Integrating *In Vitro* and *In Silico* Approaches Enables Accurate Prediction of Drug-Induced Liver Injury

René Geci
Uniklinik RWTH Aachen/ESQlabs
OSP Community Conference 2025

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ESQlabs

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Marina García de Lomana

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In silico PK predictions get more traction

Journal of Medicinal Chemistry

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Article

Application of Machine Learning and Mechanistic Modeling to Predict Intravenous Pharmacokinetic Profiles in Humans

Xuelian Jia, Donato Teutonico, Saroj Dhakal, Yorgos M. Psarellis, Alexandra Abos, Hao Zhu, Panteleimon D. Mavroudis,* and Nikhil Pillai*

Cite This: https://doi.org/10.1021/acs.jmedchem.5c00340

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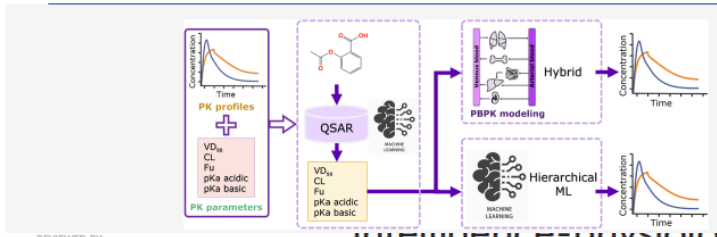

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Archives of Toxicology

...rg/10.1007/s00204-024-03764-9

...o

...natic evaluation of high-throughput PBK m...

...intelligence-physiologically based pharmacokinetic (AI-PBPK) modelling

Keheng Wu[1], Xue Li[1], Zhou Zhou[1], Youni Zhao[1], M... Zhuo Cheng[1], Xinyi Wu[1], Zhijun Huang[1], Xiong Jin... Mengjun Zhang[3], Jack Liu[1] and Bo Liu[3]*

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molecular pharmaceutics

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Evaluation of the Success of High-Throughput Physi... Pharmacokinetic (HT-PBPK) Modeling Predictions t... Drug Discovery

Doha Naga,[§] Neil Parrott, Gerhard F. Ecker, and Andrés Olivares-Morales*[,§]

Cite This: *Mol. Pharmaceutics* 2022, 19, 2203−2216

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ABSTRACT: Minimizing in vitro and in vivo testing in early drug discovery with the use ... physiologically based pharmacokinetic (PBPK) modeling and machine learning (ML) approaches h... the potential to reduce discovery cycle times and animal experimentation. However, the predictic... success of such an approach has not been shown for a larger and diverse set of compoun... representative of a lead optimization pipeline. In this study, the prediction success of the oral (PO... and intravenous (IV) pharmacokinetics (PK) parameters in rats was assessed using a "bottom-up... approach, combining in vitro and ML inputs with a PBPK model. More than 240 compounds fi... which all of the necessary inputs and PK data were available were used for this assessment. Different clearance scaling approaches were assessed, using hepatocyte intrinsic clearance and protein binding as inputs. In addition, a novel high-throughput PBPK (HT-PBPK) approach was evaluated to assess the scalability of PBPK predictions for a larger number of compounds in drug discovery. The results showed that bottom-up PBPK modeling was able to predict the rat IV and PO PK parameters for the majority of compounds within a 2- to 3-fold error range, using both direct scaling and dilution methods for clearance predictions. The use of only ML-predicted inputs from the structure did not perform... inputs, likely due to clearance miss predictions. The HT-PBPK approach produced comparable results to... approach but reduced the simulation time from hours to seconds. In conclusion, a bottom-up PBPK and ... successfully predict the PK parameters and guide early discovery by informing compound prioritization, pr... assays are in place for key parameters such as clearance.

KEYWORDS: *drug discovery, PBPK models, HT-PBPK, physicochemical properties, clearance predictions and...*

J Pharmacokinet Pharmacodyn (2017) 44:549–565
DOI 10.1007/s10928-017-9548-7

ORIGINAL PAPER

Evaluation and calibration of high-throughput predictions of chemical distribution to tissues

Robert G. Pearce[1,2] · R. Woodrow Setzer[1] · Jimena L. Davis[1,3] · John F. Wambaugh[1]

JC... AND MODELING

Cite This: *J. Chem. Inf. Model.* 2019, 59, 4893−4905

pubs.acs.org/jcim

Prediction of Oral Bioavailability in Rats: Transferring Insights from in Vitro Correlations to (Deep) Machine Learning Models Using in Silico Model Outputs and Chemical Structure Parameters

Sebastian Schneckener,[†] Sergio Grimbs,[†] Jessica Hey,[†] Stephan Menz,[§] Maren Osmers,[§] Ste...

[†]Ba... [‡]Ba... [§]Ba...

Research Article

Predictive Performance of Next Generation Human Physiologically Based Kinetic (PBK) Models Based on *In Vitro* and *In Silico* Input Data

Ans Punt[1], Jochem Louisse[1], Karsten Beekmann[1], Nicole Pinckaers[1], Eric Fabian[2], Bennard van Ravenzwaay[2], Paul L. Carmichael[3], Ian Sorrell[3] and Thomas E. Moxon[3]

[1]Wageningen Food Safety Research, Wageningen, The Netherlands; [2]BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany; [3]SEAC, Unilever, Colworth Science Park, Sharnbrook, Bedford, UK

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INTOX

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Open Access!

