

# S+ SimulationsPlus

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**



#### Let's not forget ...

In GastroPlus<sup>™</sup>, the PBPK model is linked to the ACAT<sup>™</sup> 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**

rediction Type Steady-State Prediction	© Dynamic Simulation	- Simulation Mode © Single Sim © Pop Sim	C DILlsym C Monolix	Run Baseline Simulation	Run Full Simulation Close
irrent Compound: RSV PO 5m	g DDI RIF IV_Prueksaritanont		-Interacting Compound(s): ~ 2-D	I Standard-2023-01-03.mdb	
Perpetrator (Inhibitor/Inducer) for Steady - State Predictions	<ul> <li>Victim (Substrate)</li> </ul>	Show Notes for Current Compound	Perpetrator Perpetrator Parameters	IF IV 600mg DDI RSV PO 5mg Pruek 2014	Show Notes for Interacting Compound
Enz / Trans Location CLint,u CLin JGT1A1 - Liver 4.84E-02 L	nt Units fm 1%1 fm source Turnover [1/min] Referen		rpetrator En	z / Trans Inh/Ind Const Type Const Cons Value Units	nd kinact st [min-1] Select Validated F SAdd F Zemax
JGT1A1 Gut 1.56E-03 L 2C9 Liver 1.69E-02 L	The left side of the	DDI window	FIV 600mg DDIRSV P0 5mg Pruek 0A FIV 600mg DDIRSV P0 5mg Pruek 0A	ATP1B3 Ki-rev-in vitro. U 0.07 uM ATP1B1 Ki-rev-in vitro. U 0.07 uM	0 V False - 4 Delete 0 V False - Enz/Tran
C9         Gut         6.02E-05         L           JATP2B1         Tissue         0.00E+00         L	displays the <i>current</i>	t compound –	FIV 600mg DDIRSV P0 5mg Pruek P-g FIV 600mg DDIRSV P0 5mg Pruek BC	gp Ki-rev-in vitro. U 0.49 uM XRP IC50-rev-in vitro. U 14.9 uM	0 False
DATP1B3 Tissue 0.00E+00 L DATP1B1 Tissue 0.00E+00 L	record which was a	nonod on main	FIV 600mg DDI RSV PO 5mg Pru OA	ATP2B1 IC50-rev-in vitro, T 2.1 uM ATP2B1 Kinewin vitro II 65 uM	0 False 0
letabolic profile detected from information able.	record which was o	pened on main	sing Information		Rate Constants [1/h]
5 Add Enz/Trans 6 Delete Enz/T	GastroPlus window	at the time of	Dose [mg]: 600	Int [h]: 0.5 CL [L/h]: 11.367	ka: 0 kel: 0.3826
	accessing DDI mod	ıle	Perpetrator Concs for	r Steady-State Predictions	Blood Flows [L/h]
			Concentration type [ug/mL Svs Cmax RIF IV 600mg DDI R 0	_] Select	Percents [%]
Fraction Me			Liver In Unb RIF IV 600mg DDI 0	<b>N</b>	Fup: 7 Fa: 0
(Other Syst (	You select the desig	gnation for			FDp: 0 F: 0
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ot metabolic profile in	Liver C Gut (Last dos	e in .mdd file starts at 5.5)	Show Plot Show Stat	s	
vance			Internation Concerned Informations		



#### **Basic Interface Layout**

Steady State Frediction	Oynamic Simulation	⊙ Single Sim ⊂ Pop Sim	C DILlsym C Monolix Simulation Close
Current Compound: RSV P0 5mg DDI C Perpetrator (Inhibitor/Inducer) fm for Steady - State Predictions	RIF IV_Prueksaritanont	Show Notes for Current Compound	Intera ting Compound(s): ".2-DDI Standard-2023-01-03.mdb         Perpetrator       If I I RIF IV 600mg DDI RSV P0 5mg Pruek 2014         Perpetrator Parameters       St w Notes for Interacting Compound
Enz / Trans     Location     CLint.u     CLint Units       UGT1A1     Liver     4.84E-02     LA       UGT1A1     Liver     1.56E-03     LA       2C3     Liver     1.69E-02     LA       2C3     Gut     6.02E-06     LA       OATP2B1     Tissue     0.00E+00     LA       OATP1B3     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP1B3     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP1B3     Tissue     0.00E+00     LA       OATP1B3     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP1B3     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP2B1     Tissue     0.00E+00     LA       OATP2B1     Exact     0.00E+00     LA       View     Querchant     0.00E+00     LA       US     Querchant     0.00E+00     LA       US     Querchant     0.00E+00     LA       US     Querchant     Querchant	Im [%]     Im source     Turnovet [1/min]       0.66     Cele In Five     0.0005       96.28     Cele In Five     0.0005       3.72     Cele In Five     0.0005       0.     Cele In Five     0.0005       Calculate Im values     Systemic:       Systemic:     Systemic:	Reference       Image: Constraint of the second secon	The right side of the DDI window displays the <i>interacting</i> 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 <i>interacting</i> compound as a victim or a perpetrator is automatically set depending on the user selection for the <i>current</i> compound
C Liver	C Gut (	(Last dose in .mdd file starts at 5.5)	



