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SAWP DISCUSSION MEETING

QUALIFICATION PROCEDURE - SIMCYP SIMULATOR V19

JULY 10, 2024

Attendees

Dialling in from the UK

- Karen Rowland Yeo
- Masoud Jamei

Dialling in from France

- Frederic Bois

Dialling in from Prague

- Sibylle Neuhoff

Second list of issues

1. The Applicant stated that they only optimised kinact as based on their experience no optimisation is needed for KI. This could be understood if the plasma concentration is >> KI. Ki and KI were however in the same order of magnitude, so this is unlikely to be the case. Please discuss.
2. Liver kdeg values are not identical between enzymes involved. The Applicant should discuss the robustness of kdeg values and the values that would be included in the public qualification report.
3. Please explain how Ki values lower than the lowest reported in vitro values have been selected for the PBPK model without optimisation of these parameters. There is a high variability between the median reported in vitro values and the Ki values used in Simcyp: reported values are on average 40-fold higher with a range of 0.3-690-fold. Please, discuss how in vitro data can be used for competitive inhibition. Should a positive control be included in the in vitro assays?
4. For CYP3A4, please present differential inhibition of intestinal or hepatic CYP3A4.
5. The Applicant optimised some in vitro values in compound files based on clinical data. The Applicant should discuss, in which cases optimisation was regarded necessary, i.e. how the need for optimisation was determined.
6. The applicant is invited to present model diagnostics following the principles recommended by the qualification team in the scientific discussion above.

Issue 1 – inactivation parameters

The applicant stated that they only optimised kinact as based on their experience no optimisation is needed for KI. This could be understood if the plasma concentration is >> KI. Ki and KI were however in the same order of magnitude, so this is unlikely to be the case. Please discuss.

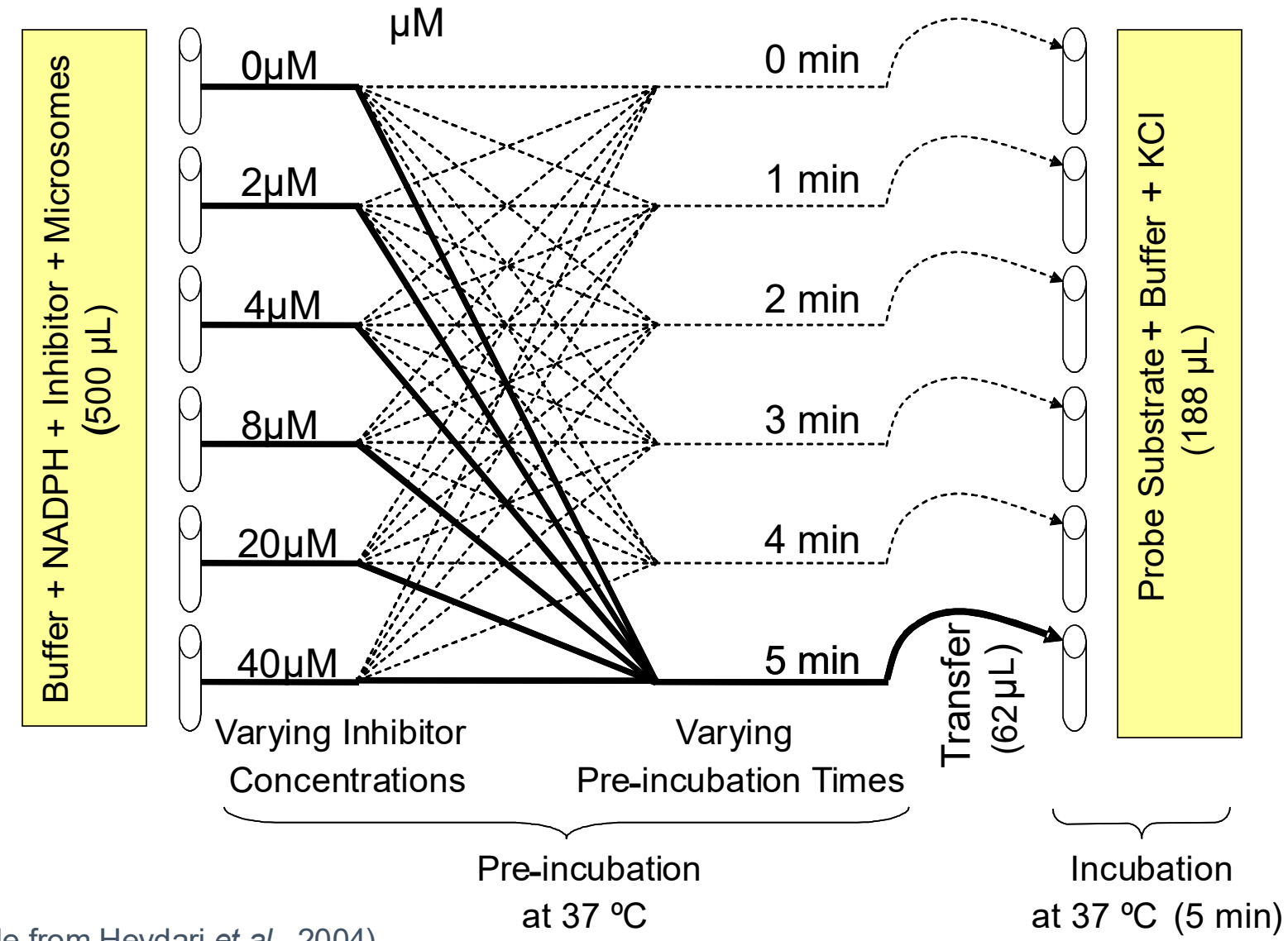
- *Relevant excerpt from the scientific discussion:* The Applicant stated that they only optimised kinact as based on their experience no optimisation is needed for KI. This may not always be the case. Therefore, two different scenarios in the clinical study (e.g. different dosing) may be required to optimise both parameters. For KI and kinact determination, standardised procedures (see e.g. Grimm et al., Drug Metabolism and Disposition, 2009, 1355) should be used. The Applicant should ensure that the KI and kinact values in the compound files are reliable. Thereby it should be demonstrated that no optimisation is needed for KI.

Predicting DDIs involving MBI

- Predicting the magnitude of time-dependent CYP-mediated DDIs from *in vitro* data requires estimates of:
  - inactivation parameters of the inhibitor (KI, k_{inact})
  - the turnover of the CYP enzyme (k_{deg})
- The latter is discussed in the next section (Issue 2).

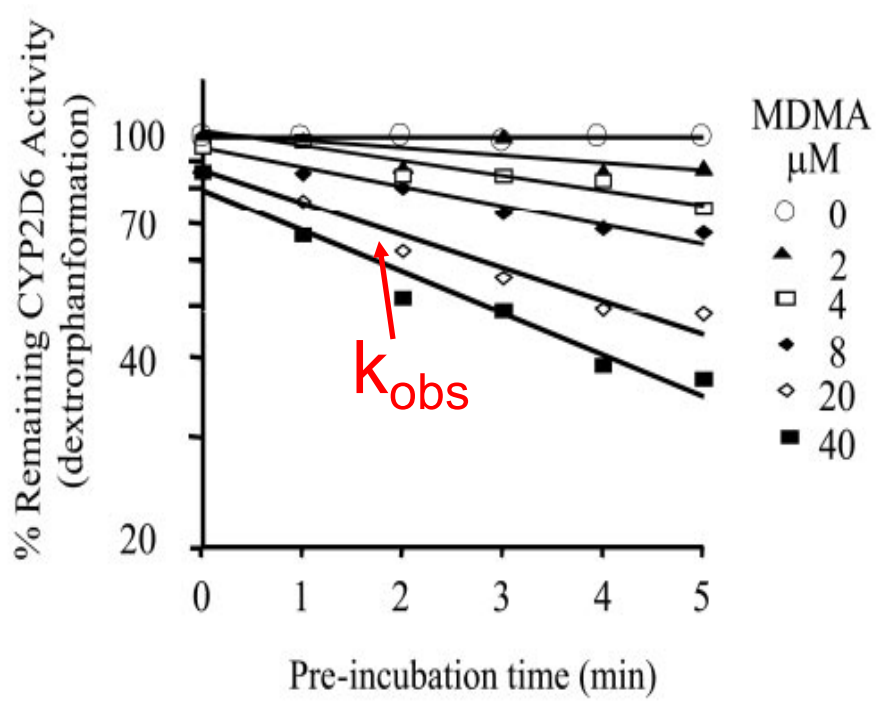
- Here, we focus on the inactivation parameters.
- Important to consider the experiment and subsequent data analysis used to derive the inactivation parameters.

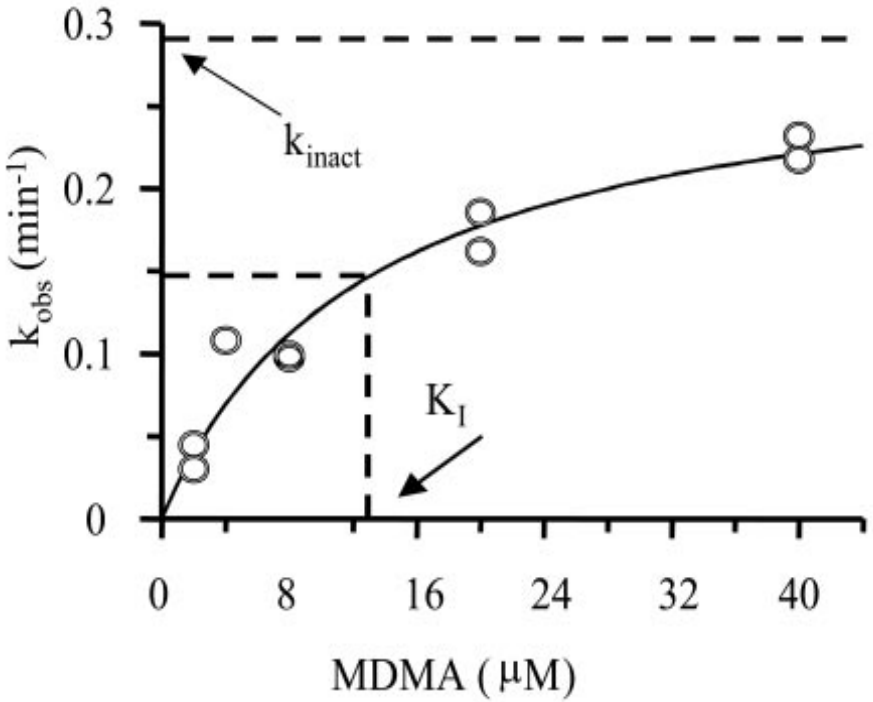
Quantitative Assessment of MBI



(Example from Heydari *et al.*, 2004)

Obtaining Kinetic Parameters of MBI





(Heydari *et al*., 2004)

$$k_{obs} = \frac{k_{inact} \times I}{K_I + I}$$

k_{inact}	= maximum inactivation rate constant
K_I	= Irreversible inhibition constant ([I] where $k_{inact}/2$)

Derivation of inactivation parameters – standard approach

Prediction of time-dependent CYP3A4 drug–drug interactions by physiologically based pharmacokinetic modelling: Impact of inactivation parameters and enzyme turnover

K. Rowland Yeo [a,*], R.L. Walsky [d], M. Jamei [a], A. Rostami-Hodjegan [a,c], G.T. Tucker [a,b]

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[b] Clinical Pharmacology, University of Sheffield, Sheffield S10 2JF, UK
[c] School of Pharmacy and Pharmaceutical Sciences, Faculty of Medical and Human Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK
[d] Pharmacokinetics, Dynamics and Drug Metabolism, Pfizer Inc., Groton, CT, USA

- Inactivation parameters determined anew in a single laboratory under standardised conditions
- The conversion of midazolam to its 10 hydroxy metabolite (1'OH midazolam) by human liver microsomes was used as a marker of CYP3A4 activity.

$$k_{obs} = k_{obs[0\mu M]} + \frac{k_{inact} \times [I]}{K_I + [I]} \quad (3)$$

where [I] represents the concentrations of the test drug in the primary incubation, k_{inact} is the maximal inactivation rate, and K_I is the inactivator concentration at half k_{inact}.

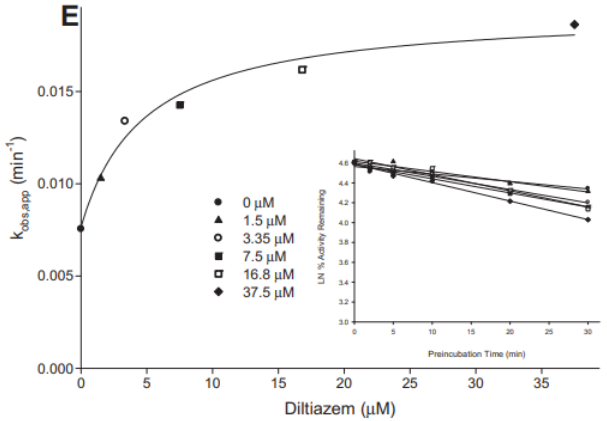


Fig. 1. Inhibitory effect of (A) azithromycin, (B) clarithromycin, (C) erythromycin, (D) microsomes.

Inactivation parameters derived using standard dilution method

Source	Hours	Hepatocytes			HLM			
		KI	kinact	kinact/KI	KI	kinact	kinact/KI	HLM/Hep
		uM	h-1	h-1/uM	uM	h-1	h-1/uM	
3	Diltiazem	8.90	1.37	0.15	1.53	1.44	0.94	6.12
3	Erythromycin	67.90	4.74	0.07	5.33	3.66	0.69	9.84
3	Verapamil	2.10	1.63	0.78	3.19	4.56	1.43	1.84
3	TAO	3.40	22.14	6.51	0.44	4.68	10.64	1.63
4	Crizotinib	0.89	0.78	0.88	0.37	6.90	18.65	21.28
5	Crizotinib	1.60	1.86	1.16	0.40	2.46	6.15	5.29
5	Erythromycin	1.60	3.72	2.33	0.40	1.92	4.80	2.06
6-Simcyp	**Clarithromycin**				**12.00**	**2.13**	**0.18**	
6-Simcyp	**Diltiazem**				**0.84**	**0.70**	**0.84**	
6-Simcyp	**NDM**				**4.75**	**1.09**	**0.23**	
6-Simcyp	**Erythromycin**				**23.20**	**3.18**	**0.14**	
6-Simcyp	**Verapamil**				**2.21**	**0.66**	**0.30**	
7	Clarithromycin	4.47	0.67	0.15	36.20	4.87	0.13	0.90
7	Diltiazem	8.96	1.30	0.15	1.32	0.65	0.50	3.41
7	NDM	2.96	0.76	0.26	0.96	0.57	0.60	2.31
7	Erthromycin	8.13	0.85	0.10	14.30	3.34	0.23	2.25
7	Verapamil	0.21	1.03	5.03	1.71	2.92	1.71	0.34

Even using the "standard" dilution method, results can be highly variable.

Not always clear if correction for nonspecific microsomal binding has been applied to KI value.

3. Chen Y, Liu L, Monshouwer M, Fretland AJ. Drug Metab Dispos. 2011 Nov;39(11):2085-92
4. Mao J, Johnson TR, Shen Z, Yamazaki S. Drug Metab Dispos. 2013 Feb;41(2):343-52.;
5. Mao J, Tay S, Khojasteh CS, Chen Y, Hop CE, Kenny JR. Pharm Res. 2016 May;33(5):1204-19.
6. Rowland Yeo K, Walsky RL, Jamei M, Rostami-Hodjegan A, Tucker GT. Eur J Pharm Sci. 2011 Jun 14;43(3):160-73.
7. Tseng E, Eng H, Lin J, Cerny MA, Tess DA, Goosen TC, Obach RS. Drug Metab Dispos. 2021 Oct;49(10):947-960.

Correction for nonspecific microsomal binding - fu_{mic}

Non-specific binding of compounds in *in vitro* metabolism assays: a comparison of microsomal and hepatocyte binding in different species and an assessment of the accuracy of prediction models

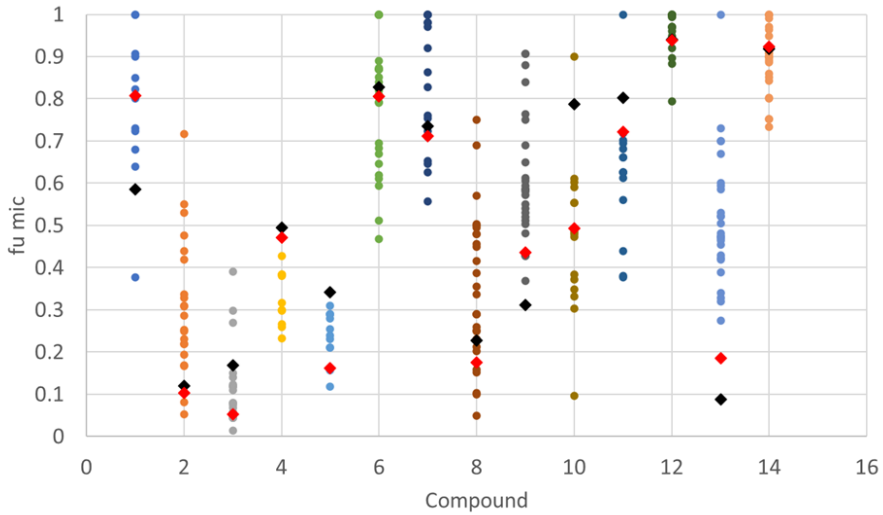
Iain Gardner[a], Mandy Xu[b], Chunyan Han[b], Yi Wang[b], Xingjin Jiao[b], Masoud Jamei[a], Hiba Khalidi[a], Peter Kilford[a,c], Sibylle Neuhoff[a], Roz Southall[a], David B. Turner[a], Helen Musther[a], Barry Jones[d] and Simon Taylor[d]

External literature compound set, N = 697

Method	Hallifax and Houston	Poulin	Austin	Turner-Simcyp
Acid				
N	164	164	164	164
R^2	0.22	0.38	0.47	0.46
RMSE	0.513	0.367	0.26	0.275
Base				
N	229		229	229
R^2	0.372		0.343	0.37
RMSE	0.264		0.291	0.271
Base (subset)				
N	44	44	44	44
R^2	0.11	0.42	0.08	0.099
RMSE	0.358	0.297	0.39	0.378
Neutral				
N	304	304	304	304
R^2	0.54	0.68	0.67	0.70
RMSE	0.304	0.195	0.19	0.143

External Pharmaron compound set, N = 53-60

- Austin model: R^2 = 0.47; RMSE = 0.23
- Hallifax & Houston: R^2 = 0.56; RMSE = 0.21
- Poulin: R^2 = 0.54; RMSE = 0.26
- Simcyp: R^2 = 0.69; RMSE = 0.198
  - Acid: R^2 = 0.28; RMSE = 0.25
  - Base: R^2 = 0.72; RMSE = 0.19
  - Neutral: R^2 = 0.84; RMSE = 0.15



1-Alprazolam, 2-Amitriptyline, 3 -Chlorpromazine, 4-Clozapine, 5-Desipramine, 6-Diclofenac, 7-Diltiazem, 8-Imipramine, 9-Midazolam, 10-Propranolol, 11-Quinidine, 12-Tolbutamide, 13-Verapamil, 14-Warfarin. Black-predicted PhysChem data in Turner-model, Red-measured PhysChem data in Turner-model.