Systematic evaluation of high-throughput PBK modelling strategies for the prediction of intravenous and oral pharmacokinetics in humans

Geci R, Gadaleta D, Lomana MG de, Ortega-Vallbona R, Colombo E, Serrano-Candelas E, Paini A, Kuepfer L, Schaller S (2024) Archives of Toxicology:1–18. doi: 10.1007/s00204-024-03764-9

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Are high-throughput PK predictions useful for real decision making?

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

DILI is hard to predict but important

- Drug-induced liver injury (DILI) can occur via many different mechanisms
- Often idiosyncratic with only 1 - 20 out of 100,000 patients affected
- Many confounding factors (genetics, comorbidities, comedications etc)
- Difficult to predict with current preclinical methods
- Can lead to liver failure and patient death
- Many drug candidates fail due to liver safety in clinical studies
- Common reason for withdrawal from market after approval

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Methodology

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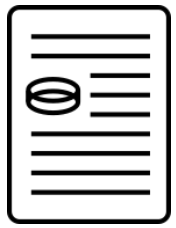
There are many *in vitro* DILI assays



Molecular assays, cell assays… different cell types… 2D, 3D…

Parameters	O'Brien et al. (2006)	Xu et al. (2008)	Dawson et al. (2012)	Tolosa et al. (2012)	Thompson et al. (2012)	Gustafson et al. (2014)	Sakatis et al. (2012)	Persson et al. (2013)	Garside et al. (2014)	Atienzar et al. (2014)	Tomida et al. (2015)
S, MF, MA	MA	MF	S	MF	MA	S	MF	MF	MA	MA	MA
No. of compounds	243	344	85	78	36	104	223	102	144	51	32
In vivo toxic	146	200	64	66	27	83	113	66	108	40	17
In vivo non-toxic	95	144	21	12	9	21	110	34	36	11	15

Raw data able	Parameters	Shah et al. (2015)	Schadt et al. (2015)	Saito et al. (2016)	Aleo et al. (2019)	Khetani et al. (2012)	Proctor et al. (2017)	Vorrink et al. (2018)	Williams et al. (2019)	Porceddu et al. (2012)	Albretcht et al. (2019)	Tolosa et al. (2019)
Longest a incubati time (hı	S, MF, MA	MA	MA	MA	MA	MA	S	S	MA	MA	S	MA
	No. of compounds	125	81	28	200	45	110	123	96	124	30	15
	In vivo toxic	70	38	11	79	35	69	70	63	87	14	11
	In vivo non-toxic	55	43	17	121	10	41	53	33	37	16	4
	Raw data available	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No

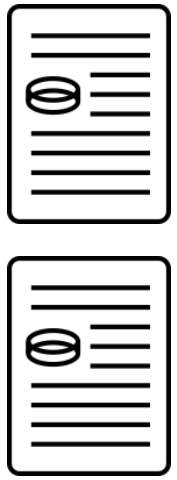
Table from: Walker, P. A., Ryder, S., Lavado, A., Dilworth, C. & Riley, R. J. The evolution of strategies to minimise the risk of human drug-induced liver injury (DILI) in drug discovery and development. Arch Toxicol 94, 2559–2585; 10.1007/s00204-020-02763-w (2020).

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

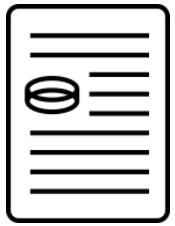
Data collection

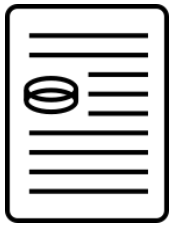
***In vitro* studies (17x)**

Cytotoxicity
Mitochondrial toxicity
BSEP inhibition
…



Strongest toxicity potency value per compound





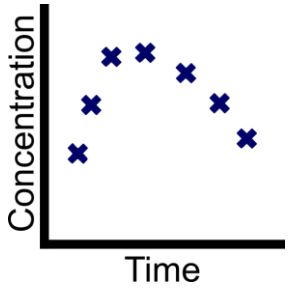
Clinical toxicity

No DILI
Less DILI
Most DILI
Clinical Failure

DILIrank
Chen et al.
(2016)