Drug-Drug Interaction Predictions			- 0 ×
File         Current Compound         Interacting Compounds         Options         Help           Prediction         Type         C         Steady-State Prediction         C         Dynamic Simulation	Simulation Mode	C DILlsym C Monolix Run Baseline Simulation Run Full	Simulation Close
Current Compound: RSV PO 5mg DDI RF IV_Prueksaritanont            Parpetrator [Inhibitor/Inducer]           Victim (Substrate)          Image: Information State Predictions          The state Predictions           Victim (Substrate)          Image: Information State Predictions          The state Predictions           The state Predictions          Image: Information State Predictions          The state Predictions           The state Predictions          Image: Information State Predictions          Enc / Trans Location CLink, CLink Units Image: Information State Predictions           The state Predictions          Image: Information State Predictions          Enc / Trans Location CLink, CLink 0.23         Cale In Five 0.0005         Output Distance 0.002:02         Left 1.582:0.0         Left 1.582:0.0         Cale In Five 0.0005         Output Distance 0.002:02         Left 1.582:0.0         Cale In Five 0.0005         Output Distance 0.002:00         Lh         0.0         Cale In Five 0.0005         Output Distance 0.002:00         Lh         0.0         Cale In Five 0.0005         Output Distance 0.002:00         DATEBLE         Tissue 0.002:00         DATEBLE         Tissue 0.002:00         DATEBLE         Tissue 0.002:00         Cale In Five 0.0005         Output Distance         State Prove 0.24         Output Distanc	Show Notes for Current Compound         im         Reference         Image: Show Notes for Current Compound         FDp [%]         FDp [%]         F0         F0	The substrate metabolic profile <i>Fg</i> values) needs to be specified steady-state predictions. <i>fm</i> values: - may be calculated from <i>in vitro</i> using built-in converter or enter manually by user and saved in converter - if K <sub>m</sub> and V <sub>max</sub> values are alread present in the database, prograve use them to calculate the <i>fm</i> variational to the <i>fm</i> variational tothe tothe <i>fm</i> variatio	(fm and for add trans coassays red latabase. dy m will lues
Plot metabolic profile in C Liver C Gut	Simulation Length (h): 24.5 (Last dose in .mdd file starts at 5.5)		
Reference		Interacting Compound Information: PK model: HumAmeMaHIthy30Y0_75kg_24BMI-Pruek(assumed) ACAT model: Human - Physiological - Fasted	



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-Current Con	npound:	RSV PO	5mg DDI R	IF IV_F	Prueksarit	anont —					-Interacting	Compound(s):	: ~.2-DDI Standard-2	023-01-03.mdb		
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E Drug-Drug Interaction Predictions						- 0
File Current Compound Interacting Compounds (	Options Help	-Simulation Mode				
C Steady-State Prediction	Dynamic Simulation	• Single Sim C Pop Sim	C DILIsym C Monolix	Run Baseline Simulation	Run Full Simulation	Close
Current Compound: RSV PO 5mg DDI RIF	IV_Prueksaritanont		-Interacting Compound(s): ~.2	2-DDI Standard-2023-01-03.mdb		
C Perpetrator (Inhibitor/Inducer)	Victim (Substrate)	Show Notes for Current Compound	Perpetrator II	RIF IV 600mg DDI RSV PO 5mg Pruek 2014	Show N	lotes for Interacting
fm for Steady - State Predictions			Perpetrator Parameters			compound
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Metabolic profile detected from information in Enzyme table.	Fa (%): 0 FDp	%): 0 Fg (%): 0	Dosing Information		Rate Constants [1/h] -	
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Fraction Metabolized by	/ CYPs					Fa: 0
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Uller Syst CL	Full Simu	lation dosing:	involving 'sim	nulation' concentr	rations	y-State Conc
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			prediction inv	volving <i>simulated</i>		
(209)			perpetrator o	concentration)		
Plot metabolic profile in	Simulation	Length (h): 24.5				•
( Liver	C Gut (Last dose	in .mdd file starts at 5.5)	Show Plot Show	Stats		
Reference			Interacting Compound Information: PK model: HumAmeMalHlthy30YO_75kg ACAT model: Human - Physiological - Fas	1_24BMI-Pruek(assumed) sted		



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#### **Perpetrator Settings**

Inhibition/Induction constants for all / 🖉 Drug-D File Cur proteins (enzymes or transporters) affected Predi C Ste by a given compound and its metabolites need to be specified for all predictions O Per (steady-state and/or dynamic simulations). fm for Enz / Multiple constants for the same compoundprotein pair may be saved in the database. Metabo table

C Liver

Only one competitive inhibition, one timedependent 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.

C Gut

toracting Compound(s): ~ 2	DDI Stan	dard_2022_01_0	3 mdb						
Bernstrator		a DDI PSV P0 5mg	Prusk 201	4			Show	Notes for	Inte acting
Perpetrator Parameters		ig DDI H3Y FO Siligi	TUEK 20	• •				Compou	ind
Perpetrator	Enz / Trans	Inh/Ind Const Type	Inh/Ind Const	Inh/Ind Const	kinact [min-1]	Select	Validated	lt^ F	3 Add nz/Tra
RIF IV 600mg DDI RSV PO 5mg Pruek	OATP1B3	Ki-rev-in vitro. U	0.07	uM	0	5	False	-	1 Delet
RIF IV 600mg DDI RSV PO 5mg Pruek	OATP1B1	Ki-rev-in vitro. U	0.07	uM	0	V	False	-	nz/Tra
RIF IV 600mg DDI RSV PO 5mg Pruek	P-gp	Ki-rev-in vitro. U	0.49	uM	0		False		
RIF IV 600mg DDI RSV PO 5mg Pruek	BCRP	IC50-rev-in vitro. U	14.9	uM	0		False	-	
RIF IV 600mg DDI RSV PO 5mg Pru	OATP2B1	IC50-rev-in vitro, T	2.1	uM	0		False	0	<b>(</b> *
RIE IV 600ma DDI RSV PO 5ma Pruek	IDATP2R1	Kirouin uitro II	65	Loka	In		Falea		~
De sine luteror stien					Π.		Laure PL Jul		
Using miomation					- ha	te cons	tants (17rij		
Dose [mg]: 600	Int [h]: [0.1	5 C	L [L/h]:	1.367		ka: 0		kel:	0.3826
Perpetrator Conce	for Steady-	State Predictions			<b>F</b> BIO	od Flow	s [L/n] —		
Concentration tupe Co	nc sala	-				Qe: 14	1.88	Qh:	87.741
Concentration type [ug	j/mL]	<u> </u>							
Sys Cmax RIF IV 600mg DDI R 0	<u> </u>				[ Pe	rcents [/	6]		
Liver In Unb RIF IV 600mg DDI 0	M					Fup: 7		Fa:	0
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lesults: Steady-state —									
Concentration Type AUC	Ratio AUC R	atio AUC Ratio Perpe	etrator						
- Gu	t - Liver	- I otal Ulass	ification	]					