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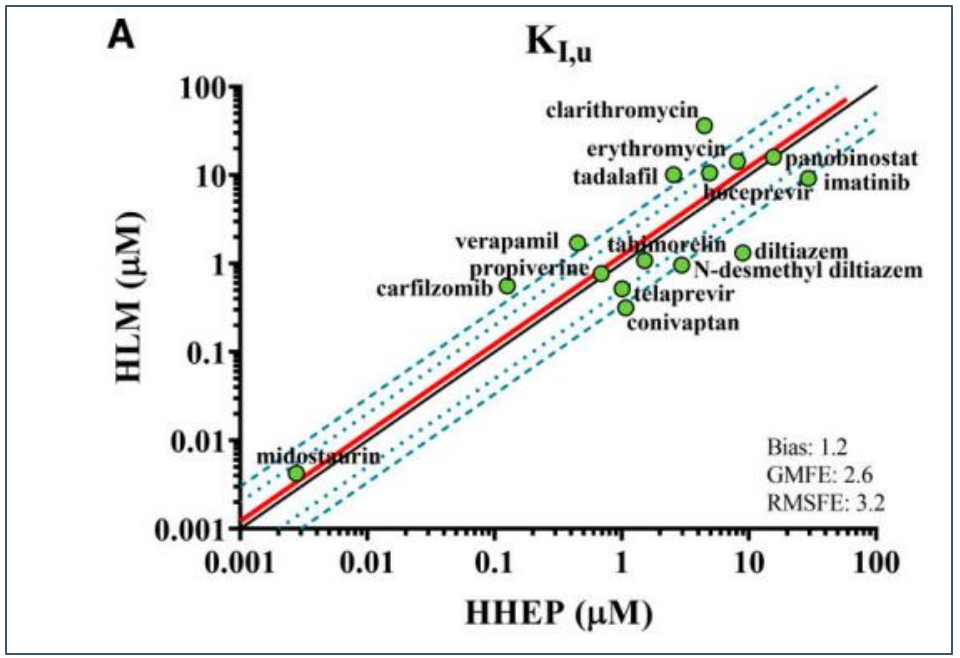
Equations for fu_{mic} predictions

- **For basic compounds**
  - $fu_{mic} = 1 / [([P]_{mic} \times 10^{0.646 * logP - 2.236}) + 1]$ r^2=0.81, RMSE=0.23
- **For neutral compounds**
  - $fu_{mic} = 1 \; [([P]_{mic} \times 10^{0.522 * logP - 1.728}) + 1]$ r^2=0.77, RMSE=0.12
- **For acidic compounds**
  - $fu_{mic} = 1 / [([P]_{mic} \times 10^{0.263 * logP - 1.804}) + 1]$ r^2 = 0.84, RMSE 0.05
- **Test set of partially ionised compounds**
  - f_i = fraction ionised (at assay pH) (Range 0 to 1)
  - $logK_{mic} = log(f_i \times$ Predicted Basic|Acidic $K_{mic} + (1- f_i) \times$ Predicted Neutral $K_{mic})$

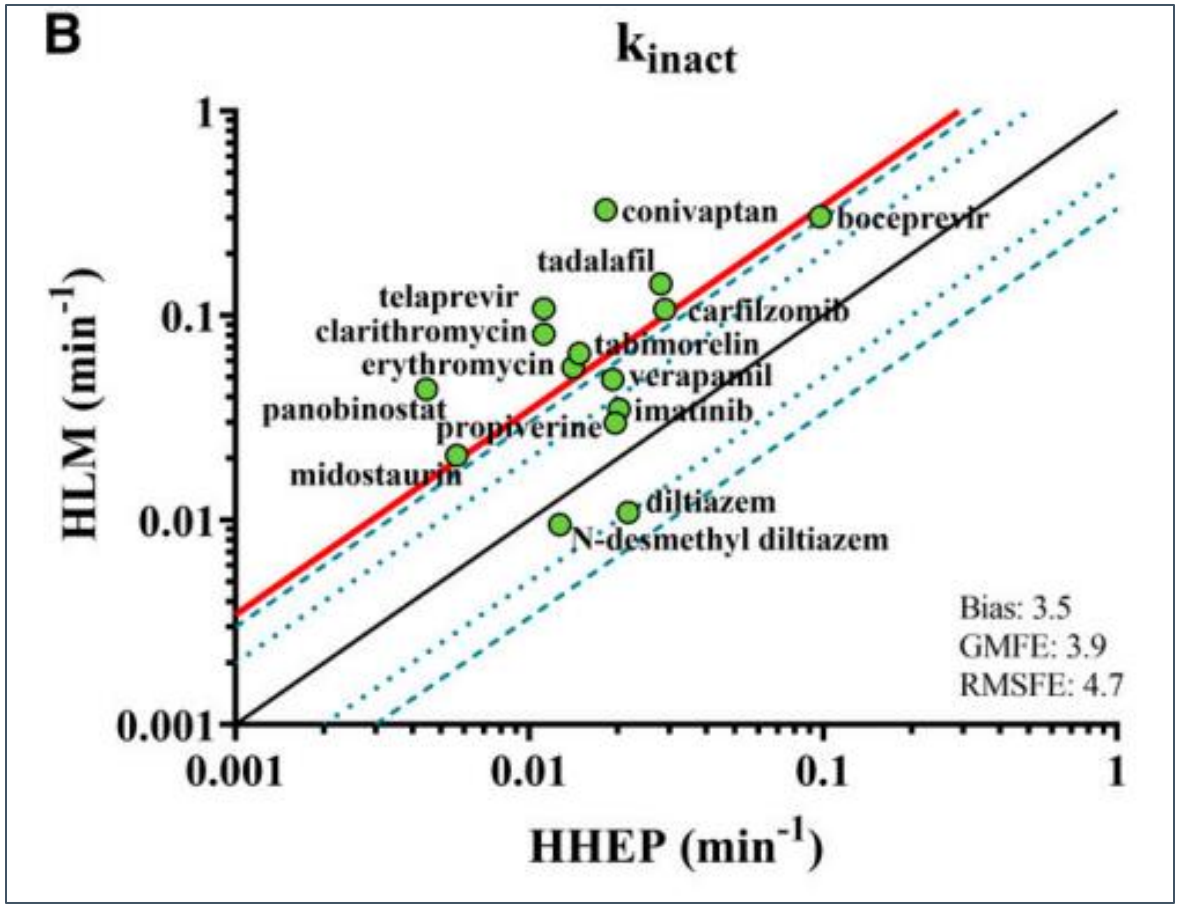
Inactivation parameters for 23 drugs – Tseng *et al.* 2021

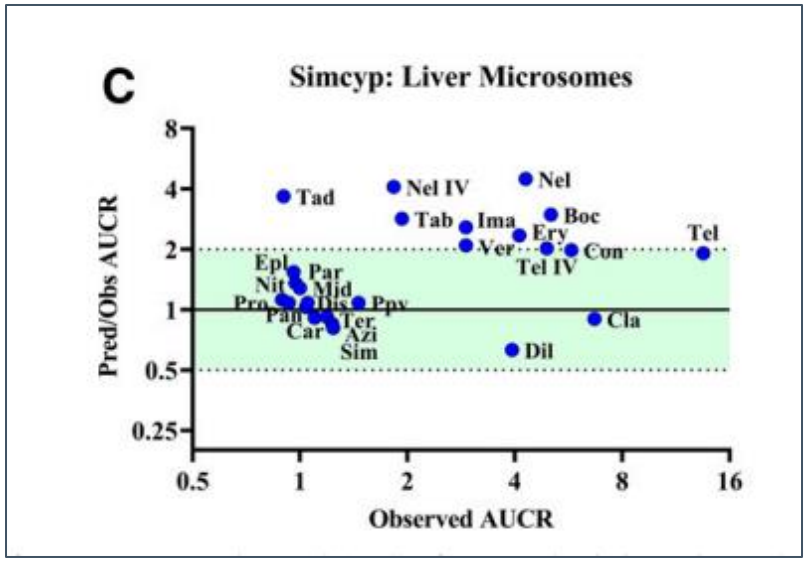
- Identified 23 drugs with clinical DDI data
- As part of the analysis, CYP3A4 inactivation parameters were generated in HLM and HH.
- KI values ranged from 0.360 to 202 µM in HLM and 0.574 to 51.2 µM in HH.

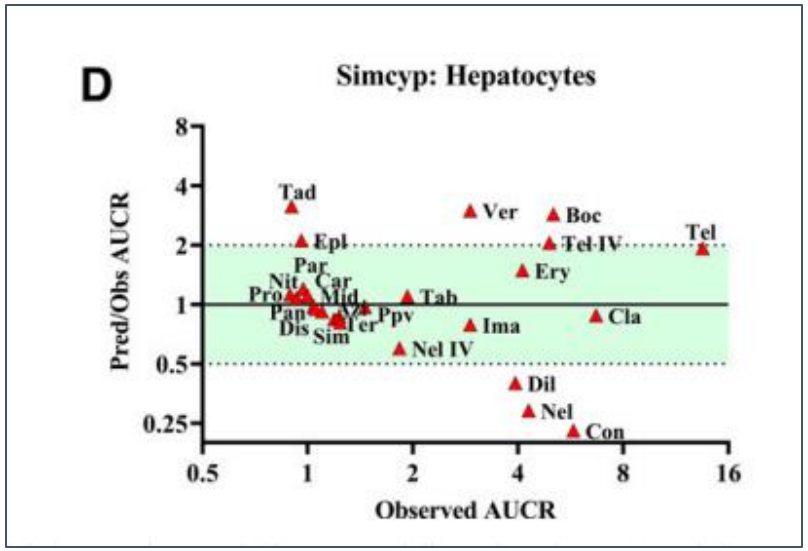
- After correcting for nonspecific microsomal binding (NSMB – application of fu_{mic}), on average, the KI,u values in HLM were 1.2-fold greater than in HH.
- Thus, it appears that applying a NSMB correction (via fu_{mic}), leads to minimal differences in KI values generated in HLM *versus* HH systems.



Inactivation parameters for 23 drugs – Tseng *et al.* 2021







On average, k_{inact} values were 3.5-fold greater in HLM than in HH.

Optimisation of inactivation parameters - k_{inact}

- In a complex but comprehensive numerical analysis of *in vitro* inactivation data and predictions of DDIs involving MBI, Yadav *et al.* (2019) concluded that as the conventional replot method considers only the *initial* rate of inactivation, ignoring the curvature in the plots at later time points, can lead to an overestimation of k_{inact}.

- Based on our experiences from Consultancy examples, optimisation of k_{inact} was performed to capture the observed DDI with midazolam. For example, tyrosine kinase inhibitors, crizotinib, ceritinib and larotrectinib.
  - KI,u values were as derived *in vitro*.
  - Multiple dose data were then used to verify the optimised parameters.

- In some cases, KI,u may have to be optimised to capture observed non-linearity.
- Of the 24 sets of inactivation parameters used for the moieties in the qualification DDI matrix, 1 k_{inact} value and 2 KI,u values were optimised (Issue 5).

Conclusions (Issue #1 – inactivation parameters)

- For a new investigational drug that is a MBI compound, it is important to correct the KI for NSMB (apply a fu_{mic} value).
- The fu_{mic} must be measured or calculated at the microsomal protein concentration used in the preincubation step.
- If the clinical DDI is overpredicted, we recommend that the k_{inact} should be optimised initially (e.g. crizotinib).
- However, in some cases the KI,u value may have to be optimised, especially to capture the non-linearity observed over multiple dosing due to auto-inhibition.
- Multiple doses conducted at various dose levels can also be used to assess the inactivation parameters.

Issue 2 – kdeg values

Liver kdeg values are not identical between enzymes involved. The Applicant should discuss the robustness of kdeg values and the values that would be included in the public qualification report.

- *Relevant excerpt from the scientific discussion*: In addition, liver kdeg values play a role and are not identical between enzymes involved. It is unclear how robust the kdeg values are. For CYP2D6, for example, the Applicant provided several values, and it is unclear, which one was used in the model. The Applicant should ensure that kdeg values and associated uncertainty should be included in the public qualification report.

Table 1. Values of kdeg used for individual CYP enzymes.

Enzyme	V19		Source	ICH M12		Source
	t1/2 (h)	kdeg (h^{-1})	references	t1/2 (h)	kdeg (h^{-1})	references
CYP1A2	38.5	0.0183	1,2	38	0.018	5
CYP2C8	23	0.0301	2	22	0.0318	6
CYP2C9	104	0.0067	2	104	0.0066	2
CYP2C19	26	0.0267	2	26	0.0264	2
CYP2D6	70	0.0099	2	51	0.0138	7,8
CYP3A4	36	0.0193	3	36	0.0192	3
CYP3A4gut	24	0.03	4	24	0.0288	4

1. Diaz, D.; Fabre, I.; Daujat, M.; Saintaubert, B.; Bories, P.; Michel, H. and Maurel, P. (1990) Gastroenterology, 99(3), 737-747. 2. Renwick, A.B.; Watts, P.S.; Edwards, R.J.; Barton, P.T.; Guyonnet, I.; Price, R.J.; Tredger, J.M.; Pelkonen, O.; Boobis, A.R. and Lake, B.G. (2000) Drug Metab. Dispos., 28(10), 1202-1209. 3. Fromm MF; Busse D; Kroemer HK; Eichelbaum M. Hepatology. 1996 Oct;24(4):796-801. 4. Greenblatt DJ, von Moltke LL, Harmatz JS, Chen G, Weemhoff JL, Jen C, Kelley CJ, LeDuc BW, Zinny MA. Time course of recovery of cytochrome p450 3A function after single doses of grapefruit juice. Clin Pharmacol Ther. 2003 Aug;74(2):121-9. doi: 10.1016/S0009-9236(03)00118-8. PMID: 12891222. 6. Backman JT, Honkalammi J, Neuvonen M, Kurkinen KJ, Tornio A, Niemi M, Neuvonen PJ. Drug Metab Dispos. 2009 Dec;37(12):2359-66. 7. Liston, H.L.; DeVane, C.L.; Boulton, D.W.; Risch, S.C.; Markowitz, J.S. and Goldman, J. (2002) J. Clin. Psychopharmacol., 22(2), 169-173. 8. Venkatakrishnan, K. and Obach, R.S. (2005) Drug Metab. Dispos., 33(6), 845-852.

Background

Enzyme	Method	n	$t_{1/2}$ (h) *
CYP1A2	*In vitro* Method 1	1	51
	In vitro Method 2	NC	43**
	In vitro Method 2	5	36 (8-58)
	In vivo Method 1	12	39 (27-54)
	In vivo Method 3	7	105
CYP2A6	*In vitro* Method 2	2	26 (19-37)
CYP2B6	*In vitro* Method 2	1	32
CYP2C8	*In vitro* Method 2	5	23 (8-41)
CYP2C9	*In vitro* Method 2	5	104
CYP2C19	*In vitro* Method 2	3	26 (7-50)
CYP2D6	*In vitro* Method 2	4	70
	In vivo Method 2	13	51
CYP2E1	*In vitro* Method 2	5	27 (7-40)
	In vivo Method 1	6	60
	In vivo Method 2	11	50 ± 19
CYP3A4	*In vitro* Method 1	1	44
	In vitro Method 2	NC	26**
	In vitro Method 2	4	79
	In vivo Method 1	15	72**
	In vivo Method 3	6	96 ± 38 (53-154)
	In vivo Method 1	7	72 (20-146)
	In vivo Method 1	3	(85-806)
	In vivo Method 1	8	(36-50)
	In vivo Method 3	13	10** (2-158)
	In vivo Method 3	35	94 (62-205)
	In vivo Method 3	7	70
	In vivo Method 3	16	85 ± 61
	In vivo Method 3	6	140 (48-284)
CYP3A5	*In vitro* Method 2	3	36 (15-70)

* $t_{1/2} = 0.693 / k_{deg}$. Values in brackets are ranges and ± indicates SD
** estimated from an analysis of the reported data

Yang J, Liao M, Shou M, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A. Cytochrome P450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. Curr Drug Metab. 2008 Jun;9(5):384-94.