PK Study data

Highest oral dose
In vivo Cmax



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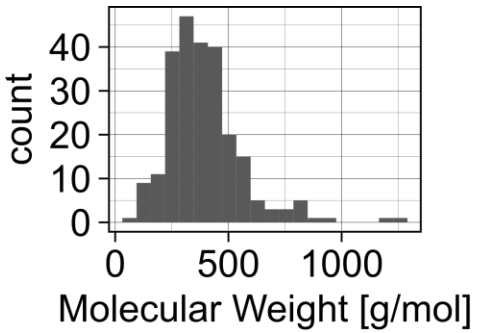
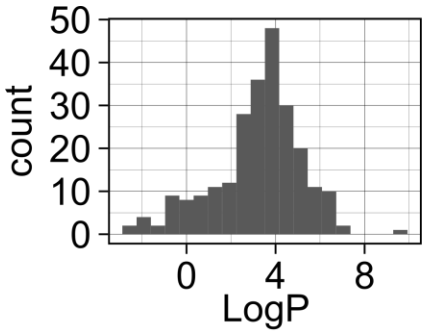
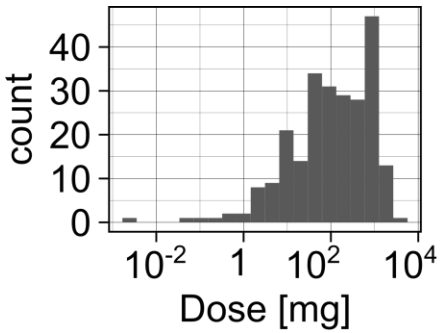
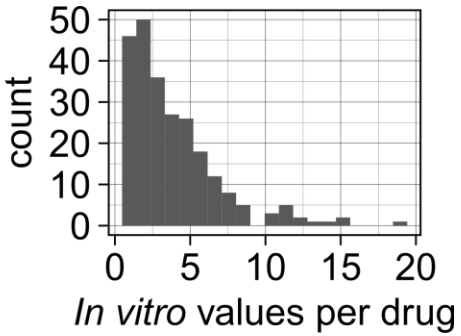
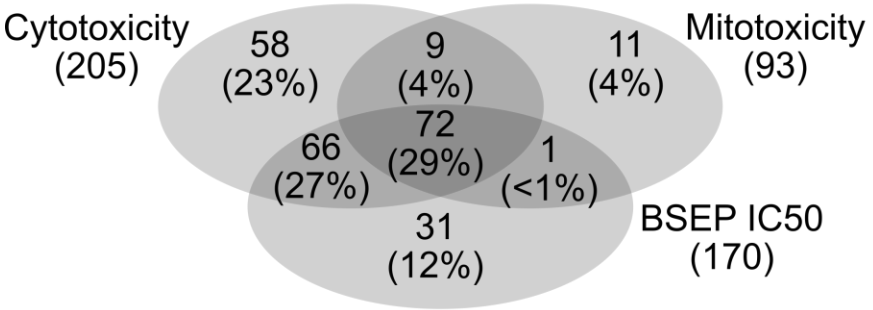
Our integrated DILI dataset: 241 drugs

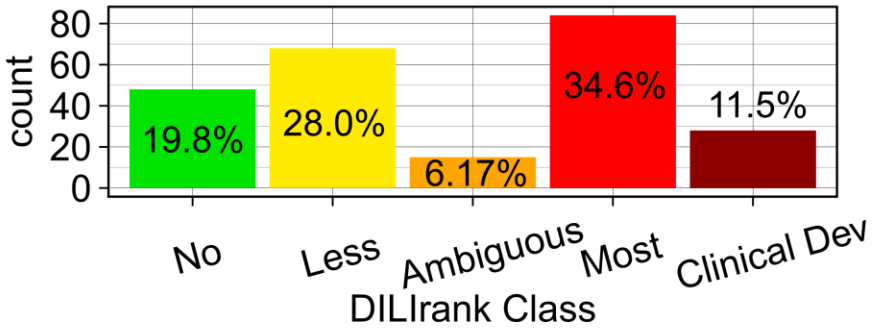












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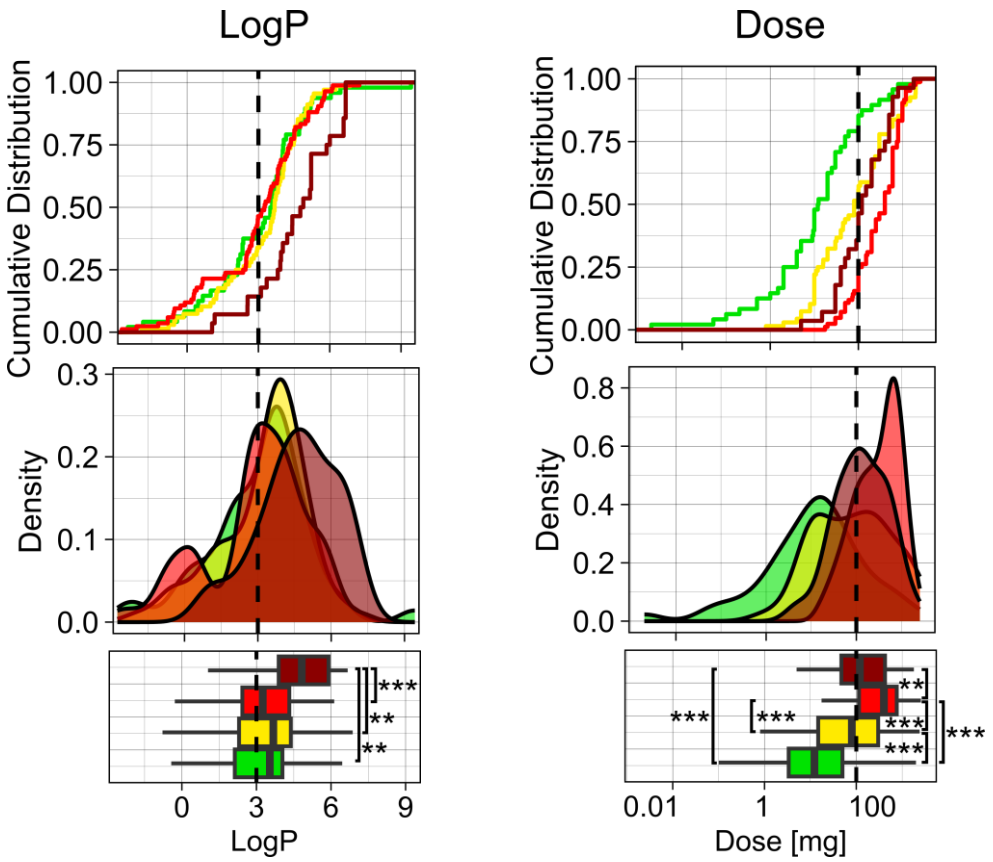
Results