Simulation Length (h):

(Last dose in .mdd file starts at 5.5)

NASDAO: SLP

24.5

Plot metabolic profile in

Reference

#### **Perpetrator Settings**

🖉 Drug-Drug Interaction Predictions

File Current Compound Interacting Compounds Options Help

C Steady-State	C Simulation Mode	C DILIsym C Monolix Run Baseline Simulation Close
- Current Comp	Perpetrator dosing information needs to	Interacting Compound(s): ".2-DDI Standard-2023-01-03.mdb
C Perpetrator (I	be specified for all predictions (steady-	Perpetrator RIF IV 600mg DDI RSV P0 5mg Pruek 2014 FI
fm for Steady - S	state and/or dynamic)	Perpetrator Parameters
UGT1A1 _ Liv	state and/or dynamic).	Perpetrator Enz / Trans Inh/Ind Const Type Const [fmin-1] Select Validated    F Enz/Trans
UGT1A1 Gu 209 Liv		RIF IV 600mg DDI RSV P0 5mg Pruek     0ATP1B3     Ki-rev-in vitro. U     0.07     uM     0     ✓     False     4 Delete       RIF IV 600mg DDI RSV P0 5mg Pruek     0ATP1B1     Ki-rev-in vitro. U     0.07     uM     0     ✓     False     4 Delete       RIF IV 600mg DDI RSV P0 5mg Pruek     0ATP1B1     Ki-rev-in vitro. U     0.07     uM     0     ✓     False     4 Delete
OATP2B1 Tis	Missing values for dose or dosing	RIF IV 500mg DDI RSV PD 5mg Pruek (R-gp RV Revin vitro. U 0.43 UM 0 Fraise - RIF IV 600mg DDI RSV PD 5mg Pruek (BCRP IC50-evin vitro. U 14.3 UM 0 Fraise - BIE IV 500mg DDI RSV PD 5mg Pruek (IC50-evin vitro. U 14.3 UM 0 Fraise -
Metabolic profile d	interval (or any of the perpetrator	
table.	nroperties) may result in failure in	Dosing Information         Rate Constants [1/h]           Dose (mg):         6600         Int [h]:         0.5         CL [L/h]:         [11:367]         ka:         0         kel:         0.3826
5 Add Enz/Tra		Blood Flows [L/h]
S 🕹 🛟	obtaining effective perpetrator	Concentration type Conc [ug/mL] Select
	concentration (the concentration value	▶ Sys Cmax RIF IV 600mg DDI R         □         □         □         □         Percents [%]           Liver In Unb RIF IV 600mg DDI 0         □
	will be 0) and calculating DDI	FDp: 0 F: 0
	predictions (the result will show $N/\Lambda$ )	Perpetrator Steady-State Conc
		- Results: Steady-state
		Concentration Type - Gut - Liver - Total Classification
Plot metabolic profi	e in Simulation Length (h): 24.5	
	C Liver C Gut (Last dose in .mdd file starts at 5.5)	Show Plot Show Stats
Reference		Interacting Compound Information: PK model: HumAmeMaHithy3010_75kg_24BMI-Pruek(assumed) ≙CAT model: Human, Phuniational: Easted



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#### **Prediction Results**

Drug-Drug Interaction	Predictions	- Ontions Help									- 0
Prediction Type -	diction	Options Help     Oynamic Simulation	Simulation Mod	C Pop Sim	C DILIsym	C Monolix		Run Baseline Simulation	Run Full Simul	ation	Close
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- Plot metabolic profile in	To obta run a fu "baselin interact automa types o availab	in the AUC ratio, all simulation as w ne" (i.e., simulati tions are ignored atically calculated f simulation resu le.	you need well as on where ). The rational when boo lts are	to the os are th	- Results: D Compound RSV P0 5mg D RIF V 600mg C RIF-Gluc Metat RSV P0 5mg D RIF V 600mg C RIF-Gluc Metat Show Pk	Dynamic Simula DI RIF IV_Prueksaritanc DI RSV PD 5mg Pruek solite-DDI DI RIV PD 5mg Pruek solite-ratio ot Show	Ition - Compet           Fa           [%]           ont-DDI           2014-DDI           99.99           0           ont-ratio           1.032           2014-ratio           1           0           / Stats	FDp         F         Cmax (ug/ml           43.32         34.42         0.01           99.38         99.38         24.88           0         0         0.931           1.032         1.776         4.367           1         1         1.001           0         0         1.121           pplete Ki Selection: Clione info         Clione info	FDp:         0           Perpe         Perpe           [htts]         [ng-k/mL] [ng-	C(0-t) h/mL] 76 100 2.8 78 21 21 08	F: 0 tate Conc
					PK model: Hum/	AmeMalHithy30Y0_75kg uman - Physiological - Fa	248MI-Pruek(assumed isted	d)			



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#### **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 on and Research Food and Drug Administration 10001 New Hampshire Ave., Hillandale Bidg, 4<sup>th</sup> Floor Silver Hampshire Ave., Hillandale Bidg, 4<sup>th</sup> Floor Silver Spring, MD 20993-0002 Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353 Email: drugnifo@ida.hks.gov https://www.file.gov/Drug-Guidance.com plance/Evaluator Information Guidancesitefuilt.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.