In vitro Method 1	Radio-Labeling of Enzyme ('Pulse-Chase' Method)
In vitro Method 2	Degradation of Enzyme in Cultured Hepatocytes or Liver Slices
In vitro Method 3	Induction of CYP Enzymes in Hepatocytes
In vivo Method 1	Recovery of Enzyme Activity After Enzyme Induction
In vivo Method 2	Recovery of Enzyme Activity After Mechanism-Based Inhibition (MBI)
In vivo Method 3	Pharmacokinetic Modeling of Auto-Induction

Hepatic CYP3A4 k_{deg} values – *in vitro* versus *in vivo*

Enzyme	Method	n	$t_{1/2}$ (h) *	Min	Max	Reference	Kdeg (1/h)
CYP3A4	*In vitro* Method 1	1	44	ns	ns	68	0.0158
CYP3A4	*In vitro* Method 2	NC	26	ns	ns	10	0.0267
CYP3A4	*In vitro* Method 2	4	79	ns	ns	74	0.0088
CYP3A4	*In vivo* Method 1	15	72	ns	ns	87	0.0096
CYP3A4	*In vivo* Method 3	6	96	53	154	140	0.0072
CYP3A4	*In vivo* Method 1	7	72	20	146	141	0.0096
CYP3A4	*In vivo* Method 1	3	ns	85	806	142	-
CYP3A4	*In vivo* Method 1	8	ns	36	50	143	-
CYP3A4	*In vivo* Method 3	13	10	2	158	144	0.0693
CYP3A4	*In vivo* Method 3	35	94	62	205	100	0.0074
CYP3A4	*In vivo* Method 3	7	70	ns	ns	101	0.0099
CYP3A4	*In vivo* Method 3	16	85	24	146	145	0.0082
CYP3A4	*In vivo* Method 3	6	140	48	284	146	0.0050

k_{deg} = 0.0267 h^{-1}
Reflects shorter half-life and quicker recovery of enzyme
Predicted DDIs tend to be lower

k_{deg} = 0.0077 h^{-1}
Reflects longer half-life and slower recovery of enzyme
Predicted DDIs tend to be higher

NC-not clear; ns – not stated

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Hepatic CYP3A4 k_{deg} value – Fromm *et al.* 1996

Differential Induction of Prehepatic and Hepatic Metabolism of Verapamil by Rifampin

MARTIN FRIEDRICH FROMM, DAGMAR BUSSE, HEYO KLAUS KROEMER, AND MICHEL EICHELBAUM

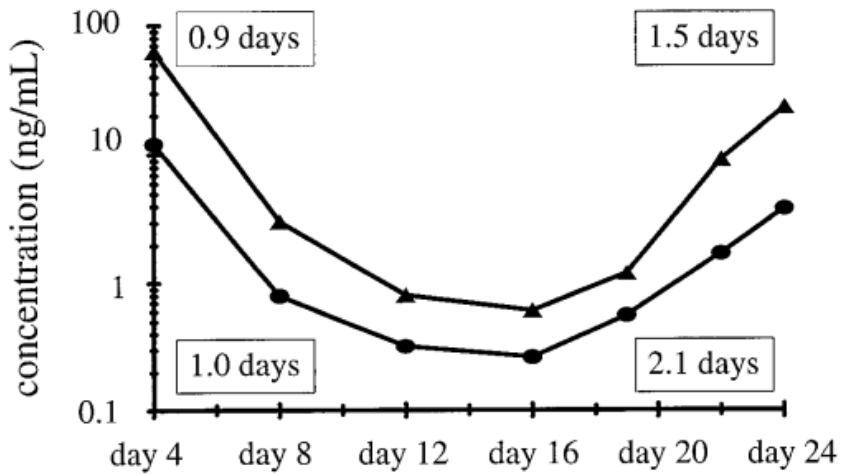

FIG. 2. Mean S-(●) and R-verapamil (▲) trough levels before (day 4), during (day 8 to day 16), and after induction (day 19 to day 24) with rifampin. Half-life of induction (day 4 to day 8) and half-life of decrease in enzyme activity (day 16 to day 24) are given for both enantiomers.

remained constant for the subsequent 8 days of rifampin treatment. After rifampin administration was stopped (day 16), verapamil trough levels slowly increased. The half-life of decrease in enzyme activity (most likely CYP3A4) averaged 1.5 to 2.1 days. These *in vivo* data are in agreement with *in vitro* experiments in human hepatocytes reporting a half-life for CYP3A in hepatocytes of 1.3 to 1.8 days.[47]

$t_{1/2}$ = 1.5 days=36 hours
k_{deg}=0.0193 h^{-1}

Hepatic CYP3A4 k_{deg} value – 0.0077 h^{-1} *versus* 0.0193 h^{-1}

Prediction of time-dependent CYP3A4 drug–drug interactions by physiologically based pharmacokinetic modelling: Impact of inactivation parameters and enzyme turnover

K. Rowland Yeo [a,*], R.L. Walsky [d], M. Jamei [a], A. Rostami-Hodjegan [a,c], G.T. Tucker [a,b]

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[b] Clinical Pharmacology, University of Sheffield, Sheffield S10 2JF, UK
[c] School of Pharmacy and Pharmaceutical Sciences, Faculty of Medical and Human Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK
[d] Pharmacokinetics, Dynamics and Drug Metabolism, Pfizer Inc., Groton, CT, USA

- Inactivation parameters determined anew in a single laboratory under standardised conditions.
- The conversion of midazolam to its 10 hydroxy metabolite (1'OH midazolam) by human liver microsomes was used as a marker of CYP3A4 activity.

$$k_{obs} = k_{obs[0\mu M]} + \frac{k_{inact} \times [I]}{K_I + [I]} \quad (3)$$

where [I] represents the concentrations of the test drug in the primary incubation, k_{inact} is the maximal inactivation rate, and K_I is the inactivator concentration at half k_{inact}.

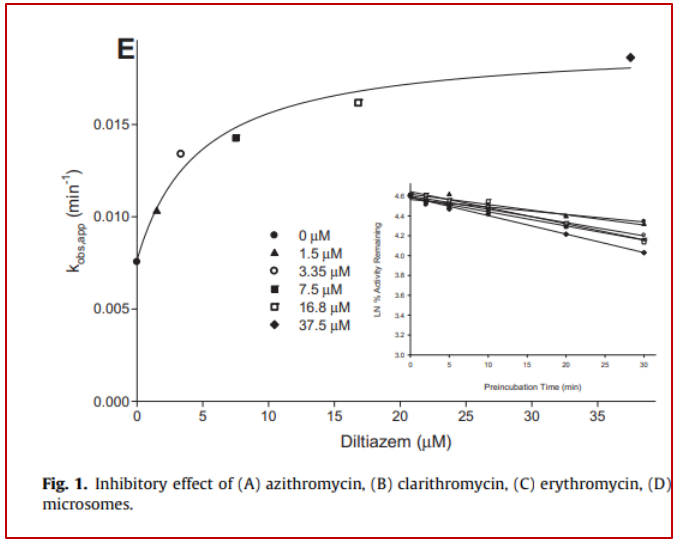
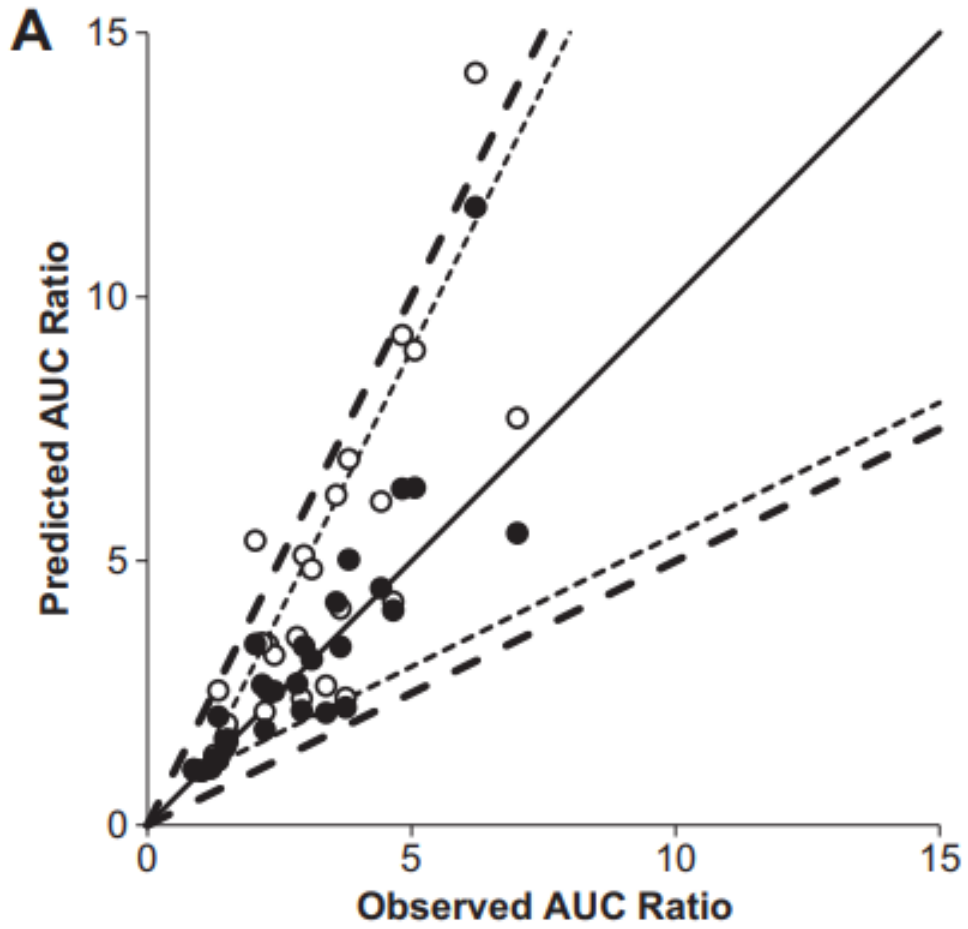


Fig. 1. Inhibitory effect of (A) azithromycin, (B) clarithromycin, (C) erythromycin, (D) microsomes.

Hepatic CYP3A4 k_{deg} value - 0.0193 h^{-1}



- Hepatic k_{deg} value of 0.0193 h^{-1} ($t_{1/2}$ – 36 h) can be used in conjunction with inactivation parameters determined by a standardised conventional experimental approach i.e. dilution approach.

○ k_{deg}=0.0077h^{-1}
● k_{deg}=0.0193 h^{-1}

Hepatic CYP3A4 k_{deg} value - 0.0193 h^{-1} - 2021

Static and Dynamic Projections of Drug-Drug Interactions Caused by Cytochrome P450 3A Time-Dependent Inhibitors Measured in Human Liver Microsomes and Hepatocytes[S]

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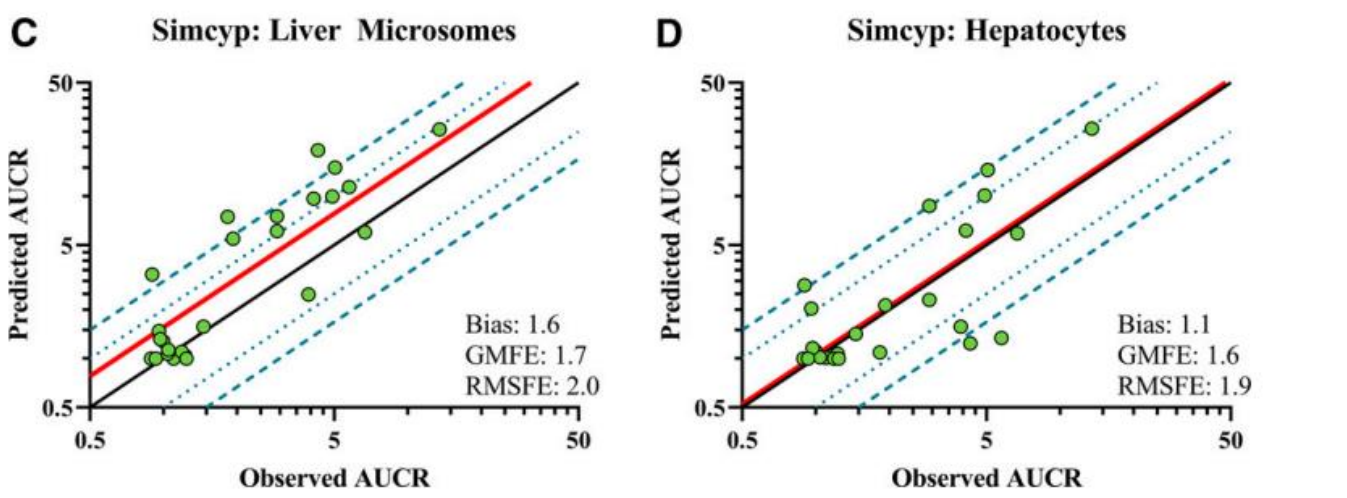


Source		HLM		
		KI	kinact	kinact/KI
		uM	h-1	h-1/uM
Simcyp	Clarithromycin	12.00	2.13	0.18
Simcyp	Diltiazem	4.75	0.70	0.15
Simcyp	NDM	1.74	1.09	0.63
Simcyp V19	Erythromycin	23.20	2.25	0.10
Simcyp	Verapamil	2.21	2.00	0.90
Tseng	Clarithromycin	36.20	4.87	0.13
Tseng	Diltiazem	1.32	0.65	0.50
Tseng	NDM	0.96	0.57	0.60
Tseng	Erthromycin	14.30	3.34	0.23
Tseng	Verapamil	1.71	2.92	1.71

Hepatic k_{deg} values for non-CYP3A4

Projections of Drug-Drug Interactions Caused by Time-Dependent Inhibitors of Cytochrome P450 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 Using In Vitro Data in Static and Dynamic Models[S]

Elaine Tseng, Jian Lin, Timothy J. Strelevitz, Ethan DaSilva, Theunis C. Goosen, and R. Scott Obach

TABLE 2

Total and free inhibition parameters determined in human liver microsomes

		Reversible Inhibition			Time-Dependent Inhibition				
P450	Drug Name	K_i[a] (μM)	$cf_{u,mic}$ (0.03 mg/ml)[b]	$K_{i,u}$ (μM)	K_I (S.E.) (μM)	$cf_{u,mic}$ (0.3 mg/ml)[b]	$K_{I,u}$ (μM)	k_{inact} (S.E.) (min^{-1})	$k_{inact}/K_{I,u}$ ($ml \cdot min^{-1} \cdot \mu mol^{-1}$)
1A2	Furafylline	16.5	0.990	16.3	2.81 (0.45)	0.910	2.56	0.175 (0.009)	68.4
	Zileuton	16.4	0.997	16.3	87.0 (16.6)	0.968	84.18	0.105 (0.006)	1.20
	Ticlopidine	4.93	0.925	4.56	21.0 (6.9)	0.554	11.63	0.0219 (0.0028)	1.90
	Isoniazid	194	0.998	193	ND	0.976	ND	ND	0.150 (0.006)[d]
	Rofecoxib	0.345	0.985	0.340	1.16 (0.23)	0.867	1.01	0.0566 (0.0032)	56.3
	Fluvoxamine	0.0110	0.971	0.011	0.0514 (0.0162)	0.768	0.039	0.0661 (0.0066)	1670
2B6	Ticlopidine	0.162	0.925	0.150	0.352 (0.051)	0.554	0.195	0.354 (0.012)	182
	Clopidogrel	0.201	0.954	0.191	0.122 (0.039)	0.673	0.082	0.243 (0.0204)	2960
2C8	Gemfibrozil acyl glucuronide	13.1	0.985	12.86	18.9 (6.0)	0.869	16.427	0.214 (0.024)	13.0
2C9	Tienilic acid	0.118	0.997	0.117	0.715 (0.184)	0.974	0.697	0.257 (0.017)	369
	Amiodarone	3.77	0.178	0.671	6.91 (4.11)	0.021	0.146	0.00772 (0.00157)	52.8
	Mifepristone	2.14	0.850	1.82	Not TDI	0.361	Not TDI	Not TDI	Not TDI
	Fluvoxamine	2.71	0.971	2.63	Not TDI	0.768	Not TDI	Not TDI	Not TDI
2C19	Ticlopidine	0.165	0.788[c]	0.130	1.09 (0.26)	0.427[c]	0.465	0.114 (0.009)	245
	Esomeprazole	1.29	0.934[c]	1.21	1.78 (0.29)	0.738[c]	1.314	0.0524 (0.0027)	39.9
	Fedratinib	>50.0	0.182[c]	9.12	19.1 (5.1)	0.043[c]	0.816	0.0212 (0.0021)	26.0
	Osilodrostat	4.45	0.973[c]	4.33	15.9 (7.32)	0.879[c]	13.969	0.00673 (0.00084)	0.500
	Fluvoxamine	0.129	0.909[c]	0.117	Not TDI	0.665[c]	Not TDI	Not TDI	Not TDI
2D6	Paroxetine	0.198	0.735	0.145	0.615 (0.130)	0.217	0.133	0.157 (0.008)	1180
	MDMA (CS)	1.92	0.991	1.89	15.4 (1.9)	0.917	14.125	0.444 (0.024)	31.4
	Amiodarone	13.1	0.178	2.32	9.76 (5.50)	0.021	0.207	0.00925 (0.00146)	44.7
	N-desethylamiodarone	1.16	0.190	0.220	2.74 (1.12)	0.023	0.063	0.0132 (0.0014)	210
	Hydralazine	>50.0	0.998	>49.9	Not TDI	0.979	Not TDI	Not TDI	Not TDI
	Mirabegron	1.63	0.985	1.61	5.61 (1.77)	0.866	4.857	0.11 (0.009)	22.6
	Dronedarone	0.351	0.083	0.029	Not TDI	0.009	Not TDI	Not TDI	Not TDI

CERTARA

Hepatic k_{deg} values for non-CYP3A4

	kdeg values (h^{-1})		
Enzyme	Default V19	Tseng (2021) V19	Tseng (2024) V20
CYP1A2	0.0183		0.0183
CYP2C8	0.0301		0.0301
CYP2C9	0.0067		0.0067
CYP2C19	0.0267		0.0267
CYP2D6	0.0099		0.0217
CYP3A4	0.0193	0.0193	

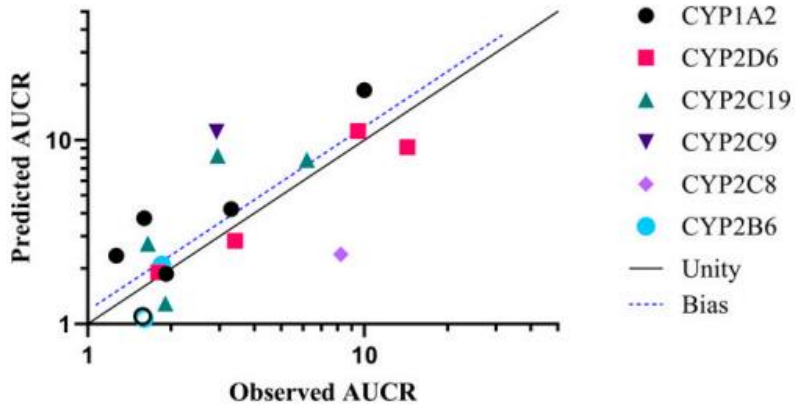


Fig. 2. Predicted versus observed AUC ratios from Simcyp modeling. Results using human liver microsome–generated inactivation parameters. Solid black lines represent unity; dotted lines represent the bias. Open symbols represent underpredictions compared with observed.