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Evaluation 1: Simple heuristic screening rules poorly predict DILI



Balanced accuracies: 50 - 70%

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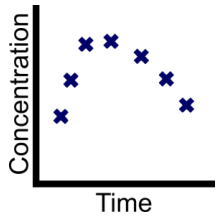
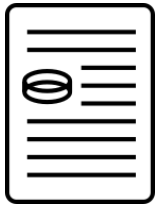
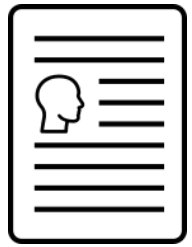
Evaluation 2: Cmax to *in vitro* toxicity ratios



$$\frac{In\ vivo\ \text{Cmax}}{(Strongest)\ in\ vitro\ \text{toxicity}} = \text{Cmax to toxicity ratio}$$



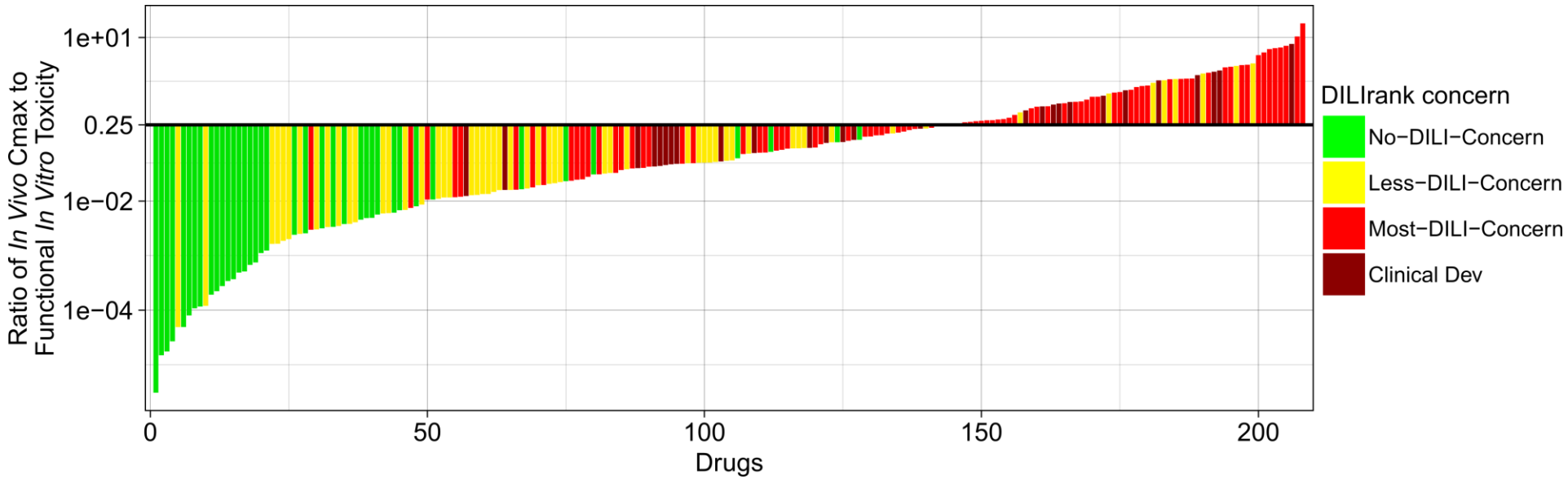
Clinical toxicity



No DILI
Less DILI
Most DILI
Clinical Failure

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Evaluation 2: Cmax to *in vitro* toxicity ratios strongly predict DILI



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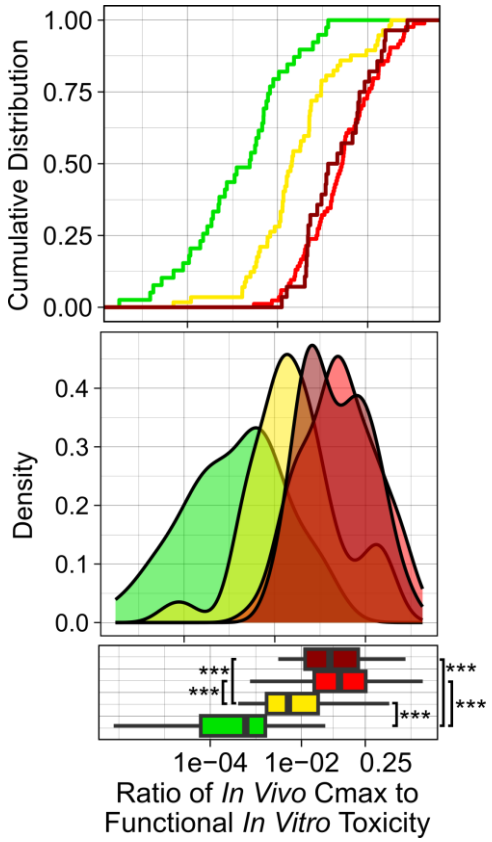
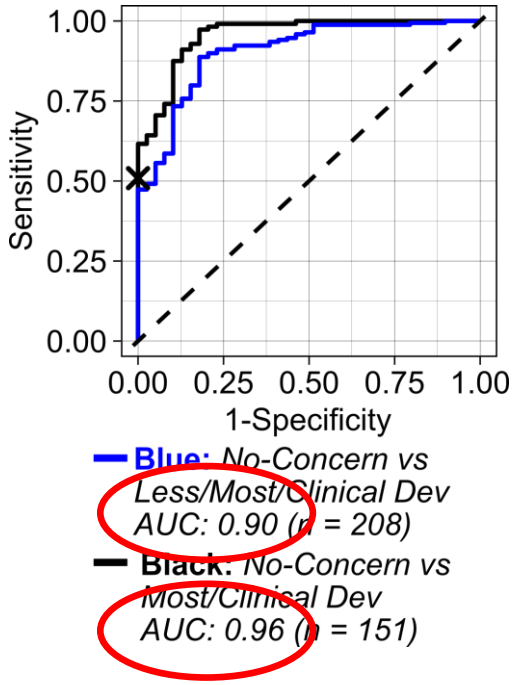
Evaluation 2: Cmax to *in vitro* toxicity ratios strongly predict DILI