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

7.3. Predictive Modeling

7.3.1 Using Mechanistic Static Models for DDI Predictions ...

7.3.2 Using PBPK Models to Predict Enzyme or Transporter-Based DDIs ....

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

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



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



EMA, Guideline on the investigation of drug interactions

#### US FDA

## 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.

<sup>(c)</sup> 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.

<sup>(d)</sup> 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).

<sup>(e)</sup> 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.



## 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)

Does the compound have active hepatocyte uptake, do the drug's physiological properties (e.g., low passive membrane permeability,<sup>(a)</sup> high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH) support importance of active uptake into liver?



<sup>(a)</sup> 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<sup>-5</sup> cm/sec (10 nm/sec) or lower is classified as "low" permeability.

<sup>(b)</sup> 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<sub>i</sub>. 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.

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



<sup>(4)</sup> 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.



#### **Regulatory** Requirements of Transporter Evaluation for Inhibition Studies

	Inhibition S	tudy		
	USFDA	NMPA	PMDA	EMA
	2020	2021	2018	2013
Efflux Trans	sporter			
P-gp (MDR1)	yes	yes	yes	yes
BCRP	yes	yes	yes	yes
BSEP	no	no	no	prefer
MRP2	no	no	no	no
Uptake Tran	nsporter			
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	yes, adjust	yes, adjust	consider
	pH	pH	pH	
MATE2K	yes, adjust	yes, adjust	yes, adjust	consider
	pH	pH	pH	
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



 $[I]_1$  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 LG<sub>0</sub> is in a molar unit). For IC<sub>50</sub> determination, a unidirectional assay (e.g., B to A) based on the probe substrate can also be considered.



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



<sup>[a]</sup> R-value = 1+ (fu x I<sub>in,max</sub>/IC<sub>50</sub>), where, I<sub>in,max</sub> is the estimated maximum inhibitor concentration at the inlet to the liver and is equal to:  $C_{max} + (k_x x Dose x F_a F_g/Qh)$ .  $C_{max}$  is the maximum systemic plasma concentration of inhibitor; Dose is the inhibitor dose;  $F_a F_g$  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<sup>-1</sup> (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.

<sup>De</sup>These are the suggested values according to the upper limit of equivalence range. We are open to discussion based on sponsors' interpretation.



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



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

<sup>(a)</sup> 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.



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



## Regulatory Guidance Assessing tDDIs PBPK Modeling Considerations

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



#### 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 <sup>a</sup>	Outcome <sup>b</sup>	Software used	Reference
Healthy population										
OAT3, MRP4	Furosemide	Substrate	DDI	Full PBPK	Uptake parameters	In vitro, PE,	3	1	PK-Sim	Britz
	Probenecid	Inhibitor			Inhibition parameters	OP, SA				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	Simvastatin acid	Substrate	DGI	Full PBPK	Literature reported uptake parameters	In vivo, PE,	3	1	PK-Sim	Wojtyniak
BCRP	Simvastatin lactone					OP				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		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)

Vijaywargi G., Biopharm Drug Dispos. 2023



## Transporter based PBPK Models in Diseased and Geriatric Population

Transporter	Molecule	Mechanism	Model objective	Minimal or full PBPK	Data input	Data source	Acceptance criterion <sup>a</sup>	Outcome <sup>b</sup>	Software used	Reference	
Diseased population	Diseased population (hepatic <sup>A</sup> , renal impaired <sup>B</sup> and cancer <sup>C</sup> )										
<sup>A</sup> OATP1B1	Pemafibrate	Substrate	Clinical PK	Full PBPK	Experimental metabolic and uptake clearances	Experimental in vitro, PE, OP	5	1	Simcyp	Ogawa et al. (2020)	
<sup>B</sup> 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 <sup>a</sup>	Outcome <sup>b</sup>	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)


# **Transporter Mediated DDI in Regulatory Submissions**

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

Dn	ıg	Key t level) a	heme (impact and question(s)	Victim/perpetr	ator? Brief description	Internal impact	Qualification dataset	FDA/EMA response	
Tra (m 20	ametinib arketed) en et al., 115 <sup>61</sup>	DDI (hi Requee clinical investij tion of <i>In vitro</i> data fie tial risk accord regulat	gh) studies to gate the inhibi- intestinal BCRP. BCRP inhibition gged the poten- of <i>in vivo</i> DDI ng to the EMA ory guidelines.	Perpetrator: Wee BCRP inhibitor	In vitro Trametinib is a wea BCRP inhibitor, however based upon the EMA DDI guidance criteria the in vitro risk in the gut could not be excluded using in vitro data alone. Predicted intestinal concentrations were simu- lated using GastroPlus. Co plete inhibition was predict for the first 40 minutes pos dose and partial inhibition was predicted up to 1.6 hours post dose and restricted to the duodenum and jejunum. Recommenda tion was to limit the co- administration of sensitive BCRP substrates to 2 hour post trametnib administration	<ul> <li>k 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.</li> <li>Absorption was simulated</li> <li>m and the outputs of the model (predicted concentrations vs. st time) along the intestinal track were used as input in the DDI prediction guide lines, internal static modeling as well as cross</li> <li>a referencing data in the Washington database to inform concomitant medications at sits. No clinical BCRP DDI study was conducted</li> </ul>	In vitro BCRP inhibition data. Sponsor was requested to fur- ther 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.	
Ta	able 3 Ex	xamples of	transporter	r-mediated D	DI PBPK analyses and th	eir impact on drug deve	lopment and regulatory	decision	
E: ni 4	xample umber (	Drug Axitinib (marketed)	Key t Transport fund Inhibit Substra Intestinal tr P-gp (apical inhibitor	theme er (location ction) or – inh ate - sub ransporter: I efflux)	Victim/perpetrator/ and question(s)? Does P-gp inhibition <i>in vitro</i> translate to clinical DDI liabili unbound C <sub>max</sub> of 0.0008 µM,	Brief description ACAT model using Gastro was built to simulate axi concentrations in segme of GI tract	Impact <sup>a</sup> oplus tinib ents DI trial with P-gp substrate is needed	Qualification datase	t FDA/EMA response 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.