TABLE 4

Numerical accuracy of DDI predictions using Simcyp

Performance	Human Liver Microsomes
Bias (CI90%)	1.13 (0.91–1.4)
GMFE (CI90%)	1.61 (1.4–1.8)
RMSFE	1.83
% within 2-fold	80
% within 3-fold	90
% outside 10-fold	0

Conclusions (Issue #2 – k_{deg} values)

- Values of k_{deg} for CYP enzymes are variable depending on the method used to determine them.
- Along with our analysis and the simulation results published by Tseng *et al.* (2021) and Tseng *et al.* (2024), the k_{deg} values presented below give reasonable predictions, when used in combination with inactivation parameters generated *in vitro* in HLM (or HH) using the standard dilution approach.

Enzyme	V19 t1/2 (h)	V19 kdeg (h^{-1})	Source references	ICH M12 t1/2 (h)	ICH M12 kdeg (h^{-1})	Source references
CYP1A2	38.5	0.0183	1,2	38	0.018	5
CYP2C8	23	0.0301	2	22	0.0318	6
CYP2C9	104	0.0067	2	104	0.0066	2
CYP2C19	26	0.0267	2	26	0.0264	2
CYP2D6	70	0.0099	2	51	0.0138	7,8
CYP3A4	36	0.0193	3	36	0.0192	3
CYP3A4gut	24	0.03	4	24	0.0288	4

Issue 3 – Ki values

Please explain how* Ki values lower than the lowest reported in vitro values have been selected for the *PBPK model without optimisation of these parameters. There is a high variability between the median reported in vitro values and the Ki values used in Simcyp: reported values are on average 40-fold higher with a range of 0.3-690-fold. Please, discuss how in vitro data can be used for competitive inhibition. Should a positive control be included in the in vitro assays?

- *Relevant excerpt from the scientific discussion:* For application of PBPK model, it is preferred that the Ki values are determined using the same standardised *in vitro* method for a rank order approach of a new drug as reversible inhibitor of multiple enzymes. There is more uncertainty when the Ki values are obtained from literature from various sources. The Applicant admits that the uncertainty and its relevance will vary on a case-by-case basis. **Therefore, the in vitro data for the compounds qualified should be included in the public qualification report.** Moreover, for a new drug a clinical study with a sensitive substrate / strong inhibitor will always be necessary to verify in vitro parameters.

Lower Ki values than reported range - UOW

Two possible reasons why Simcyp Ki values fall below the lowest reported *in vitro* values for perpetrators.

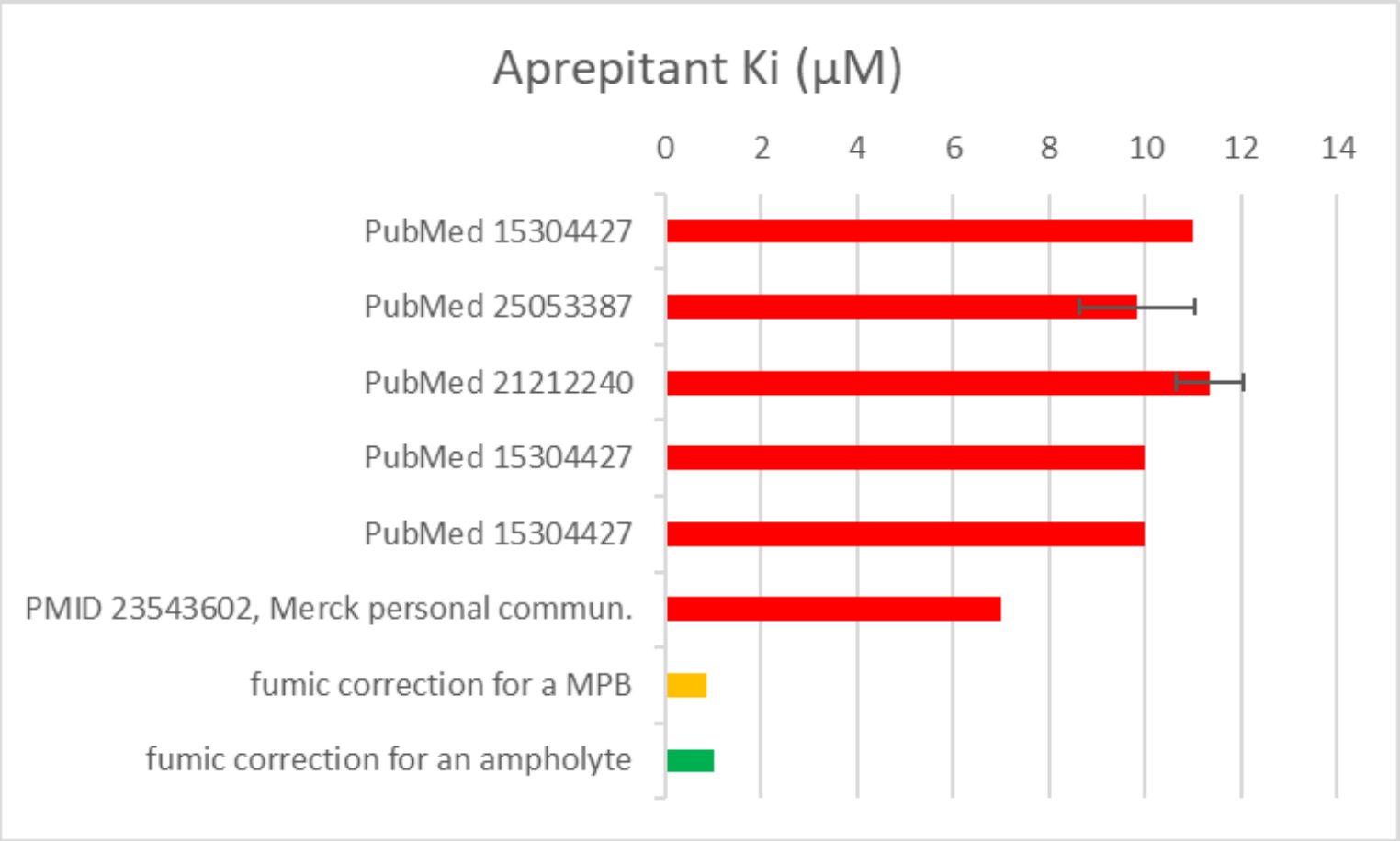
- Firstly, Simcyp Ki values could have been optimised to capture clinical DDI data.
- Secondly, Ki values could have been corrected for non-specific microsomal binding (NSMB) which may occur in incubations during experimental determination of the inhibitory potency of the perpetrator.
  - Ki values reported in the CDID (formerly UOW database) are not always corrected for NSMB (by application of fu_{mic}) and may reflect Ki not Ki,u (fu_{mic} x Ki) values.

Case Study 1 - Aprepitant

Inhibitor		FDA Classification		$K_{i,u}$	$K_{i,u}$	fumic	fumic	nge of Ki values in vit			reported values		median reference Ki/Simcyp Ki
				(µM)	source		source	Min (µM)	Max (µM)	fold	n	median (µM)	
Aprepitant	CYP3A4	Moderate		1	**In vitro** - Incorporating fumic recalculated in Simcyp as ampholyte compound type (Prueksaritanont et al., 2013, PMID: 23543602)	1	default	9.82	11.34	1.2	5	10	10.0

- The Simcyp Ki value, once corrected for NSMB (fu_{mic}) is 10-fold lower than the median reference value.
- Falls outside the reported range.

Case Study 1 - Aprepitant



- Contacted Merck to confirm the compound type.
- Corrected fu_{mic} value applied to Ki values.
- Median Ki,u value was 1 µM.

Total

fu_{mic} corrected, but the incorrect compound type assumed, hence the acidic pKa was not accounted for in the logP estimation

fu_{mic} corrected, accounting for the basic and acidic pKa values in the logP estimation

Case Study 2 - Paroxetine

Inhibitor		FDA Classification		$K_{i,u}$	$K_{i,u}$	fumic	fumic	nge of Ki values in vit			reported values		median reference Ki/Simcyp Ki
				(µM)	source		source	Min (µM)	Max (µM)	fold	n	median (µM)	
Paroxetine	CYP2D6	**Strong**		0.46	In vitro meta-analyis of 20 HLM values from Otton et al Br J. Clin.Pharmacol (1996)41: 149-156; Otton et al Clin.Pharmacol & Therap (1994)55:2 PI 71 ; Skjelbo et al Br. J. clin. Pharmac. (1992), 34, 256-261; Crewe et al Br J. Clin.Pharmacol (1992)34: 262-265; Hemeryck et al J Clin Psychopharmacol (2000) 20:4 428-434; Fogelman et al EURO PSYCHO PHARMACOLOGY (1999) 20:5 480-490; Hemeryck et al DMD (2001) 29:5 656-663; Belpaire et al Eur J Clin Pharmacol (1998) 54 261-264). --> See 'Paroxetine-Ki' tab	0.2	weighted mean of in vitro fu mic --> See 'Paroxetin e-Ki' tab	0.028	4.5	160.7	33	0.38	0.8

- Simcyp Ki value, corrected for fu_{mic} remains within range.
- 33 reported *in vitro* Ki values; only 8 corrected for fu_{mic}.

Application of fu_{mic}

System	Enzyme	fu_{mic}	Ki,u [uM]	Ki [uM]
HLM	2D6	0.25	0.0175	0.07
HLM	2D6	0.17	0.01105	0.065
HLM	2D6	0.17	0.0612	0.36
HLM	2D6	0.17	0.0255	0.15
HLM	2D6	0.51	0.2754	0.54
HLM	2D6	0.51	0.3111	0.61
HLM	2D6	0.51	0.2754	0.54
HLM	2D6	0.2	0.034	0.17
HLM	2D6	0.09	0.0405	0.45
HLM	2D6	0.09	0.0396	0.44
HLM	2D6	0.09	0.0252	0.28
HLM	2D6	0.09	0.0477	0.53
HLM	2D6	0.09	0.081	0.9
HLM	2D6	0.09	0.0396	0.44
HLM	2D6	0.09	0.117	1.3
HLM	2D6	0.09	0.09	1
HLM	2D6	0.09	0.117	1.3
HLM	2D6	0.09	0.0342	0.38
HLM	2D6	0.09	0.126	1.4
HLM	2D6	0.09	0.108	1.2
	mean=	0.2	0.0876	0.46

For basic compounds

$$fu_{mic} = 1 / [([P]_{mic} \times 10^{0.646 * logP - 2.236}) + 1]$$

fu_{mic} varies because of different microsomal protein concentration [P]

	Ki range before fu_{mic} correction	Ki range after fu_{mic} correction
Min [µM]	0.065	0.01105
Max [µM]	1.4	0.126
fold	21.54	11.4

Application of *in vitro* derived Ki values

- *In vitro* derived Ki values for a new drug should always be corrected for NSMB – Ki,u.
- A positive control is typically included – Ki,u values should be determined.
- Compared against the Ki,u values of positive control if available in Simcyp Simulator V19.
- Scalar can be derived and applied to the Ki,u for the new drug.
- Thus, initial simulations can be run using the *in vitro* Ki,u value and the updated value after application of the scalar.

7.6.1.2 CYP Enzymes Inhibitors/inducers for In Vitro Studies

The enzyme inhibitors and inducers are used to phenotype individual CYP enzymes involved in the drug candidate metabolism *in vitro*. In general, the inhibitors/inducers should be selective at the concentration used. The following tables are provided to help sponsors design *in vitro* studies and to evaluate the interaction potential (Tables 5-7). These tables are not exhaustive, and sponsors can use other inhibitors/inducers with appropriate justification.

Table 5: Examples of inhibitors for CYP enzymes (*in vitro* studies)

CYP Enzyme	Inhibitor
CYP1A2	α-Naphthoflavone, Furafylline*
CYP2B6	Clopidogrel*, Ticlopidine*, Thiotepa*
CYP2C8	Gemfibrozil glucuronide*, Montelukast, Phenelzine*
CYP2C9	Sulfaphenazole, Tienilic acid*
CYP2C19	Loratadine, Ticlopidine*
CYP2D6	Paroxetine*, Quinidine
CYP3A	Azamulin*, Itraconazole, Ketoconazole, Troleandomycin*

** Designated as time dependent inhibitor. When used, those inhibitors should be pre-incubated with the experimental system.*

Contexts of Use Statements

- **COU2** - The Simcyp Simulator (V19 R1) can be used to predict the CYP-mediated inhibitory effect of a drug on the exposure of other CYP substrates administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a sensitive CYP substrate has been conducted (and used to verify the competitive inhibition effect in vivo).

- **COU 4** - For scenarios where no clinical studies have been conducted, the Simcyp Simulator (V19 R1) can be used to predict the CYP-mediated inhibitory effect of a drug on the exposure of relevant CYP substrates (V19 files) to waive a clinical DDI study if a significant interaction is not predicted. Sensitivity analyses on relevant parameters including unbound fraction (fu) and inhibitory potency of the drug should be conducted to assess the risk of predicting a false negative.
  - For fu, the range should be determined by the variability associated with the *in vitro* determination of the parameter.
  - For the inhibitory potency, the range can be determined by a comparison of the inhibitory potency of the positive control from the *in vitro* study versus that of the compound file used in the Simcyp Simulator (V19).

Conclusions (Issue #3 – Ki values)

- A clinical study will be available to optimise the *in vitro* Ki,u value if necessary.
- The optimised Ki,u value can then be applied in simulations for untested scenarios (less-sensitive substrates of the same CYP).

- The scalar derived from the *in vivo* Ki,u value relative to the *in vitro Ki,u* value can be applied to *in vitro* derived Ki,u values for other CYP enzymes if generated using the same experimental conditions.

Issue 4 – Gut versus liver

For CYP3A4, please present differential inhibition of intestinal or hepatic CYP3A4.

- For assessment of differential inhibition in the gut and liver, require studies where a substrate has been given as IV and oral administration
- IV – hepatic CYP3A4 assessment
- Oral – intestinal CYP3A4 assessment - assuming there is no effect on absorption

Table I. Protocol design

CYP3A activity	*Hepatic CYP3A assessment*	*Intestinal CYP3A assessment*
Control (no pretreatment)	1 mg IV MDZ, wait 1 h, then 15 μg/kg IV ALF	3 mg oral MDZ, wait 1 h, then 23 μg/kg oral ALF 3 mg oral MDZ, wait 1 h, then 60 μg/kg oral ALF
Liver and intestine induction (600 mg rifampin daily for 5 d)	1 mg IV MDZ, wait 1 h, then 15 μg/kg IV ALF	3 mg oral MDZ, wait 1 h, then 60 μg/kg oral ALF
Liver and intestine inhibition (500 mg troleandomycin, repeat 6 h later)	1 mg IV MDZ, wait 1 h, then 15 μg/kg IV ALF	3 mg oral MDZ, wait 1 h, then 23 μg/kg oral ALF
Selective intestine inhibition (grapefruit juice at bedtime and next morning)	1 mg IV MDZ, wait 1 h, then 15 μg/kg IV ALF	3 mg oral MDZ, wait 1 h, then 23 μg/kg oral ALF

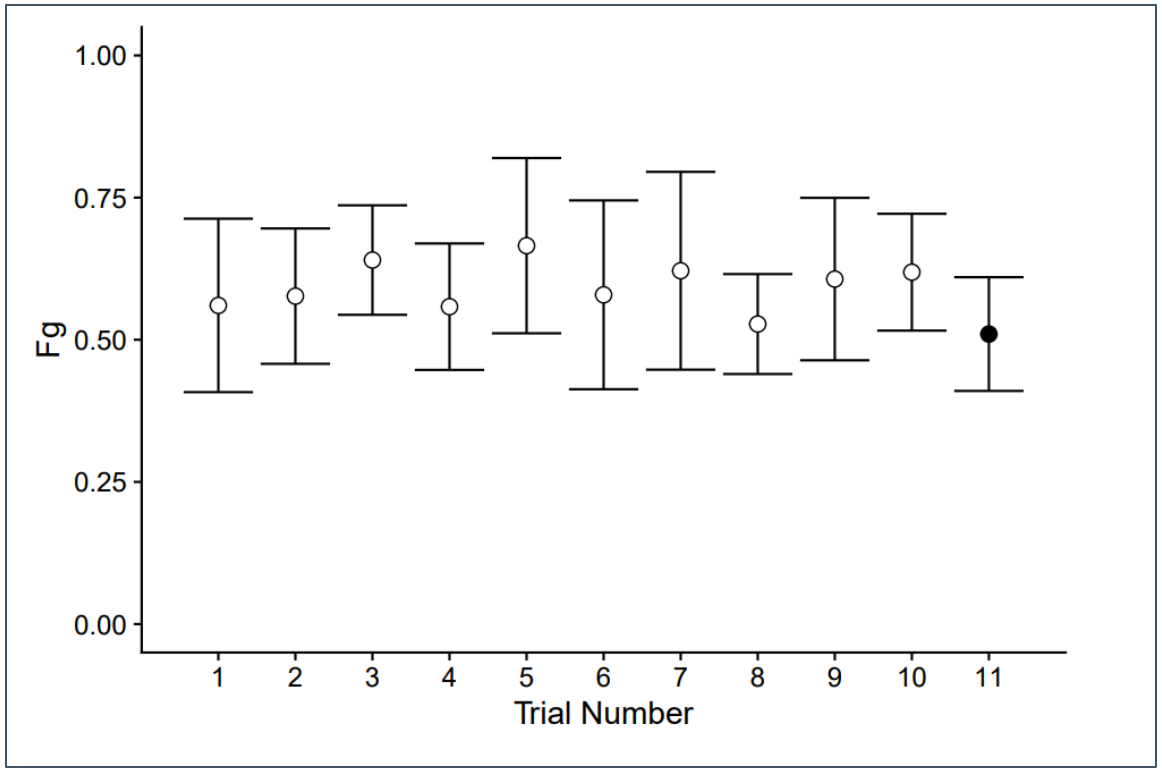
MDZ, Midazolam; IV, intravenous; ALF, alfentanil.