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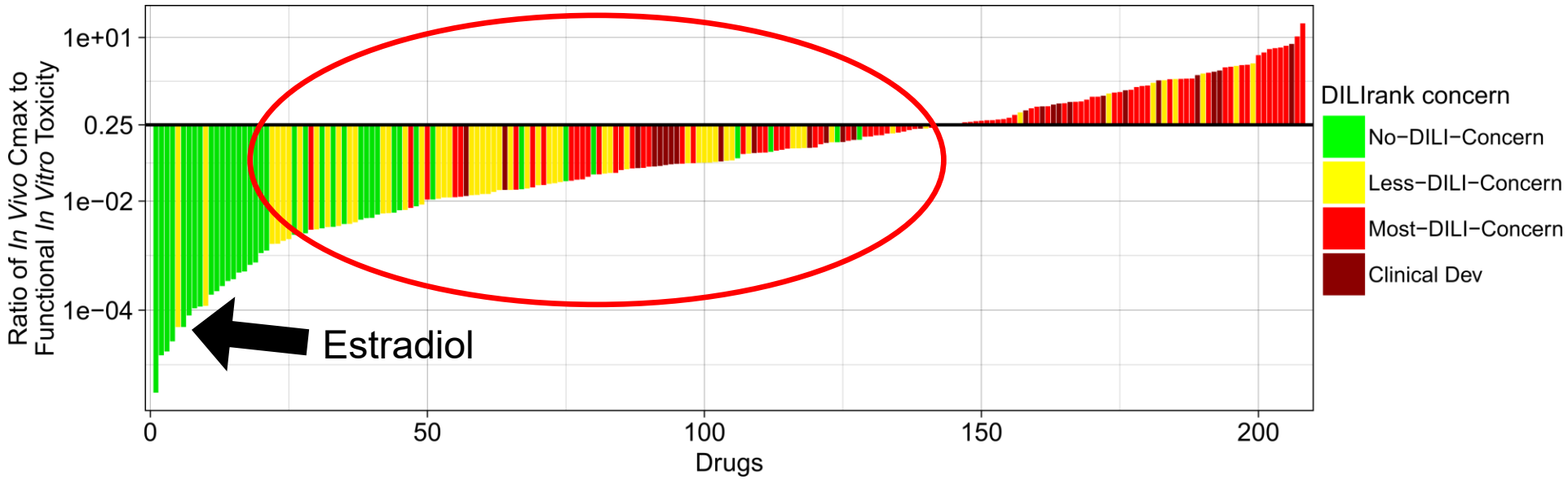
Evaluation 2: Cmax to *in vitro* toxicity ratios strongly predict DILI



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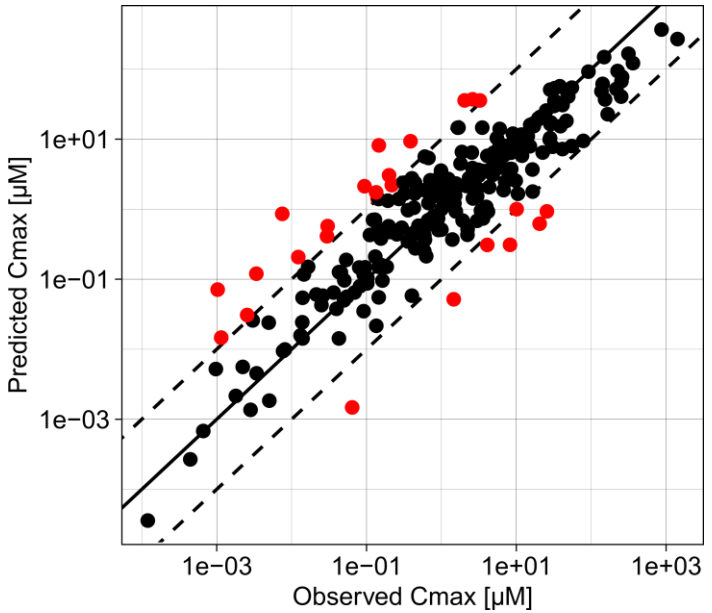
In silico predicted Cmax is similar to *in vivo* observed values