# **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<sub>m</sub>, J<sub>max</sub>, CL<sub>PD</sub>),
  - 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.

<u>Guest EJ</u>, 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 <sub>obs</sub> /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**

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1 Digoxin Physicoche	mical Properties			MWt		Estir	mated free base s	solubility using GSE	ef. Sanghvi-Yalk	owsky-QSARCombSci-22-2-25	8-2003-Estimation-Aqueous-Solubil	
2 MBB, 7/23/2019				780.96		logs	s = 0.5 - 0.01(m.p.	°C - 25) - log P so	lution logS= 0.5 -	0.01(122-25)-3.97=-4.45		
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4						-2	2.81E+00 M					
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2		S+pH	7	'	ADMET Predictor ver. 9.5	#		$\sim$		150	20100	
3	S+Sol	ublity Factor	N/A		ADMET Predictor ver. 9.5	+		CH,		r		
4	Aq. Solubility (mg/mL)	@ 25 Deg. C	0.058	mg/mL	Florence-JPharmPharmacol-28-637-1976-Solubility-of-Digoxin-Spironolactone-and-Estradiol	<u>±</u>	но 💡			Compound_Name	CAS_RegistryNumber	
15	Aq. S	Sol from GSE	1.210	mg/mL	GSE Ref. Sanghvi-Yalkowsky-QSARCombSci-22-2-258-2003-Estimation-Aqueous-Solubility-by-Gener	ral-Solu	$\parallel$	CH,		Digoxin	20830-75-5	
6	S+FaS	SIF @ pH 6.5	0.0864	mg/mL	ADMET Predictor ver. 9.5	[[013		F -	0_0			
	S+FeS	SIF @ pH 5.0	0.0783	mg/mL	ADMET Predictor ver. 9.5			, 		*fmla Structure	*mol.weight Structure	
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Physicochemical	^	Papp B->A	7.58E-06	i cm/s	Absorption Systems Lighthouse Database		11		он ~~	Selected	Therapeutic_Category	
Quotient Caco Data Digoxin Metab.		of AB & BA	7.56E-07	cm/s	Geometric mean of A->B and B->A		11				Cardiotonic	
Sheet4		1->A / A->B	100.5				11	A				
Jounela PO PSD		Hum Peff	5.59E-05	cm/s	Converted from Geo. Mean			1				
Greenblatt 0.75mg Eriefalt 1mg PO		of AB & BA	3.83E-06	cm/s	Average of A->B and B->A	Ŷ	PctBound_HumanPlas	smaProt_Log PctBound	RatPlasmaProt_Log	OralBioavailability_LitValue	MoWeight_LDS_File	
Johnson 1mg Fast & Fed	I PO	Hum Peff	1.34E-04	cm/s	Converted from Average		DatDomoiolog Human	UU DetBerne	LOCC.I	70.0000	760.9500	
Tayrouz 0.5mg PO		. Ratio							100 0000	1 app_accor	- opp_oator_cog	
Rengelshausen 0.75mg P Greiner IV&PO 1mg DDI F	PO RIF 1999	S+Rbp	0.73		ADMET Predictor ver. 9.5		BrainPlasmaRatioRat	Log Clearance	LitValue (L/h)	Papp_NDR_MDCK_AB_Log	Papp_MDR_MDCK_BA_Log	
Sheet6		Ex Rbp	1.00		Hinderling-JPharmSci-73-8-1042-1984-Digoxin-binding-and-distribution-in-blood		-1.05	06		-1.1223	0.8798	
Guney PO DDI RIF 2008 Sheet9		Ex Rbp	0.96	5	Imawaka-PharmRes-26-8-1881-2009-Predict-Human-Fb-from-Animal-PK-no-human-IV		DoseNumber_LitValue	e EffluxRatio	_Caco2_Log	pKa1	pKa2	
Kirby PO DDI RIF 2012		Rbp	0.55		Fitted to match the IV and PO Plasma profiles		0.10	00				
Sheet7		Plasma					EffluxRatio_MDR_MDR	CK_Log HIA_LIVa	e 91.0000	pKa3	HA_LIValue	
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# **Overview of Modeling Strategy of Digoxin**