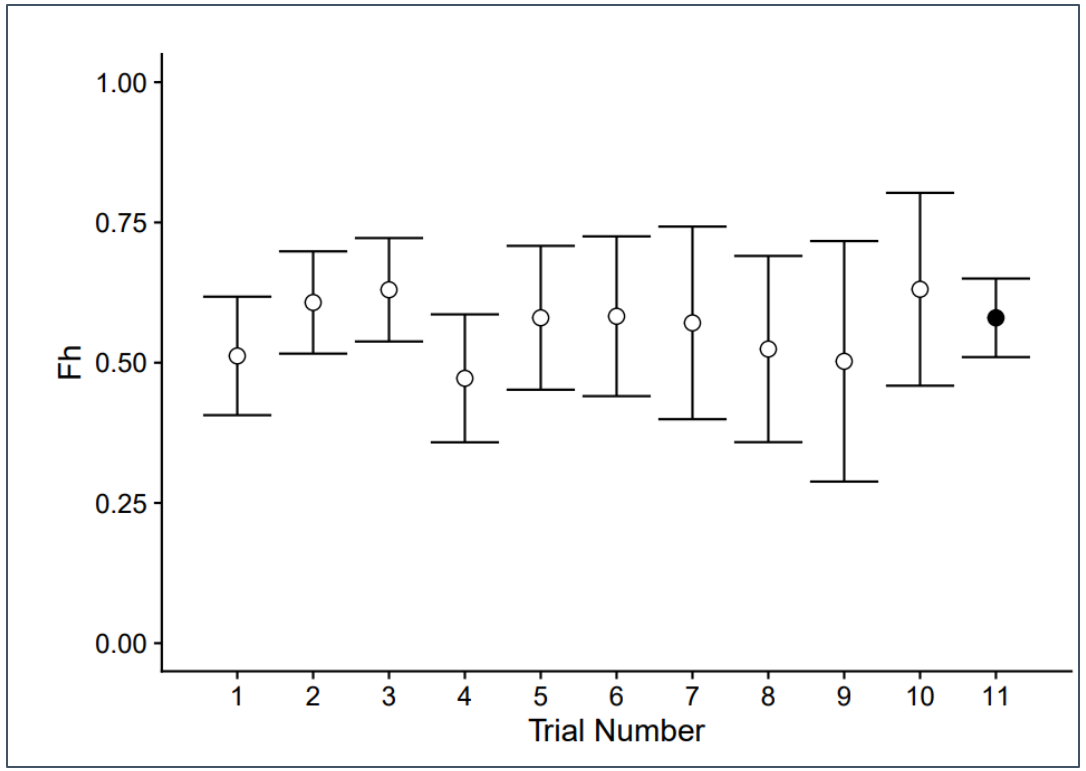
Alfentanil
F=0.42
E_H=0.28
F_G=0.56

Midazolam
F=0.26
E_H=0.52
F_G=0.56

Kharasch *et al.* CPT 2006

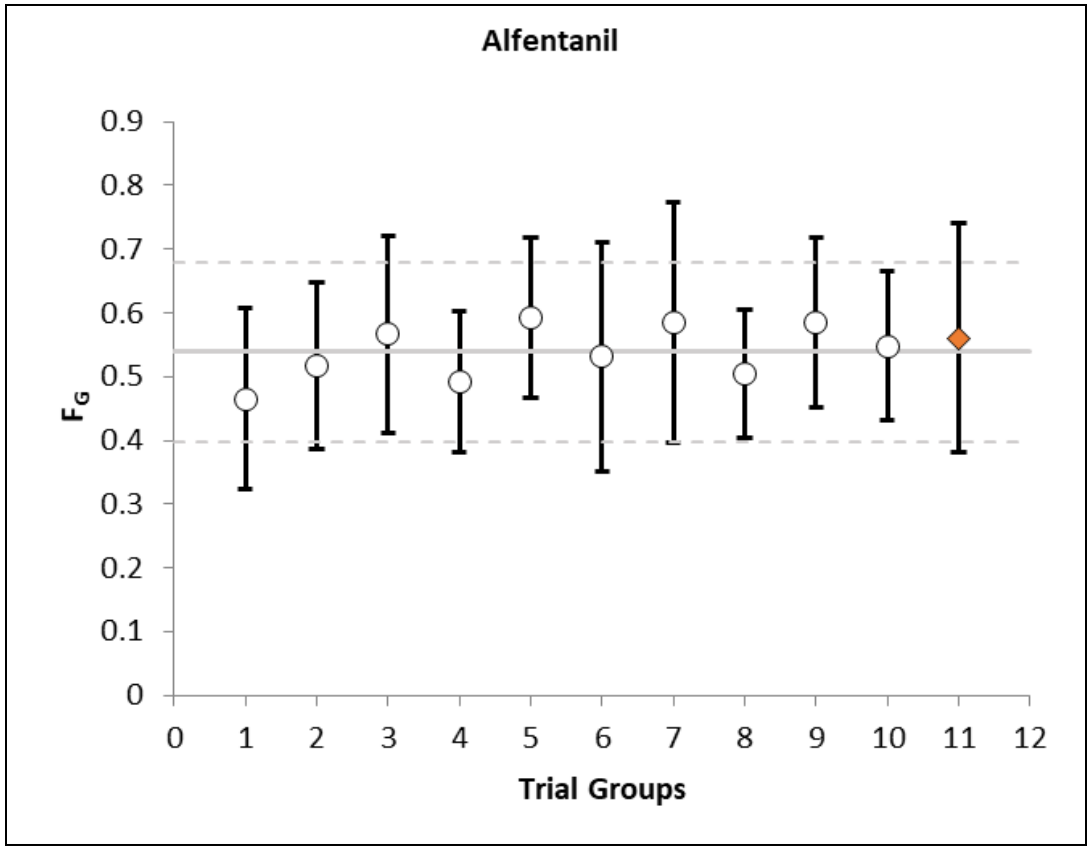
Midazolam compound file – F_G and F_H

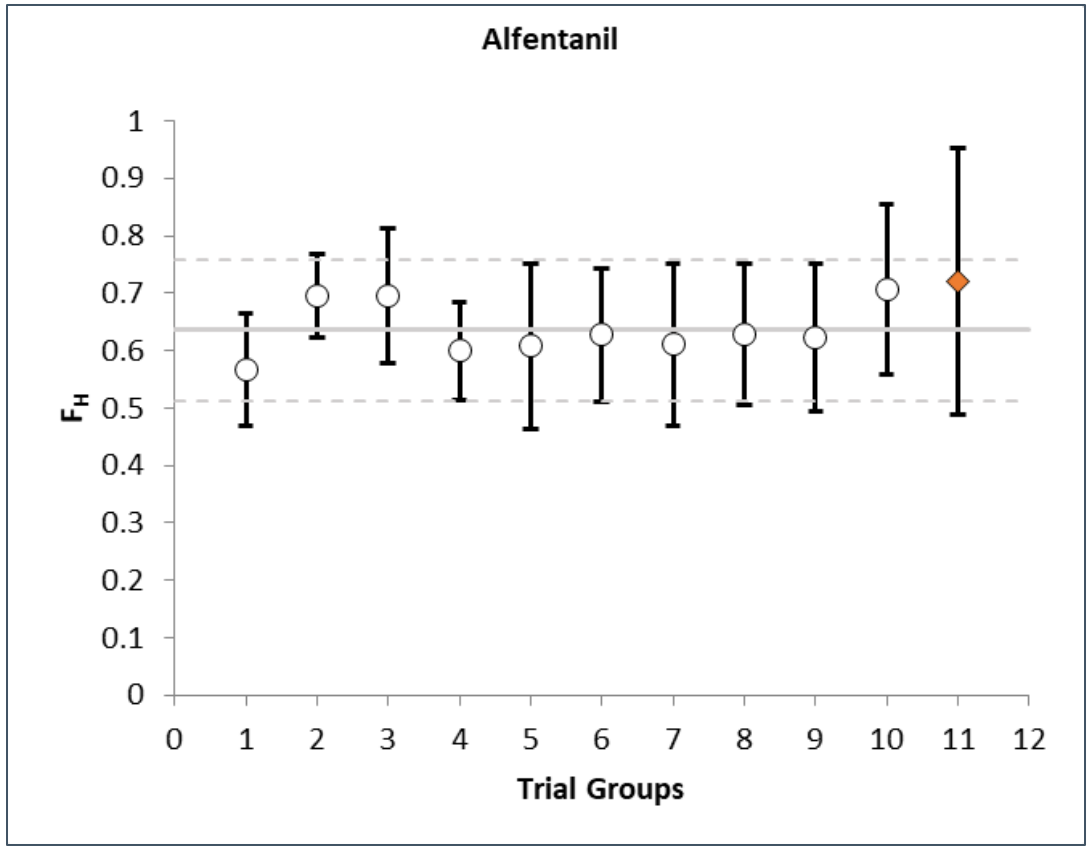




Simulated (mean +/- SD) F_G and F_H values for individual trials and observed data

Alfentanil compound file – F_G and F_H





Simulated (mean +/- SD) F_G and F_H values for individual trials and observed data (orange diamond)

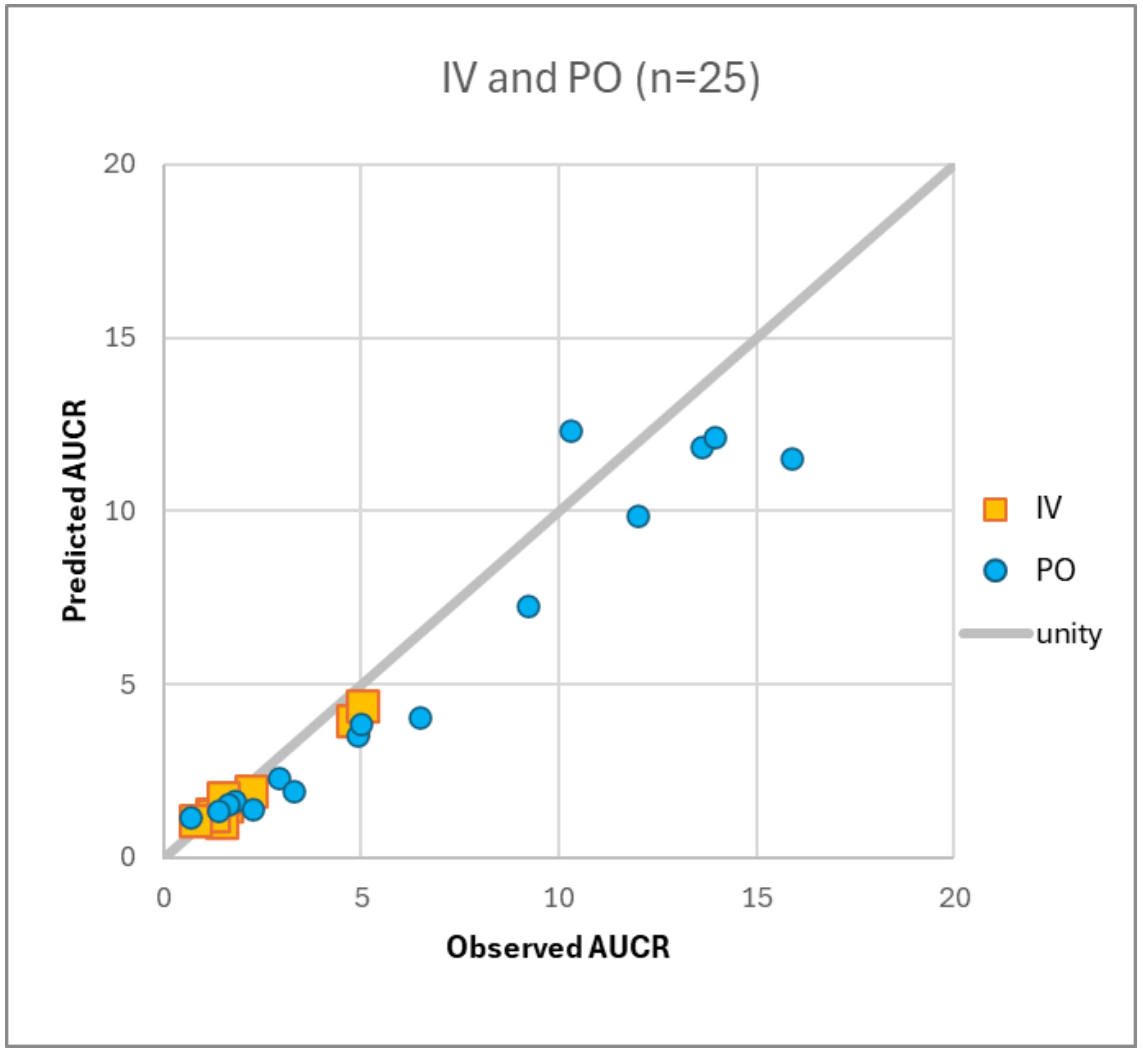
Qualification matrix – DDI studies with CYP3A4 drugs after IV/oral

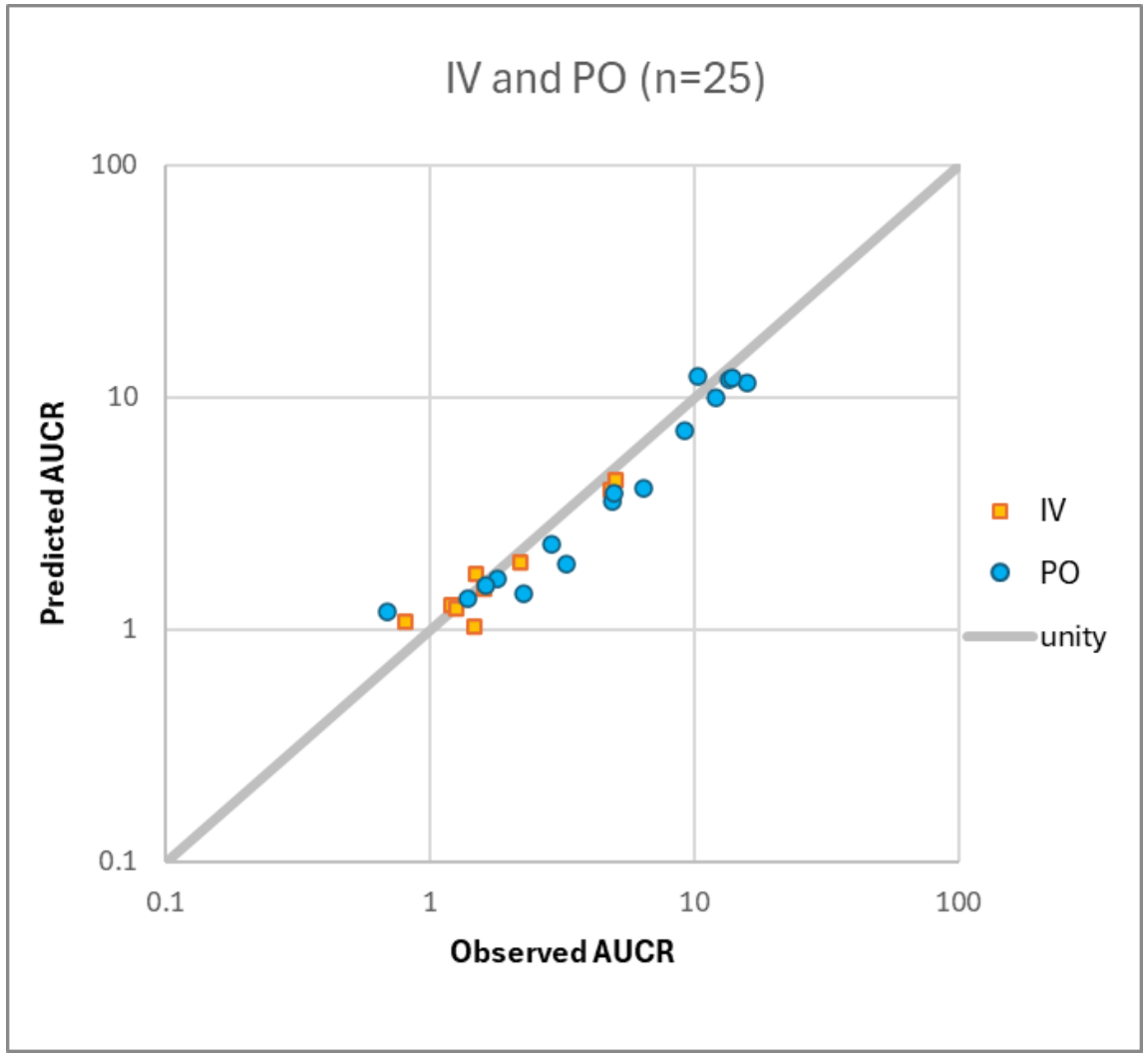
- Our DDI qualification matrix, which was used to simulate 263 clinical studies.
- There were 25 studies involving CYP3A4 drugs with DDI data for IV and oral administration of the substrate; inhibitors were dosed orally.
- Amongst these 25 studies there were 6 substrate/inhibitor pairs (strong and moderate).
- The two substrates were midazolam and alfentanil.