90% of Cmax values predicted within 10-fold

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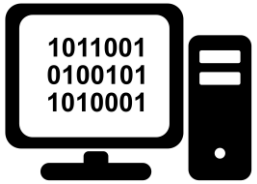
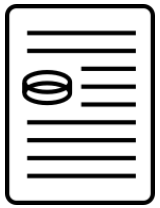
Evaluation 3: *In silico* predicted Cmax

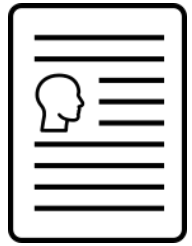


$$\frac{\sout{\textit{In vivo}}\ \textit{In silico}\ \text{Cmax}}{(\text{Strongest})\ \textit{in vitro}\ \text{toxicity}}$$

= Cmax to toxicity ratio



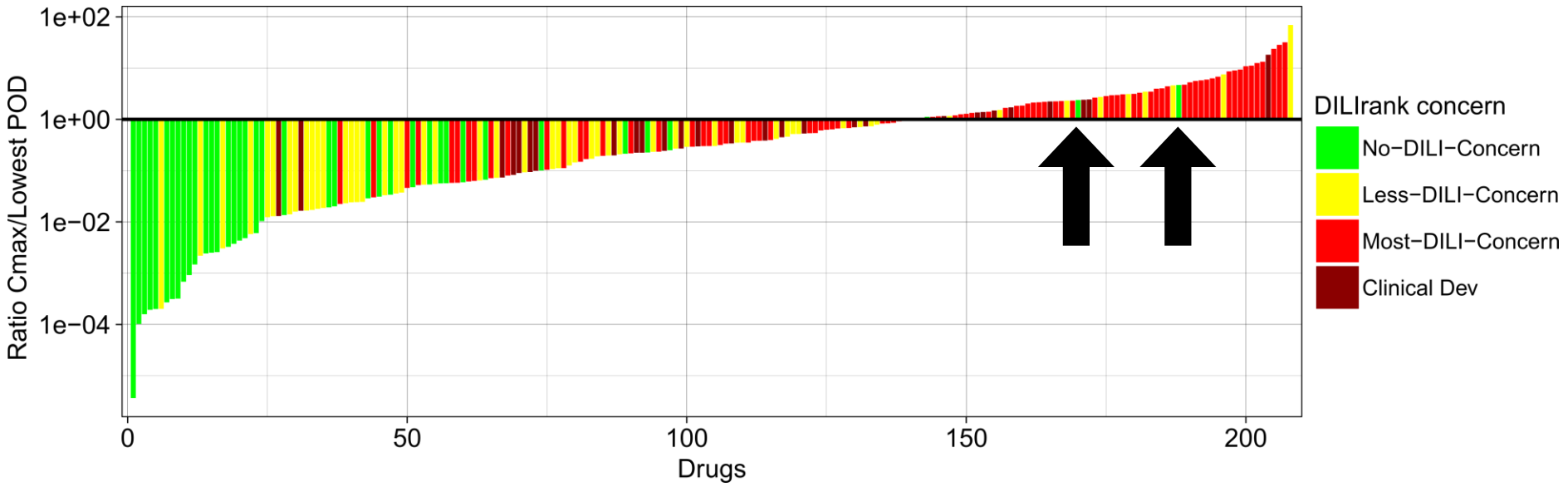
Clinical toxicity



No DILI
Less DILI
Most DILI
Clinical Failure

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Evaluation 3: *In silico* predicted Cmax enables prospective DILI evaluation



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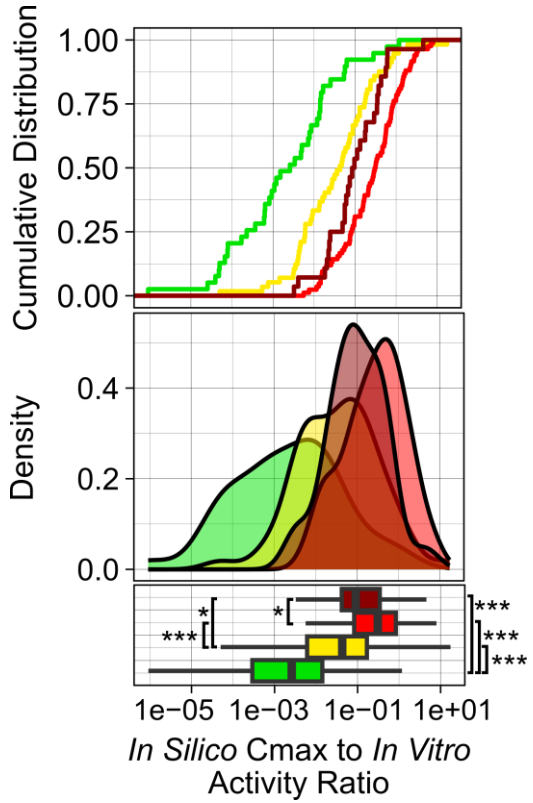
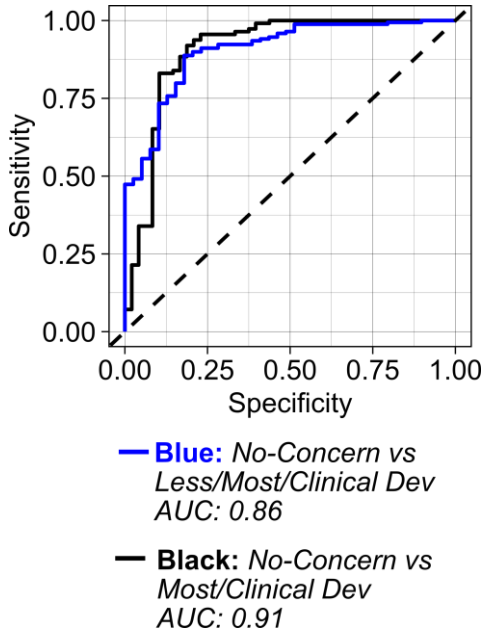
Evaluation 3: *In silico* predicted Cmax enables prospective DILI evaluation