# Key Physicochemical and Biopharmaceutical Parameters for Digoxin Used in GastroPlus Simulations

Parameter	Value	Reference	Parameter	Value	Reference
logP	1.26	[ PUBCHEM]	OATP4C1 (kidney)	CAUSE I	
Diffusion coefficient	0.44x10-5 cm2/s	ADMET Predictor	K <sub>m</sub> (μM)	7.8	[Mikkaichi et al. 2004]
рКа	NA (None in		V <sub>max</sub> (mg/s/mg trans protein)	0.1	Optimized value
Reference solubility	Physiological Range) 0.058 mg/mL @ pH =	[Florence et al. 1976]	Na⁺/K⁺-ATPase <b>(muscle</b> )		
Dissolution Model	7.0	Costro Dius default	K <sub>m</sub> (mg/L)	6.2	Assumed
Dissolution Model	size of 5 µM	(Lu et al. 1993)	V <sub>max</sub> (mg/s/mg trans protein)	0.03	Optimized value
Precipitate radius	1 μm	GastroPlus default	Р-gp <b>(РВРК</b> )		
Drug particle density	1.2 g/mL	GastroPlus default	K <sub>m</sub> (μM)	177	(Troutman et al. 2003)
Mean precipitation time	900 s	GastroPlus default	V <sub>max</sub> (mg/s/mg trans protein) P-gp ( <b>gut-apical</b> )	0.018	Optimized value
Human Jejunal P <sub>eff</sub> (×10 <sup>-4</sup> )	1.765 cm/sec	Assumed to be 10 times of rat permeability 0.4 ×10 <sup>-4</sup> cm/s	K <sub>m</sub> (μM)	177	(Troutman et al. 2003)
Blood: plasma	0.55	[Varma et al. 2005]	V <sub>max</sub> (mg/s/mg trans protein)	0.15	Optimized value
concentration ratio (Rbp)	0.55	ADMET Fredictor	Liver Apical PStc	0.5 (mL/s)	Optimized value (MDR3 P-gp
Plasma protein binding	75 %	US FDA	SpecPStc	0.35 (mL/s/mL)	Optimized value
Adjusted plasma fraction	69.249	GastroPlus algorithm	Renal Clearance Estimation method	f <sub>up</sub> * GFR	

<sup>a</sup> Predicted using ADMET Predictor<sup>®</sup> v10.0

# **Digoxin Characteristic Properties**

- P-gp substrate that reaches C<sub>max</sub> 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<sub>ss</sub> 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





# **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	
	<u>Brain</u>	<u>Nishimura</u>	0,000887	
	<u>Kidney</u>	<u>Nishimura</u>	0.000425	
	Liver*	<u>Nishimura</u>	0.150	
<u> </u>	Placenta	<u>Nishimura</u>	0.000122	
<u>Sm</u>	all Intestine	<u>Nishimura</u>	0.000614	
	<u>Kidney</u>	Mean across all PMT Sampler	BLQ	
	Liver*	<u>Mean across all PMT Samples</u>	5.061	
Note	that relative	expression values should only b	e compared between	entries of the same sou





# **Model Validation of Digoxin**

### **Observed vs Predicted Values for C**<sub>max</sub> and AUC of Digoxin



Purple Circles and Blue Circles represent C<sub>max</sub> and AUC<sub>0-inf</sub>, respectively.

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



# **DDI Accuracy**



#### **Observed vs Predicted AUC**<sub>0-t</sub> and C<sub>max</sub> 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**

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4	АВ	C D		E				F	G	H I	J	K L	м	N	0	Р
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4	111.	.9 0.0932 55.9	9 167.8				-4	4.43E+00 M								
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NASDAQ: SLP

SimulationsPlus

# **Overview of Modeling Strategy of Fexofenadine**





# Key Physicochemical and Biopharmaceutical Parameters for Fexofenadine Used in GastroPlus Simulations

Parameter	Value	Reference	Parameter	Value	Reference
logP	0.5	(Chen chen et al. 2007)	Blood:plasma concentration ratio	0.74	(Takano et al.,2016)
Diffusion coefficient	0.53x10 <sup>-5</sup> cm <sup>2</sup> /s	ADMET Predictor <sup>a</sup>	(R <sub>bp</sub> ) Plasma protein binding (F <sub>up</sub> )	31 %	(NDA-FDA-Alegra-Fexofenadine-
рКа	9.462 (base)	Based on fitting to Sol. vs. pH profile		22% (R-Fexo)	20872-label)
	3.931 (acid)			40% (S-Fexo)	(Kusuhara et al. 2013)
Reference solubility	0.14 mg/mL @ pH = 6.0	(NDA-FDA-Alegra-Fexofenadine-	Spec PStc	6.0 x 10 <sup>-4</sup> mL/s/mL tissue	Fitted
		20872-label)			
Solubility Factor	59.31 (base)	Based on fitting to Sol. vs. pH profile	Transporters		
	14.76 (acid)		P-gp K <sub>m</sub>	25.9 μM	(Takano et al. 2016)
FaSSIF solubility	0.14 mg/mL	ADMET Predictor <sup>a</sup>		20 µM	Fitted
EoSSIE colubility	0.21  mg/ml	ADMET Prodictor <sup>a</sup>	P-gp V <sub>max</sub>	0.05 mg/s (Gut)	(Fitted)
ressir solubility	0.21 mg/mL	ADMETFredictor		0.02 mg/s/mg-trans (PBPK)	
Bile salt solubilization ratio	1802.1	GastroPlus algorithm			
Human effective permeability	0.626 x 10 <sup>-4</sup> cm/s	(Absorption Systems Lighthouse	OATP2B1 K <sub>m</sub>	428 μM	(Shirasaka et al. 2014)
(P <sub>eff</sub> )		Database)	OATP2B1 V	4.2 nmol/min/mg protein	(Fitted)
(derived from Caco-2 assay)			in the second se	0.06 mg/s	
Particle radius	25 mm	GastroPlus default		0.00	
Precipitate radius	1 mm	GastroPlus default	OAT3 K <sub>m</sub>	70.2 μM	(Tahara et al. 2006)
Drug particle density	1.2 g/mL	GastroPlus default	OAT3 V <sub>max</sub>	0.12 nmol/min/mg protein	(Tahara et al. 2006)
Mean precipitation time	20000 s	Fitted		0.012 mg/s/mg-trans	

<sup>a</sup> Predicted using ADMET Predictor<sup>®</sup> v10.0



# **GastroPlus PBPK Model : Fexofenadine**

- Used the  $\log P = 0.5$ .
- Changed the kidney and muscle model to ٠ Permeability-limited with SpecPstc =6E-4 mL/s/mL
- PBPK Vmax values (P-gp & OATP2B1) and SpecPStc ٠ were optimized against IV dose
- Gut transporter Vmax values were fitted against the ٠ PO dose



# **Model Validation of Fexofenadine**

### **Observed vs Predicted Values for C**<sub>max</sub> and AUC of Fexofenadine</sub>



Purple Circles and Blue Circles represent C<sub>max</sub> and AUC<sub>0-inf</sub>, respectively.