Substrate	Inhibitor	IV	PO	total
Alfentanil	Fluconazole	3	3	6
Alfentanil	Ketoconazole	1	3	4
Midazolam	Ketoconazole	1	5	6
Midazolam	Aprepitant	3	4	7
Midazolam	Fluvoxamine	1	1	2

Predicted versus observed AUC ratios – 25 studies

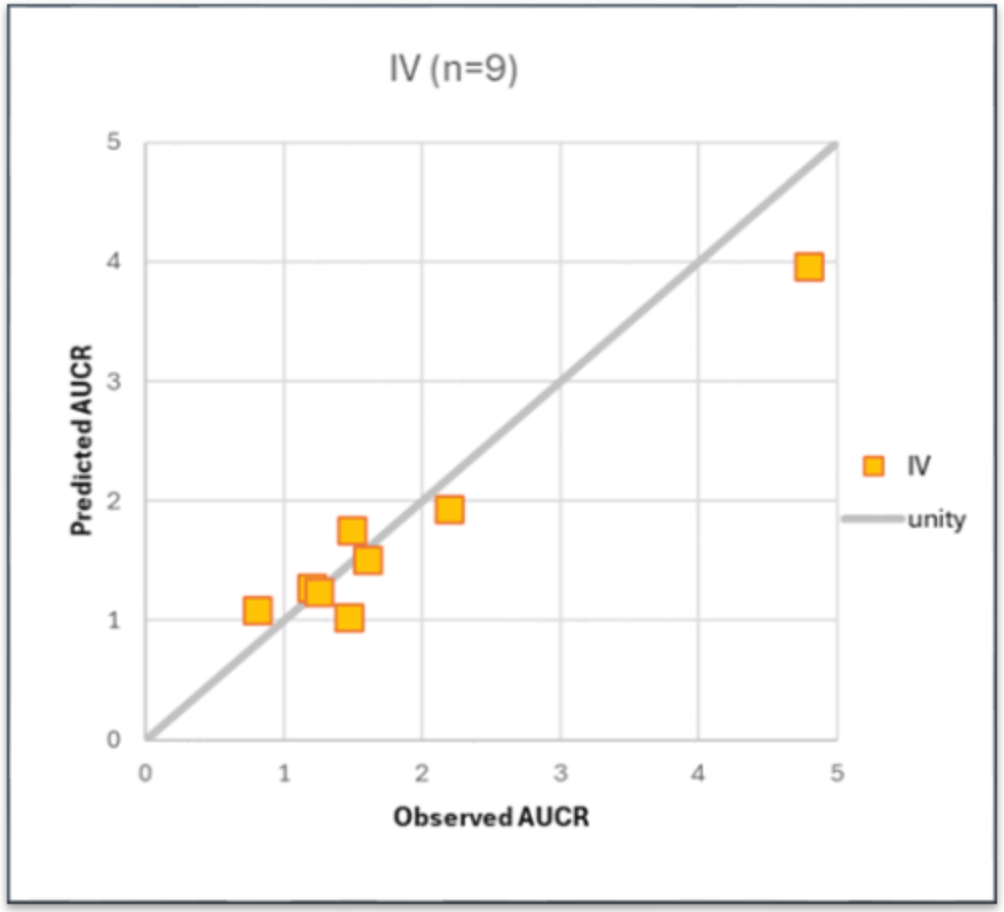
AFE (bias) = 0.90 AAFE (precision) = 1.23 52% within 1.25-fold; 88% within 1.5-fold



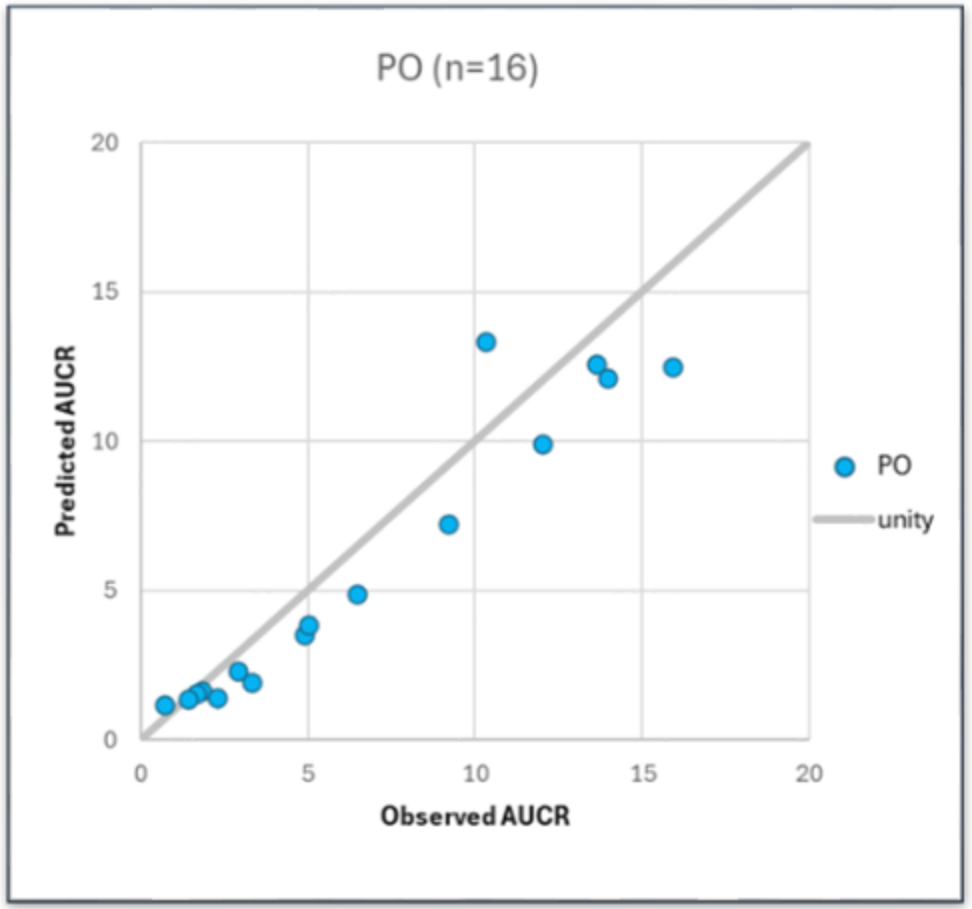


Predicted versus observed AUC ratios – IV/oral

AFE (bias) = 0.97
AAFE (precision) = 1.16
78% within 1.25-fold
100% within 1.5-fold



AFE (bias) = 0.87
AAFE (precision) = 1.28
38% within 1.25-fold
81% within 1.5-fold

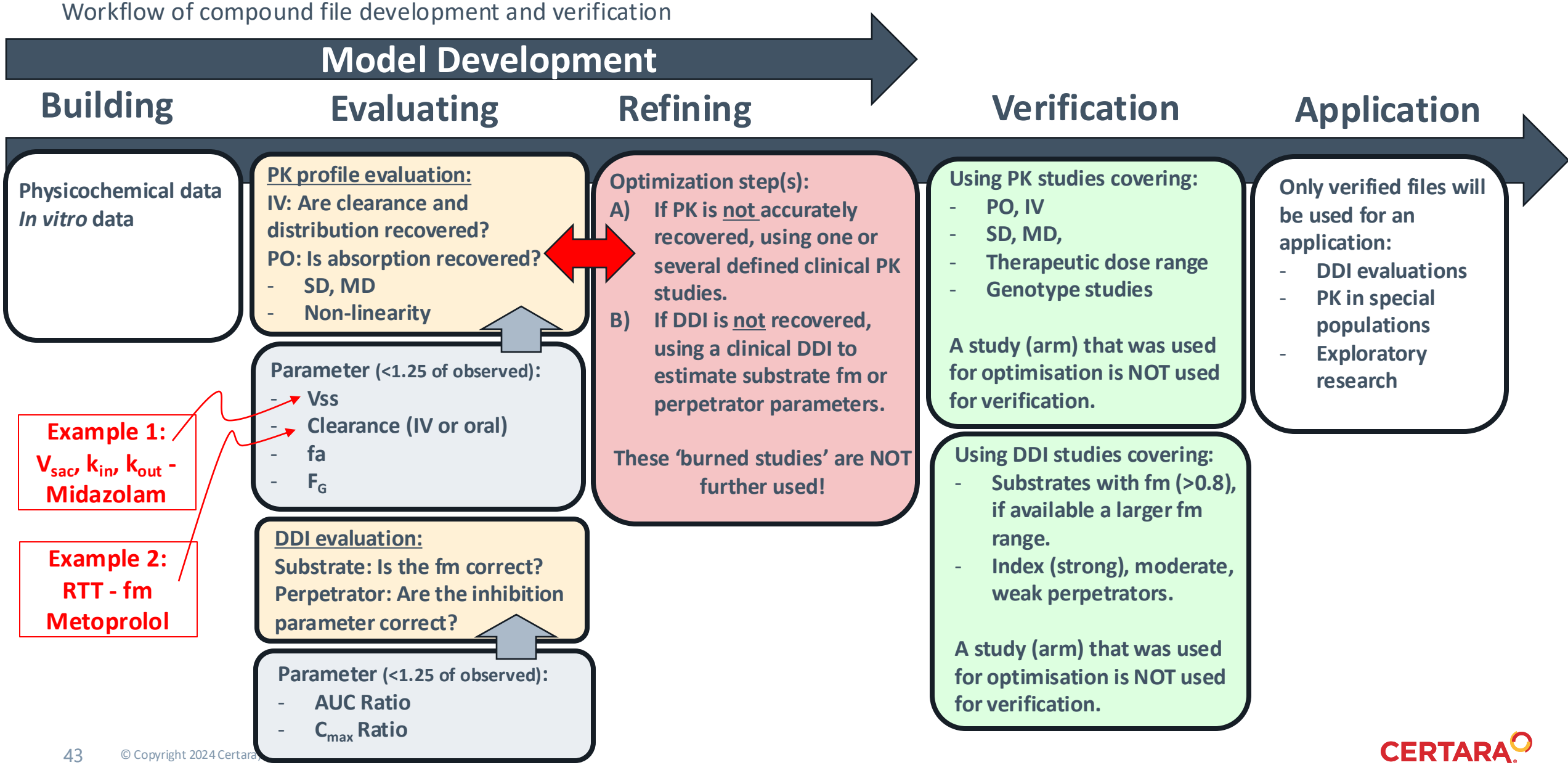


Conclusion (Issue #4 – Gut versus liver)

- The alfentanil and midazolam files that are available in V19 for IV and oral administration can differentiate CYP3A4-mediated inhibition in the liver *versus* gut.
- This was demonstrated using 4 different inhibitors of moderate to strong potency for CYP3A4.

Issue 5 – Optimisation of Parameters

Workflow of compound file development and verification



Midazolam example – recovery of profile

- The compound file summary for midazolam indicates which parameters have been optimised in the development of the file.
- Accurate prediction of CL (following IV and oral administration) from *in vitro* data was demonstrated.
- However, the distribution was not accurately described using the predicted Vss value and the inputs (V_{sac}, k_{in} and k_{out}) had to be optimised to better capture the profile as seen in the extract below and the table of input parameters.

DISTRIBUTION

Following intravenous administration, midazolam is rapidly and widely distributed. In the V15 update to the Sim-Midazolam file, distribution was described using a SAC compartment within the minimal PBPK model. The parameters were fitted using IV data reported by Kupferschmidt et al. 1995. Values of kin = 0.2 1/h, kout = 0.25 1/h, Vsac 0.23 L/kg and Vss = 0.88 L/kg are used in the Sim-Midazolam file. Midazolam is significantly bound to plasma protein (fu 0.032).

Distribution Model	Minimal PBPk model	
Vss (L/kg)	0.88	(Kupferschmidt *et al.*, 1995)
Kin (1/h)	0.2	Derived from (Kupferschmidt *et al.*, 1995)
Kout (1/h)	0.25	Derived from (Kupferschmidt *et al.*, 1995)
V_{SAC} (L/kg)	0.23	Derived from (Kupferschmidt *et al.*, 1995)

Metoprolol example – metabolic intrinsic clearance

- One of the more common *in vitro* parameters that requires optimisation is the metabolic intrinsic clearance obtained from *in vitro* data.
- If the *in vitro* metabolic data ($CLu_{H,int}$) underpredicts the observed CL (for either IV or oral), a retrograde model implemented within the Simcyp Simulator is used to estimate the hepatic intrinsic clearance ($CLu_{H,int}$) from an observed IV (reverse well stirred model) or oral clearance (Equation 1).

$$CL_{H,int} = \frac{Q_H \times CL_{H,met}}{fu_b \times (Q_H - CL_H)}$$

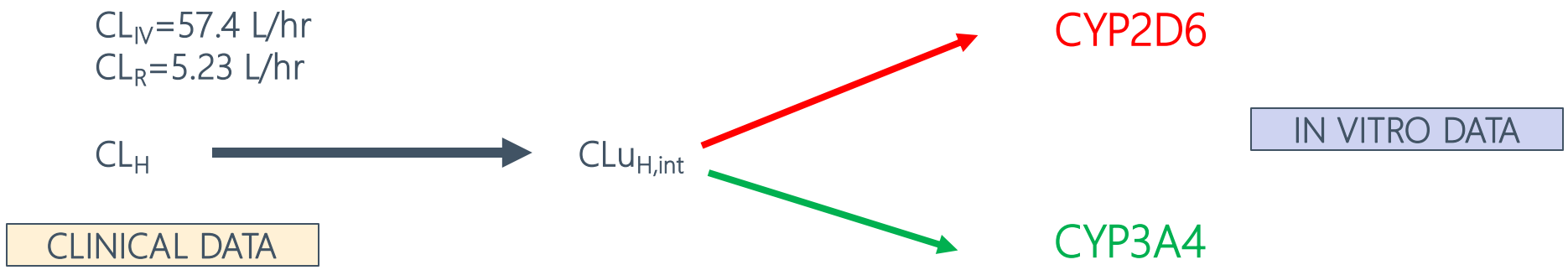
$$CL_{H,met} = \frac{CL_{iv}}{B:P} - \frac{CL_R}{B:P}$$

$$CL_H = \frac{Q_H \times fu_b \times CLu_{int}}{Q_H + fu_b \times CLu_{int}}$$

Where B:P is the concentration ratio of drug in blood to plasma;
fu_b is fraction of unbound drug in blood (calculated from fu_p/B:P);
Q_H is the blood flow in the hepatic vein;
CL_R is the renal clearance;
Uptake is a factor that accounts for any active hepatic uptake.

$$CLu_{H,int} = \frac{\frac{CL_{po}}{B:P} \times fa \times F_G - \frac{CL_R}{B:P}}{Uptake \times fu_b \times \left(1 + \frac{\frac{CL_R}{B:P}}{Q_H}\right)}$$

Metoprolol – compound file summary



Enzyme	CYP2D6	
Pathway	Pathway 1	
CL_{int} (μL/min/pmol)	3.61	Back calculated from meta analysis of CL_{iv} (see text) fm 2D6 from meta analysis of *in vitro* data including Madani *et al.* (1999), Otton *et al.* (1988)
Enzyme	CYP3A4	
Pathway	Pathway 1	
CL_{int} (μL/min/pmol)	0.02	Back calculated from meta analysis of CL_{iv} (see text) fm 2D6 from meta analysis of *in vitro* data including Madani *et al.* (1999), Otton *et al.* (1988)
CL_R (L/h)	5.23	Regardh *et al.* (1974), Hamelin *et al.* (2000)

Optimised Ki values

- Ki values were optimised using clinical DDI studies or *in vitro-in vivo* scalars, if the *in vitro* Ki even after correction for fu_{mic} was not recovering the observed DDI (18/55).

Inhibitor	Enzyme	FDA Classification	$K_{i,u}$ (µM)	$K_{i,u}$ source	fu_{mic}
Cimetidine	CYP1A2	-	4.58	*In vitro-in vivo* scalar for CYP3A4 Ki value applied to CYP1A2 Ki (PMID: 10223772).	1
Ciprofloxacin	CYP1A2	Strong	2	A clinical DDI study with caffeine (PMID: 12908854) was used to derive an optimised CYP1A2 Ki value for ciprofloxacin.	0.92
Fluvoxamine	CYP1A2	Strong	0.002	A clinical DDI study with caffeine using the fluvoxamine 100 mg BID dosage regimen (PMID: 16236038) was used to optimise the CYP1A2 Ki value.	1
Propranolol	CYP1A2	-	0.011	A clinical DDI study with theophylline using the propranolol 40 mg TID dosage regimen (PMID: 4041342) was used to derive an optimised CYP1A2 Ki value for propranolol.	1
Cimetidine	CYP2C19	-	8.2	**In vitro** value from (PMID: 11334262) with an applied *in vitro-in vivo* scalar of 19.2 (CYP3A4 Ki in vitro-in vivo scalar).	1
Fluvoxamine	CYP2C19	Strong	0.006	**In vitro** HLM value lowered 10-fold as 10-fold difference between in vitro and in vivo Ki for fluvoxamine was reported (PMID: 12695344; PMID: 11719727).	1
Voriconazole	CYP2C19	Moderate	0.1	**Optimised** using a DDI with Omeprazole in CYP2C19 EMs (PMID: 33236774); 10-fold reduction from *in vitro* Ki data reported by (PMID: 31853755).	1
Fluvoxamine	CYP2C9	Weak	0.126	Original value derived from meta-analysis of **in vitro** HLM data (PMID: 9384467, PMID: 10192756, PMID: 11719727). Value lowered 10-fold as 10-fold difference between *in vitro* and *in vivo* Ki for fluvoxamine reported (PMID: 12695344; PMID: 11719727).	1
Sulphaphenazole	CYP2C9	-	1.5	**Optimised** with the clinical study (PMID: 2311340).	1
Bupropion	CYP2D6	-	0.084	**Optimised** using DDI with Atomoxetine (PMID: 27518170)	1
OH-Bupropion	CYP2D6	-	0.0532	**Optimised** using DDI with Atomoxetine (PMID: 27518170)	1
Cimetidine	CYP2D6	-	3.5	**Optimised:** manual sensitivity analysis using a clinical DDI with Metoprolol as substrate (PMID: 7176462)	1
Norfluoxetine	CYP2D6	-	0.012	**Optimised t**o same **in vitro** value as fluoxetine (PMID: 11876575) shows that fluoxetine and Nor-fluoxetine are equal.	1
Fluvoxamine	CYP2D6	Weak	0.189	Meta-analysis of **in vitro** HLM data (PMID: 1389951, PMID: 9681670, PMID: 9205822, PMID: 10192828, PMID: 8838442, PMID: 8477556, PMID: 8667235, PMID: 7782485, PMID: 11719727). Value lowered 10-fold as 10-fold difference between in vitro and in vivo Ki for fluvoxamine reported (PMID: 11719727).	1
Duloxetine	CYP2D6	Moderate	0.005	Optimised using (PMID: 12621382).	1
Cimetidine	CYP3A4	Weak	11	**Optimised** using a DDI with Midazolam (PMID: 10223772), PMID: 6137021 was then used to optimize the CYP3A4 Ki value to capture the DDI with triazolam and improve the predictions overall.	1
Fluvoxamine	CYP3A4	Moderate	0.789	10-fold reduction on **in vitro** Ki obtained from a meta-analyse (PMID: 8846621, PMID: 8889898, PMID: 8690825, PMID: 9565774).	1
Fluvoxamine	CYP3A5	Moderate	5.82	10 -fold reduction on **in vitro** Ki (PMID: 11719727).	1

Optimised K_{app} and k_{inact} values

- K_{app} values were optimised using clinical DDI studies, if the *in vitro* K_{app} even after correction for fu_{mic} was not recovering the observed DDI (2/24).