ROC AUC for ***in vivo*** Cmax		**ROC AUC** for ***in silico*** Cmax
90%	→	86%
96%	→	91%

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Evaluation 4: *In vitro* BSEP inhibition data

JOURNAL ARTICLE

Measures of BSEP Inhibition *In Vitro* Are Not Useful Predictors of DILI FREE

Rosa Chan, Leslie Z Benet

Toxicological Sciences, Volume 162, Issue 2, April 2018, Pages 499–508,
https://doi.org/10.1093/toxsci/kfx284
Published: 20 December 2017

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Evaluation 4: *In vitro* BSEP inhibition data

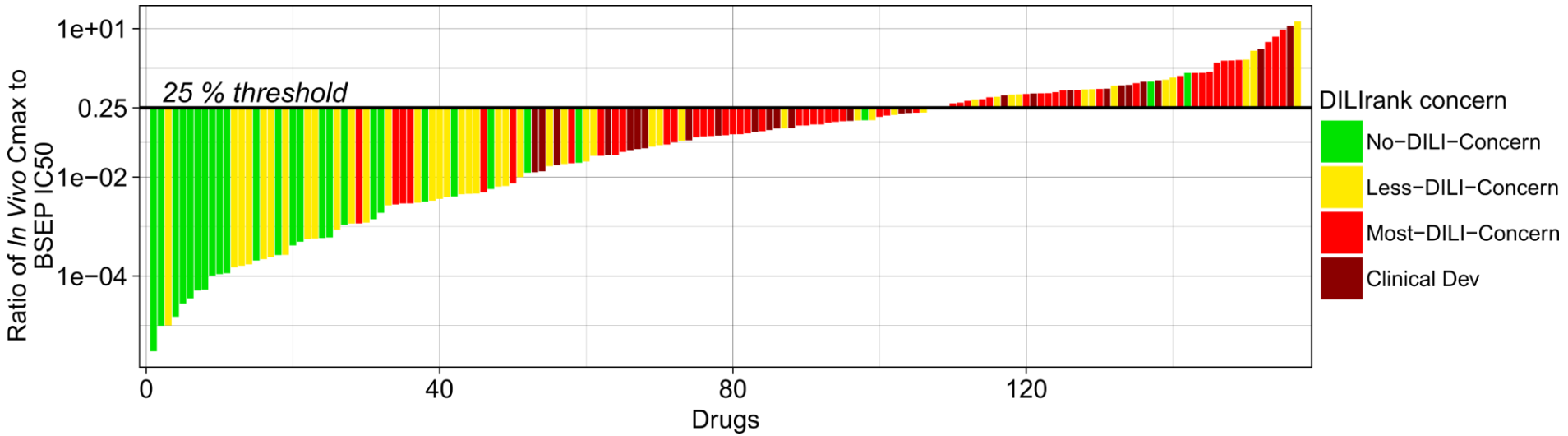
$$\frac{In\ vivo\ \text{Cmax}}{In\ vitro\ \text{BSEP IC50}} = \text{Cmax to toxicity ratio}$$

Clinical toxicity

No DILI
Less DILI
Most DILI
Clinical Failure

This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Evaluation 4: Inclusion of BSEP inhibition improves DILI predictivity



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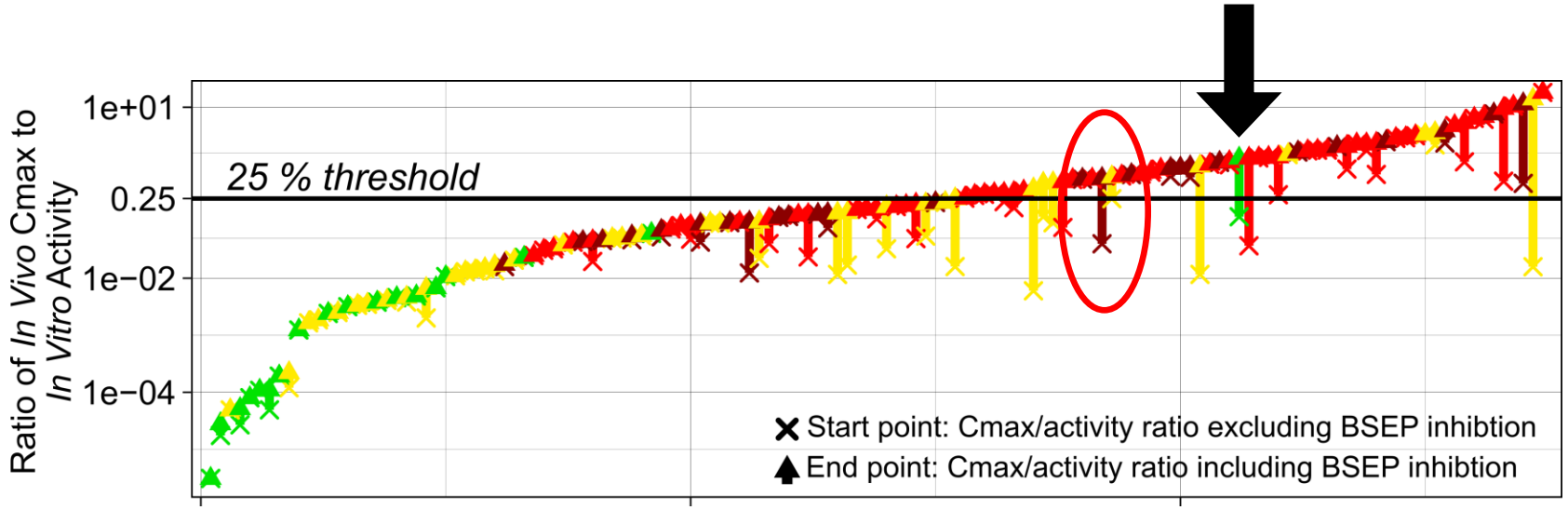
Evaluation 4: Inclusion of BSEP inhibition improves DILI predictivity



