isoherranen et al. 2008)

"rev" represents reversible inhibition and "T" represents total binding.