Inhibitor	Enzyme	FDA Classification	K_{app} (µM)	K_{app} source	fu_{mic}
Nor-Fluoxetine	CYP2C19	-	6	Manually optimised to Omeprazole DDI (PMID: 24688155, PMID: 23785064)	1
Fluoxetine	CYP3A4	-	6.9	Optimised as NOT corrected for fu_{mic} (PMID 10950845, PMID: 22490230); Verified with PMID: 14709940	1

- The k_{inact} value for Acyl-clopidogrel was optimised using clinical DDI study, as the *in vitro* k_{inact} was not recovering the observed DDI (1/24).

Inhibitor	Enzyme	FDA Classification	k_{inact} (1/h)	k_{inact} source
Acyl-Clopidogrel	CYP2C8	Moderate	16	Optimised using a DDI between Pioglitazone and Clopidogrel (PMID 27260150). PMID 24971633, reported a value of 2.82 h-1.

Conclusion (Issue #5 – Optimisation of Parameters)

- A clinical study used for refinement of a parameter cannot be used to verify the model.
- The retrograde model (Revers translational tool, RTT) can be used to assign the quantitative elimination pathways (Metoprolol example).
- Competitive interaction parameters require correction for fu_{mic} (See Issue 3).
- If a competitive inhibitor inhibits several enzymes and a significant reduction of the *in vitro* Ki is required towards one of the enzymes, the same *in vitro-in vivo* scalar can be explored for all other enzymes.
- Only a small number of MBI compounds required optimisation of the inactivation parameters in the qualification data set (See Issue 1).

Issue 6 - Simcyp Model-Based Bias and Uncertainty Analyses

Outline

- Introduction
- The data
- Preliminary visual analysis
- The model
- Results
- Conclusions

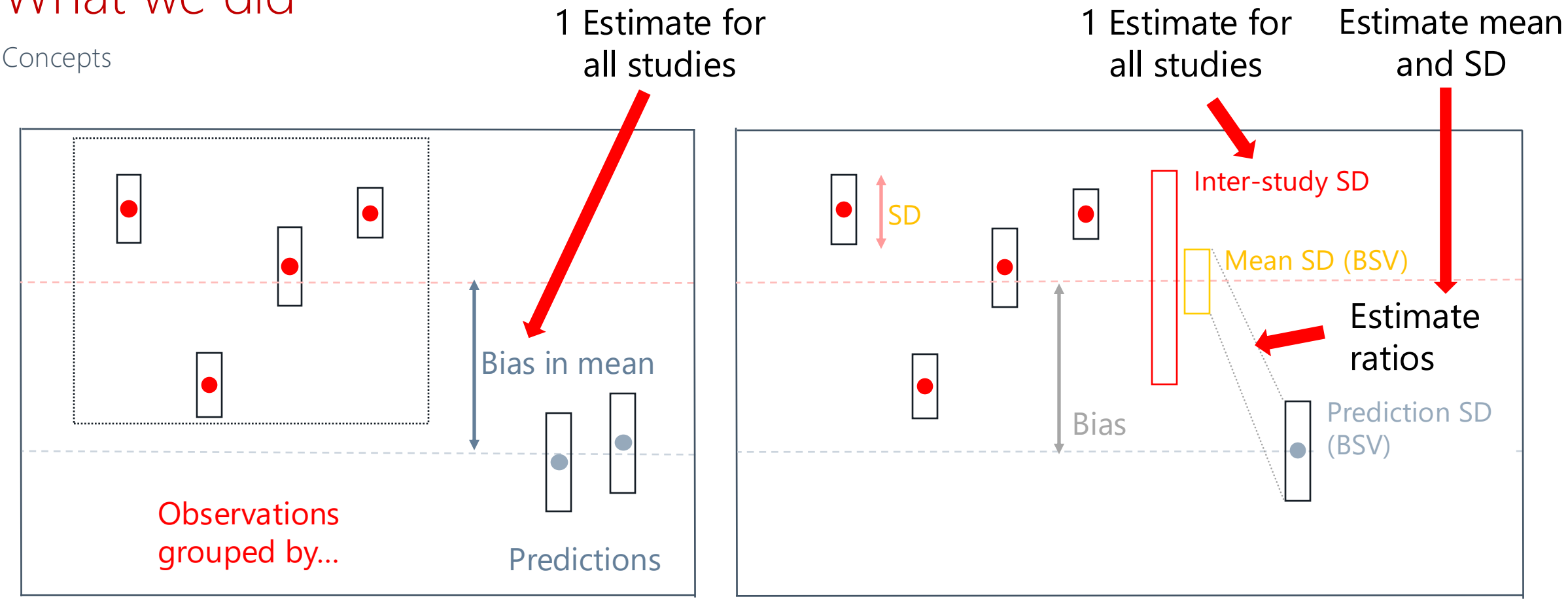
What EMA suggested we look at

Edited for conciseness

- SAWP suggested to further characterise the uncertainty of the model.
- A Bayesian uncertainty quantification model, fitted to the DDI qualification matrix, could overcome some of the limitations of AFE, AAFE…
- For example, a Bayesian meta-regression model on the fold-error with parameters expressing the degree of bias and imprecision for different CYPs…
- A graphical evaluation of this model could help to better understand the accuracy and uncertainty of the DDI predictions across different CYPs.

What we did

Concepts



- Inter-study SD is an upper-bound for imprecision. Imagine that there is no inter-study variability, then Simcyp predictions should be perfect after bias correction. If they are not, it should be because of Simcyp "imprecision". But there is inter-study variability, and we did not estimate it separately, as it would require identifying near-exact replicate studies.

What we did

Details

- We estimated 4 main parameters: a bias of predicted GMR, an inter-study variability (upper bound of imprecision), mean and SD of predicted / observed between-subject variance.
- We stratified and did separate analyses for the data by CYP, and type of inhibition (CI, MBI).
- For any stratum, we estimated the parameters jointly (imprecision requires calculation of GMR bias first).
- The “data” consist in “reported GMRs” and “predicted GMRs”, with the corresponding population geometric SDs.
- The model “bridges” reported and predicted with correction factors that bring them in sync. We want to see those corrections and understand in which cases they are the largest.

The published DDI data

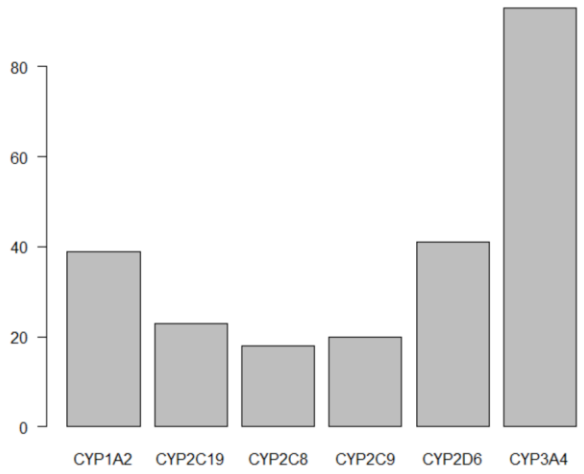
Description

- We selected 268 clinical DDI study reports involving CYP1A2, 2D6, 2C8, 2C9, 2C19, and CYP3A4 from CDIS, with 46 victim drugs and 30 perpetrator drugs.
- They reported:
  - Name of victim drug and route of administration (oral or IV).
  - Name of inhibitor, class (weak, moderate, strong) and route of administration (always oral).
  - Type of inhibition (CI or MBI).
  - Number of study subjects.
  - Whether reported GMR was a ratio of means (RoM) or the mean of individual ratios (MoR).
  - One or several of : arithmetic mean of ratios, GMR, upper and lower CI bounds of GMR, CI coverage (90% or 95%), SD of individual ratios, SE of arithmetic mean of the ratios.

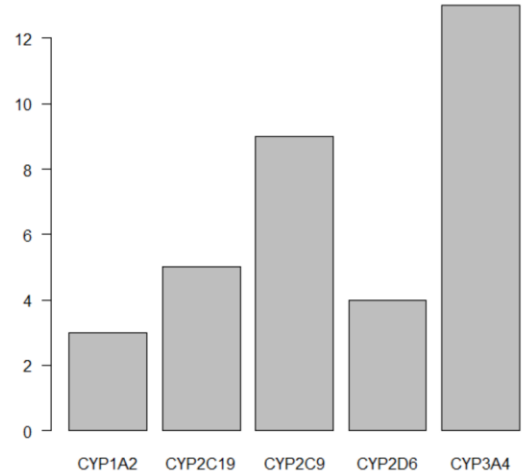
The published DDI data

Pre-treatment

- Data were preprocessed to get GMRs and GSDs:
  - Missing SD of individual ratios: we used GMR and confidence limits to compute it, compute geometric SD of the ratios and their arithmetic mean
  - Removed the 34 studies for which the arithmetic mean or SD of ratios were still missing
  - Compute geometric SDs of ratios still missing
  - Compute GMRs still missing
  - Gives 234 studies with GMRs and GSDs



Kept



Excluded

CERTARA

The Simcyp predicted data

Description and processing

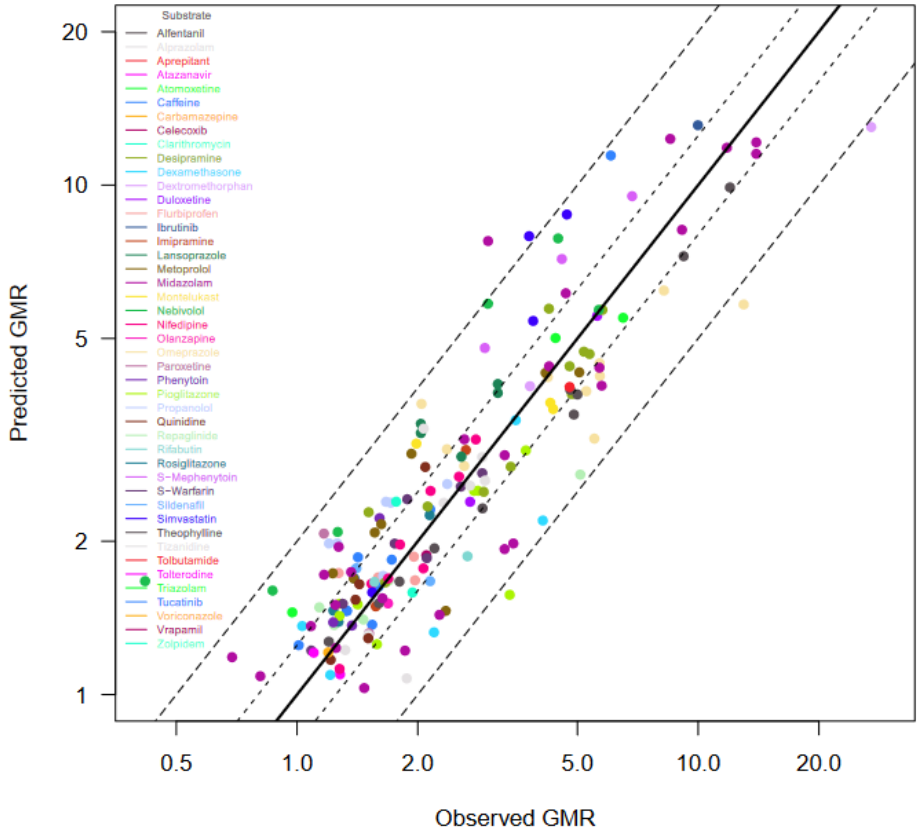
- For each reported studies, we ran Simcyp simulations for cross-over trials with 10 or 20 times the number of subjects:
  - We used the predicted GMRs and the geometric SD of the predicted individual ratios.
  - We checked that the predicted geometric SDs were not null.
  - The predicted ratios have BSV, but no within-subject variability and measurement error.

The next results will show

- Bias in GMR predictions
- Imprecision and inter-study variability
- Between-subject variability estimates

Results 1: Preliminary visual data analyses of GMRs

- We plotted Simcyp-predicted *vs*. observed GMRs and GSDs for all data colored by:
  - Type of inhibition (CI or MBI).
  - CYP enzyme.
  - Number of subjects (less than 7, or more than 6).
  - Type of GMR reported (MoR or RoM).
  - Inhibitor tested.
  - Strength of the inhibitor.
  - Substrate tested.
  - Substrate route of administration.

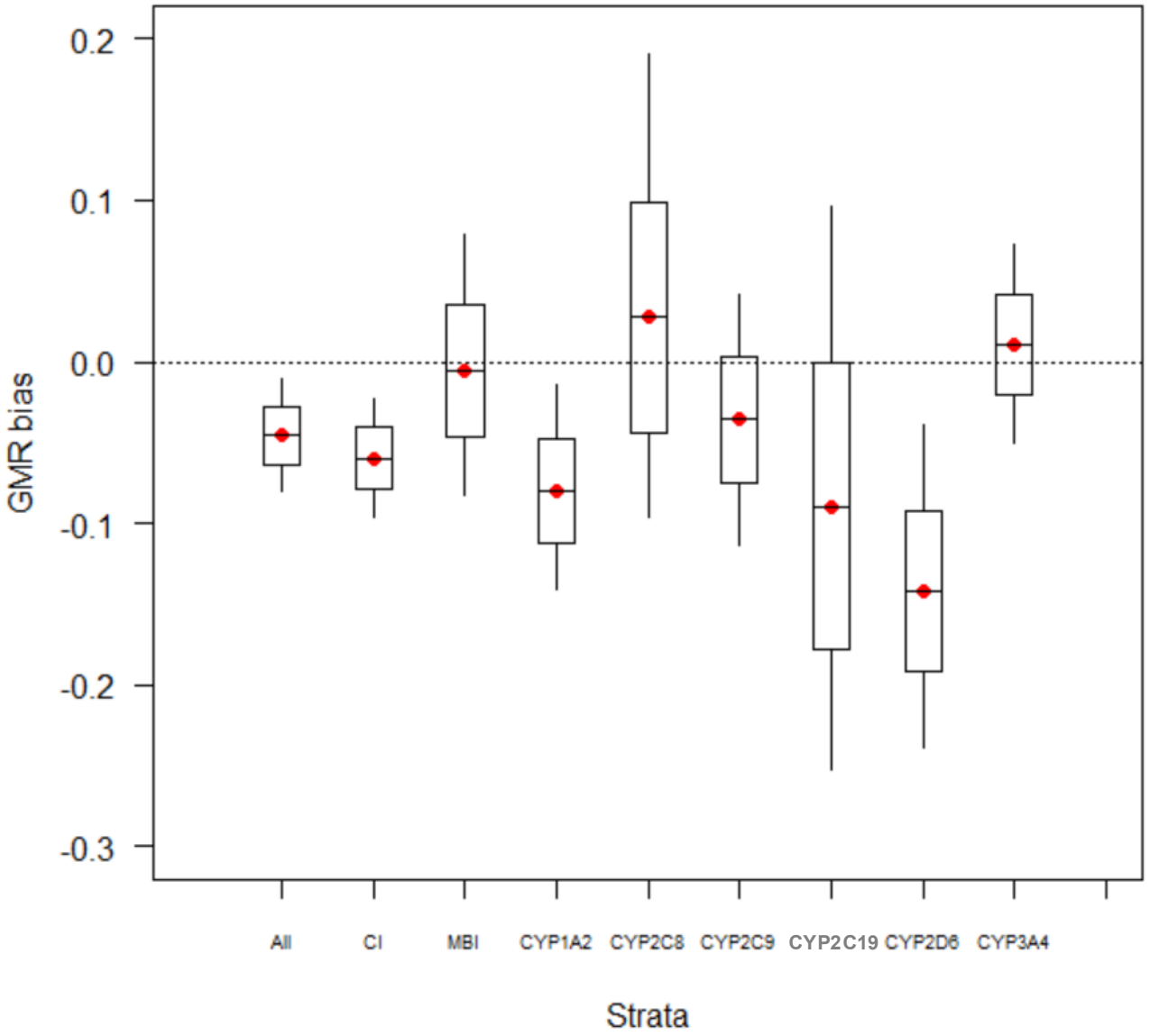


- Deviations do not seem clearly explained by the above covariates.