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



# **DDI Accuracy**



**Observed vs Predicted AUC**<sub>0-t</sub> and C<sub>max</sub> 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



ТСР	sodium-taurocholate co-transporting polypeptide
АТЗ	organic anion-transporter
CRP	breast cancer resistance protein
ATP1B1	organic anion-transporting polypeptide 1B1
ATP1B3	organic anion-transporting polypeptide 1B3
ATP2B1	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	Parameter	Value	Reference
logD	-0.33 @ pH=7.4	(Jones et al. 2012)	Transporters	5.5	
Diffusion coefficient	0.57x10 <sup>-5</sup> cm <sup>2</sup> /s	ADMET Predictor <sup>a</sup>	Influx: Basolateral side		
2/2	4.329 (acid),	(Jamei et al. 2014), ADMET	OATP1B1 (liver)		
рка	2.26 (base)	Predictor <sup>a</sup>	K <sub>m</sub> (mM)	4	(Ho et al. 2006)
Reference solubility	0.5 mg/mL @ pH = 1.2	(FDA 2003)	V <sub>max</sub> (mg/s/mg-trans) OATP1B3 (liver)	0.069	Optimized value
		(Human jejunal P <sub>eff</sub> value is	K <sub>m.u</sub> (mM)	9.8	( <u>Ho et al. 2006</u> )
		estimated from geo mean of	V <sub>max</sub> (mg/s/mg-trans)	0.086	Optimized value
Human effective permeability (P	1.02 x10 <sup>-4</sup> cm/s	$P_{app(A-B)}$ and $P_{app(B-A)}$ data in Caco-	NTCP (liver)		
Human enective permeability (F <sub>eff</sub> )		2) using built-in ABSCa	K <sub>m.u</sub> (mM)	65	( <u>Ho et al. 2006</u> )
		conversion.	V <sub>max</sub> (mg/s/mg-trans)	0.261	Optimized value
		( <u>Li et al. 2012</u> )	OATP2B1 (liver)		
Particle radius	25 mm	GastroPlus default	K <sub>m.u</sub> (mM)	2.4	( <u>Ho et al. 2006</u> )
Precipitate radius	1 mm	GastroPlus default	V <sub>max</sub> (mg/s/mg-trans)	0.0054	Optimized value
Drug particle density	1.20 g/mL	GastroPlus default	OAT3 (kidney)		
Mean precipitation time	900 s	GastroPlus default	К <sub>т.ч</sub> (тМ)	7.4	(Windass et al. 2007)
Blood: plasma concentration ratio (R <sub>bo</sub> )	0.625	(Jamei et al. 2014)	V <sub>max</sub> (mg/s/mg-trans) Efflux: Apical side	0.08	Optimized value
Plasma protein binding (F <sub>up</sub> )	10.7 %	(Jamei et al. 2014)	BCRP (liver, kidney)		
Adjusted Fun	10.697 %	GastroPlus algorithm <sup>b</sup>	K <sub>m.1</sub> (mM)	307	(Huang et al. 2006)
Metabolism			V <sub>max</sub> (mg/s/mg-trans)	0.012	Optimized value
CYP2C9 K <sub>m II</sub> (mM)	23.03	ADMET Predictor <sup>a</sup>	BCRP (gut)		. c
CYP2C9 V <sub>max</sub> (nmol/min/mg protein)	0.0001	Optimized value	K <sub>mu</sub> (mM)	307	( <u>Huang et al. 2006</u> )
UGT1A1 K <sub>m,u</sub> (mM)	16	(Schirris et al. 2015)	BCRP V <sub>max</sub> (mg/s)	0.11	Optimized value
UGT1A1V <sub>max</sub> (nmol/min/mg protein)	0.0002	Optimized value	CL <sub>PD</sub> (mL/min/million cells)	0.0264	Optimized value



# **Model Validation of Rosuvastatin**

### Goodness-of-Fit Plots Showing Observed vs Predicted Values for C<sub>max</sub> and AUC of Rosuvastatin





# Model Validation of Rosuvastatin: DDI Accuracy

Observed vs Predicted DDI Ratios for C<sub>max</sub> 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
DATP1B3	organic anion-transporting polypeptide 1B3
ОАТЗ	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	Parameter	Value	Reference
Molecular weight	424.5	ADMET predictor	Transporters		
LogD at pH 7	0.59	( <u>FDA</u> )	Influx: Basolateral side		
		Optimized to describe IV C <sub>p</sub> -	OATP1B1 (liver)		
LogP	1.8ª	time profile and to better	Km (μM)	27	( <u>Izumi et al. 2015</u> )
		capture observed V <sub>ss</sub>	V <sub>max</sub> (mg/s/mg-trans)	0.023	Optimized
Ionization constant (pKa)	4.92 (acid)	ADMET predictor	OAT3 (kidney)		
		7	Km (μM)	27.7	(Nakagomi-Hagihara et al. 2007a)
Reference solubility (mg/mL)	479.6 @ pH 6.8	( <u>Ruiz-Picazo et al. 2019</u> )	V <sub>max</sub> (mg/s/mg-trans)	0.1	Optimized
Papp (10 <sup>-5</sup> cm/s, Caco-2)	0.3	( <u>Varma et al. 2012</u> )	Efflux: Apical side		
Peff (10 <sup>-4</sup> cm/s)	1.18	ABSCa conversion <sup>b</sup>	MRP2 (liver, kidney)		
Mean precipitation time (s)	900	GastroPlus default value	Km (μM)	7.2	(Ellis et al. 2013)
Blood to Plasma concentration ratio	0.56	(Watanabe et al. 2009)	V <sub>max</sub> (mg/s/mg-trans)	0.1	Optimized
Plasma fraction unbound (%)	50	(FDA)	MRP2 (gut)		
Adjusted plasma fraction unbound	49.96	GastroPlus algorithm <sup>c</sup>	Km (μM)	7.2	( <u>Ellis et al. 2013</u> )
(%)			V <sub>max</sub> (mg/s/)	0.002	Optimized
Metabolism			CL <sub>PD</sub> (µL/min/million cells)	0.5	(Varma et al. 2012)
CYP3A4 (gut and liver)					
Km (μM)	3480	(Jacobsen et al. 1999)			
V <sub>max</sub> (nmol/min/mg-enz)	75	Optimized			