Results: GMR prediction biases

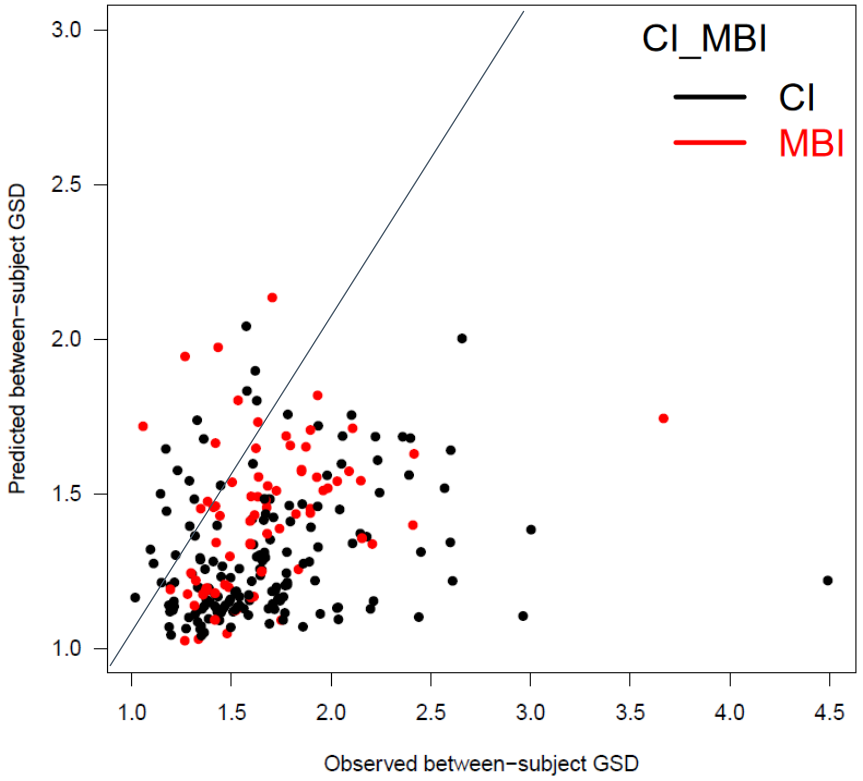
Moderate

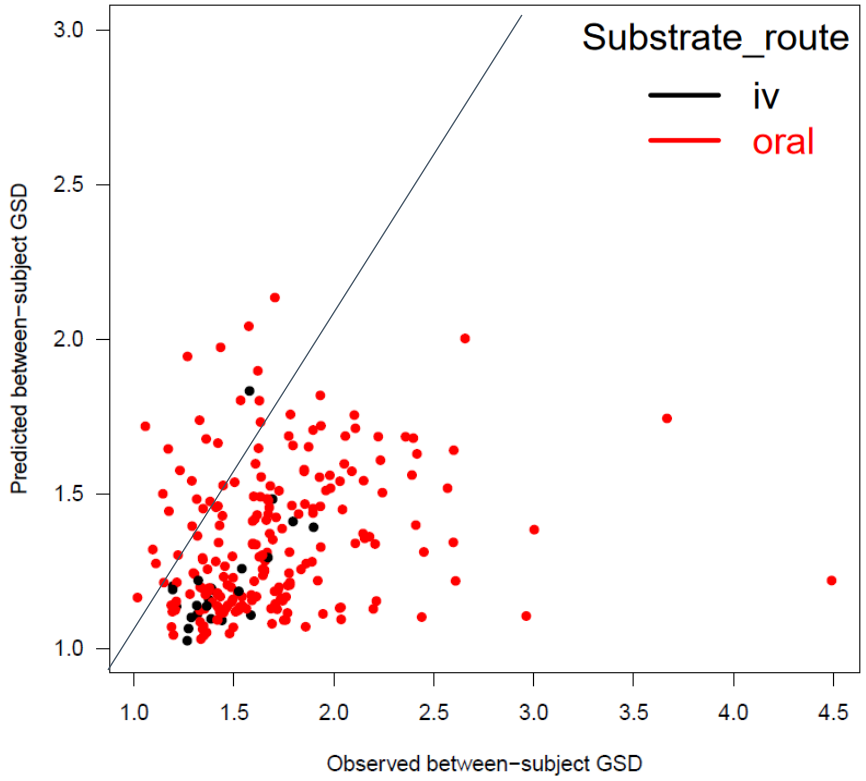
- GMR bias is mostly small and not-significantly different from zero (in 5 of 9 strata). It is significantly different from zero for stata
  - All, CI, and CY1A2, but less than 10%
  - CYP2D6, at minus 14%
- We could do a covariate analysis of bias.



Preliminary visual data analyses of GSDs

- For the geometric SDs of ratios, MBI looks a bit better than CI*, and in the case of substrate administration route: i.v. seems to give less variable and better predicted values†.



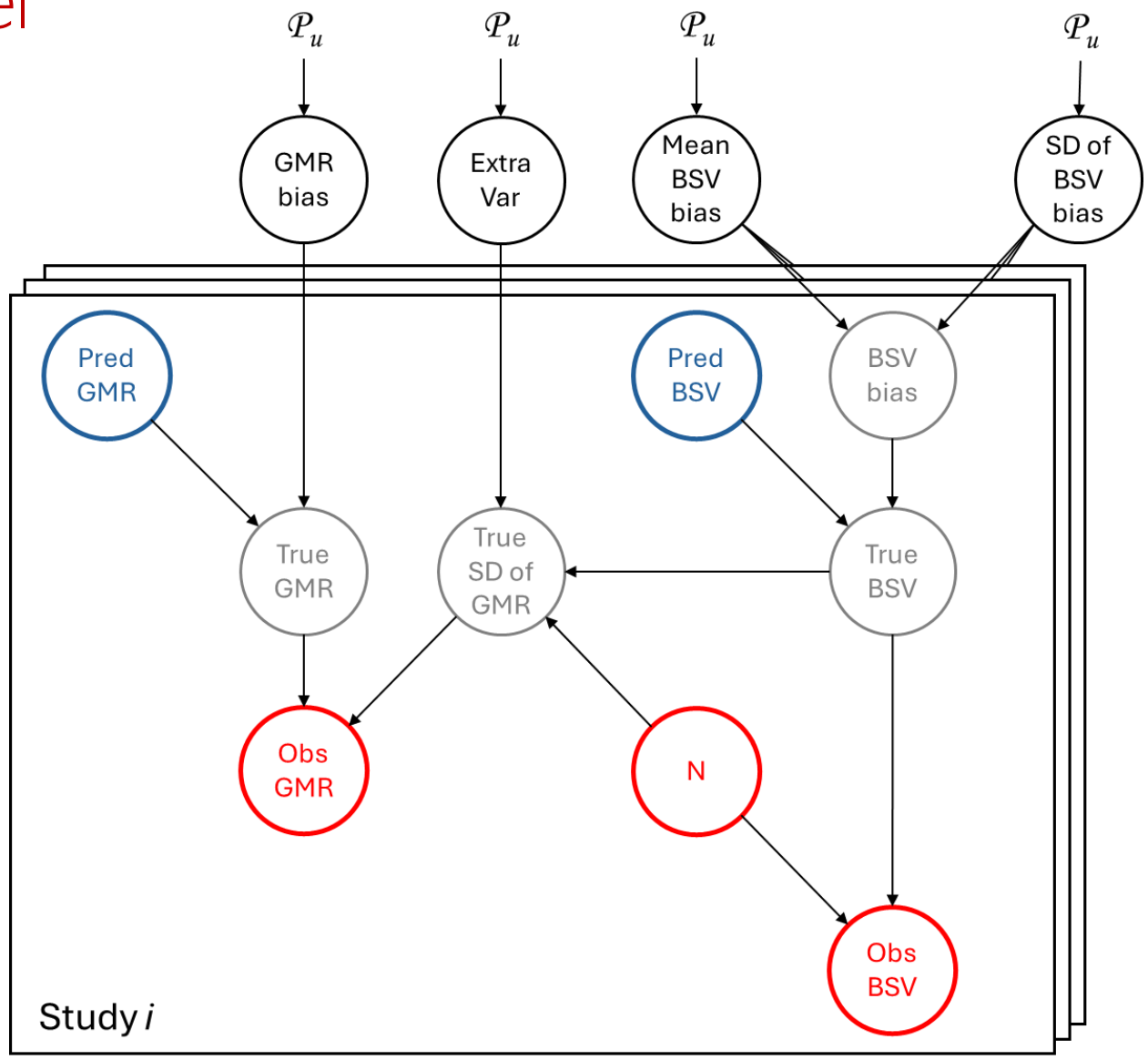


* more on that later; † not fully explored at this stage..

The bias and uncertainty model

- The model is multilevel (hierarchical) and has variables at the top level and study level. Inference was performed using MCMC simulations with the R package Nimble. We give scripts.

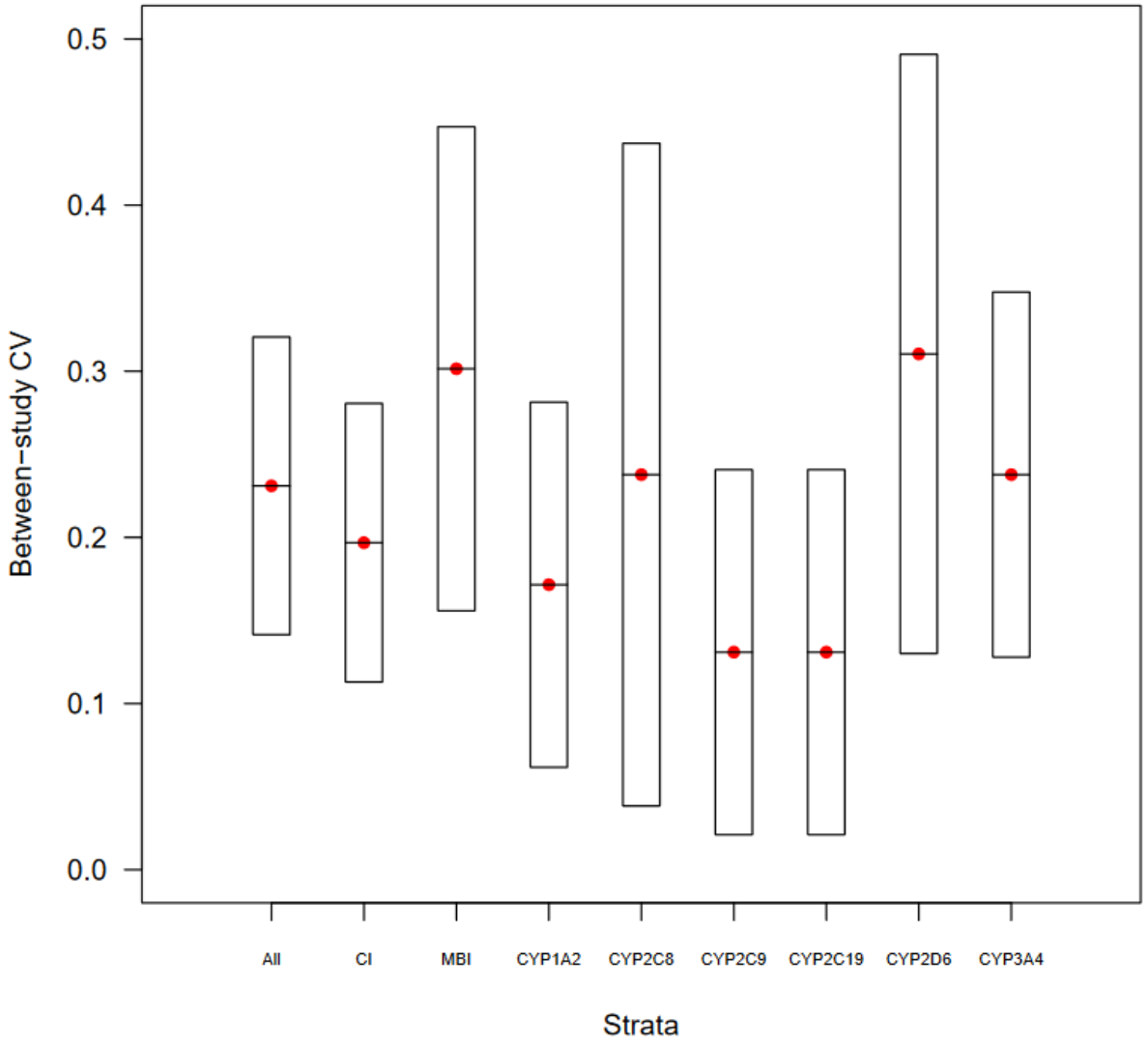
- Observations and predictions are data. The other nodes are random variables for which we want the posterior distribution given uninformative priors, $\mathcal{P}_u$, and data.



Results 2: Between study variability and imprecision

Difficult to estimate

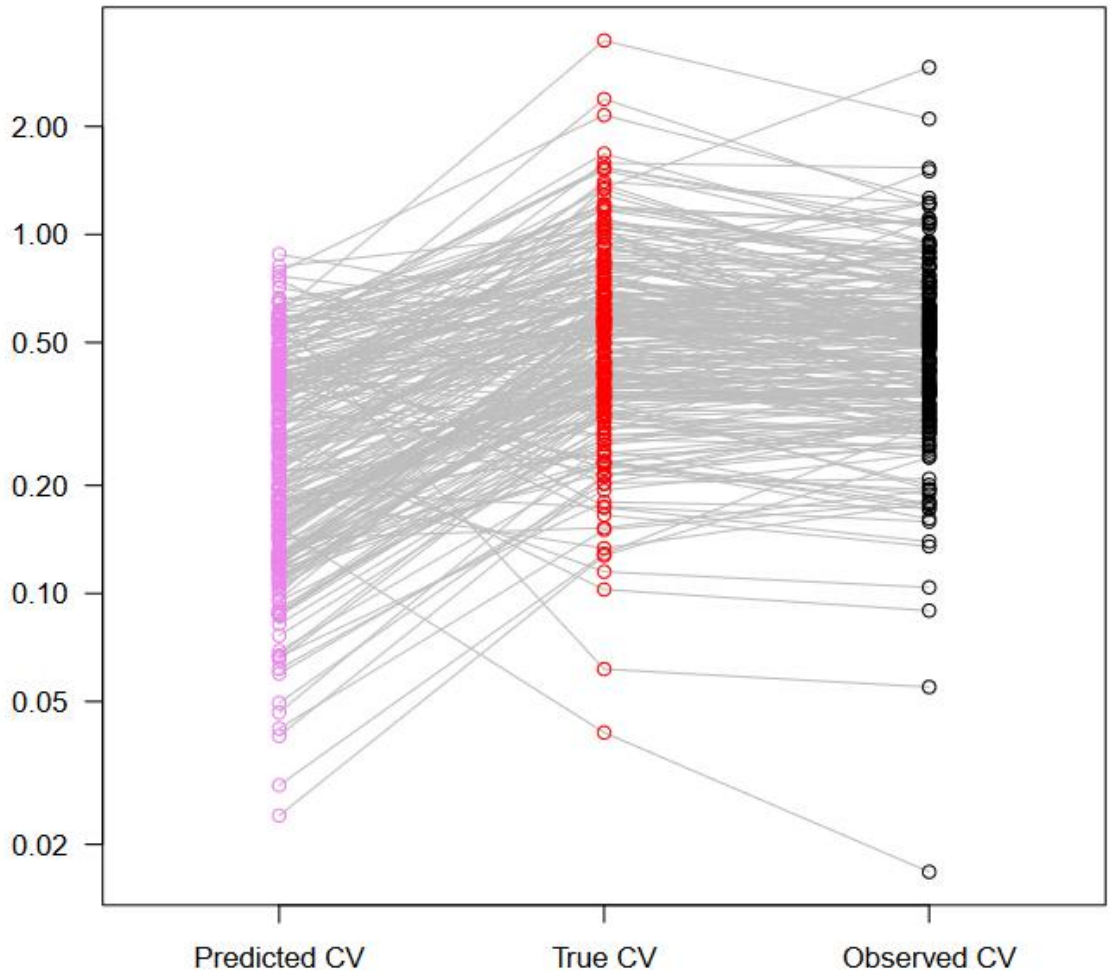
- Total variance of GMRs around their true values (bias-corrected Simcyp prediction) is due to
  - GMR sampling error (small trials),
  - Imprecision,
  - True inter-study variability.
- Only total inter-study variability can be estimated with our data and model. So, we only have an upper-bound on imprecision.
- Imprecision is at most 13 to 30%. Note that in our discussion of imprecision, lowering the upper bound by 7%, is incorrect, because sampling error was already accounted for.



Results: BSV prediction biases

Sizable and explainable

- Overall factor 2 (lognormal distribution) or 2.5 (normal) underestimation of total between-subject CVs. Total CV lumps between-subject, within-subject variability and measurement error. Initially, a normal distribution for BSV bias was assumed but is more likely to be lognormal.
- Simcyp V19 predictions were made without within-subject variability nor measurement error. Also, better genotype information has been added in V21.
- We have another data problem: All reported studies are cross-over but a large fraction of them report their results as RoM, as if they were parallel studies.



Conclusion on GMRs

- GMR prediction bias is overall -4.5%, and ranges from 1% to -14% depending on the CYP and mode of inhibition considered. CYP2C19 GMRs (bias of -9% on average but statistically not significant) and CYP2D6 GMRs (bias of -14% on average) are the most under-predicted. Those findings should be quite robust.
- The bias (CYP2C19 and CYP2D6), to some extent, may be due to the lack of detail relating to specific genotypes in the clinical studies – thus assumptions had to be made.
- Alternatively, if detailed information was provided, only UM, PM, and EM genotypes were accommodated within the Simcyp Simulator (V19). This has been updated for subsequent versions.
- Simcyp imprecision of GMR predictions upper bound should be around 23%, and in the range of 13% to 30%. Imprecision seems highest for CYP2D6.

Conclusion on BSV

- Simcyp tends to under-estimate total between-subject variability (CVs) by a factor of 2 approximately.
  - This is mostly true for CYP3A4.
  - This seems strongly affected by clinical data quality and reporting.
  - Note that we capture well the between-subject variability of AUC (with and without inhibitor).
  - We do not know how this impacts confidence intervals around predicted GMRs.

We all have difficulties grasping such numbers: If we state: “The average observed between-subject GSD is 1.7 and the average predicted between-subject GSD is 1.3”, it looks much better, but it says the same thing (a factor 2 for CVs).

THANK YOU

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