# **Model Validation of Pravastatin**

Goodness-of-Fit Plots Showing Observed vs Predicted Values for C<sub>max</sub> and AUC of Pravastatin





# **Model Validation of Pravastatin: DDI Accuracy**

Observed vs Predicted DDI Ratios for C<sub>max</sub> and AUC of Pravastatin with Perpetrators (Rifampicin, Gemfibrozil, and Its Metabolite, Itraconazole, and Fluconazole)





# **Investigational Drugs as Victim Drug-Findings/Guidance**

For Investigational drugs, initial simulations can be carried out using *in vitro* metabolism and transporter data and DDI potential as victim drug can be carried out Based on our findings, it is likely that a clinical DDI study with strong inhibitor and or mass balance study is warranted to define the relative contribution of enzymes/transporters for the total clearance of the drug Thereafter, we can test the untested scenarios like the effect of moderate or weak inhibitors of relevant transporters using the DDI Qualification matrix



# 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.
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- 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<sup>™</sup> 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
- <u>Clinical Drug Interaction Studies Cytochrome P450 Enzyme- and Transporter-Mediated Drug</u> <u>Interactions Guidance for Industry | FDA</u>
- 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 <sub>i</sub> -rev- <i>in vitro</i> , u = 18.5 $\mu$ M	(Kajosaari et al. 2005)
MRP2	Inhibition: Competitive, K <sub>i</sub> -rev- <i>in vitro</i> , u = 0.87 $\mu$ M	(Yoshikado et al. 2016)
OATP1B1	Inhibition: Competitive, $K_i$ -rev- <i>in vitro</i> , $u = 0.07 \ \mu M$	(Morse et al. 2019)
OATP1B3	Inhibition: Competitive, K <sub>i</sub> -rev- <i>in vitro</i> , u = 0.07 $\mu$ M	(Morse et al. 2019)
OATP2B1	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 65 \ \mu M$	(Karlgren et al. 2012)
BCRP	Inhibition: Competitive, IC50- <i>in vitro</i> , u = 14.9 $\mu$ M	(Costales et al. 2021)
NTCP	Inhibition: Competitive, IC50- <i>in vitro</i> , u = 127 $\mu$ M	(Zhang et al. 2019)
OAT3	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 33 \ \mu M$	(Parvez et al. 2016)
СҮРЗА4	Induction: EC <sub>50</sub> (unbound) = 0.064 $\mu$ M E <sub>max</sub> = 15	(Asaumi et al. 2018) Fitted value#
UGT1A3	Induction: $EC_{50}$ (unbound) = 0.064 $\mu M$ $E_{max} = 4.4$	(Asaumi et al. 2018) Fitted value <sup>#</sup>
UGT1A1	Induction: $EC_{50}$ (unbound) = 0.064 $\mu M$ $E_{max} = 4.4$	(Asaumi et al. 2018) Fitted value <sup>#</sup>
CYP2C9	Induction: EC <sub>50</sub> (unbound) = 0.064 $\mu$ M E <sub>max</sub> = 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<sub>max</sub> 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- <i>in vitro</i> , $u = 7.4 \ \mu M$	(Säll 2013)
Gemfibrozil	OATP1B3	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 10 \ \mu M$	(Yoshida et al. 2012)
Gemfibrozil	OAT3	Inhibition: Competitive, K <sub>i</sub> -rev- <i>in vitro</i> , $u = 3.4 \mu M$	(Nakagomi-Hagihara et al. 2007)
Gemfibrozil	NTCP	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 23 \ \mu M$	(Ho et al. 2006)
Gemfibrozil	CYP2C9	Inhibition: Competitive, $K_i$ -rev- <i>in vitro</i> , $u = 4 \ \mu M$	(Wang et al. 2002)
Gemfibrozil	UGT1A1	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 110 \ \mu M$	(Gan et al. 2010)
Gemfibrozil- glucuronide	OATP1B1	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 4.3 \ \mu M$	(Säll 2013)
Gemfibrozil- glucuronide	OATP1B3	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 74 \ \mu M$	(Yoshida et al. 2012)
Gemfibrozil- glucuronide	OAT3	Inhibition: Competitive, K <sub>i</sub> -rev- <i>in vitro</i> , $u = 9.9 \ \mu M$	(Nakagomi-Hagihara et al. 2007)
Gemfibrozil- glucuronide	UGT1A1	Inhibition: Competitive, IC50- <i>in vitro</i> , $u = 130 \ \mu M$	(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- <i>in vitro</i> , u = 1.3 nM	(Isoherranen et al. 2004)
Hydroxy itraconazole (OH-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev- <i>in vitro</i> , u = 14.4 nM	(Isoherranen et al. 2004)
Keto itraconazole (Keto-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev- <i>in vitro</i> , u = 1.4 nM	(Isoherranen et al. 2004)
N-desalkyl itraconazole (ND-ITZ)	CYP3A4	Inhibition: Competitive Ki-rev- <i>in vitro</i> , 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- <i>in vitro</i> , $T = 15 \mu M$	(Isoherranen et al. 2008)

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