This project received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 963845.

Mifepristone

Case Reports > Hepatology. 2019 Jun;69(6):2704-2706. doi: 10.1002/hep.30465.
Epub 2019 Mar 8.

Cholestatic Drug-Induced Liver Injury Caused by Mifepristone

Katalina Funke [1], Don C Rockey [1]

Affiliations + expand

PMID: 30561784 DOI: 10.1002/hep.30465

Case report | Open access | Published: 03 February 2023

Mifepristone induced liver injury in a patient with Cushing syndrome: a case report and review of the literature

Taylor A. Ault, David R. Braxton, Rebecca A. Watson, Alan O. Marcus & Tse-Ling Fong

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Mifepristone: An Uncommon Cause of Drug-Induced Liver Injury

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Abstract

Mifepristone is frequently used in large doses for management of Cushing's syndrome. This is a case of a 35-year-old woman with Cushing's syndrome, who presented with abdominal pain and jaundice. A month prior to admission, she had been started on a daily dose of 1,200 mg mifepristone. After evaluating for various other causes of liver injury, biopsy revealed cholestatic pattern of liver disease, likely associated with drug-induced hepatotoxicity. Mifepristone was discontinued and her symptoms resolved. We believe this is one of the first few reported cases of drug-induced liver injury (DILI) associated with mifepristone use.

Keywords: Chemical and drug-induced liver injury; Mifepristone; Cushing's syndrome

Case Report

A 35-year-old woman presented to our hospital with generalized abdominal pain for a month. Pain was described as dull, constant and unrelated to eating or activity. She also complained of nausea, vomiting, yellowing of her skin and generalized itching. Her medical history was significant for Cushing's syndrome, for which she was being treated with mifepristone. She was initially started on 300 mg of mifepristone 3 months prior to presentation, which was increased to 600 mg after 3 weeks, followed by 900 mg 3 more weeks later, and most recently 1,200 mg about a month prior to presentation. Her only other medication was levothyroxine, which she had been taking at a stable dose for more than 10 years. She denied tak-

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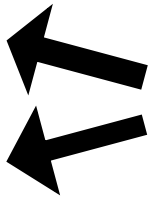
Evaluation 5: Dynamic effect modelling

$$\frac{\text{Cmax}}{\text{(Lowest) } \textit{in vitro} \text{ toxicity}}$$



Static representations of the dynamic *in vivo* situation

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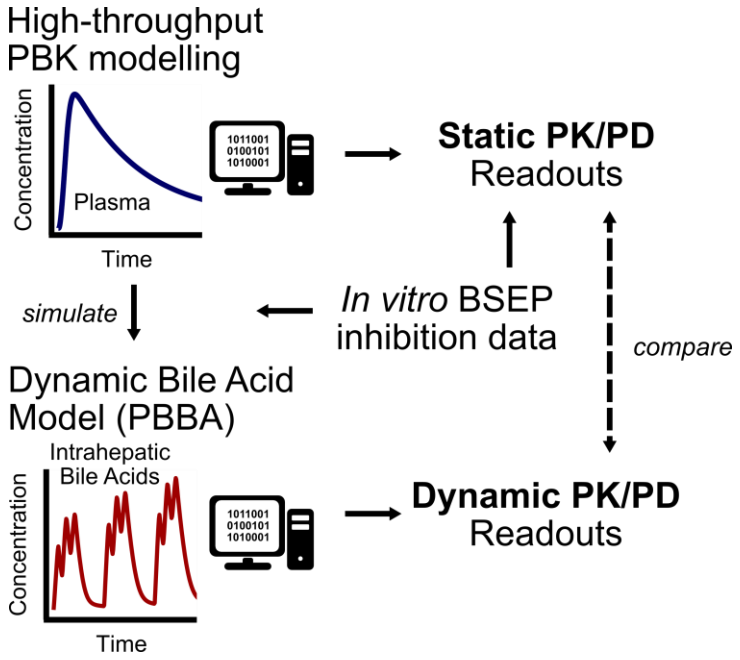
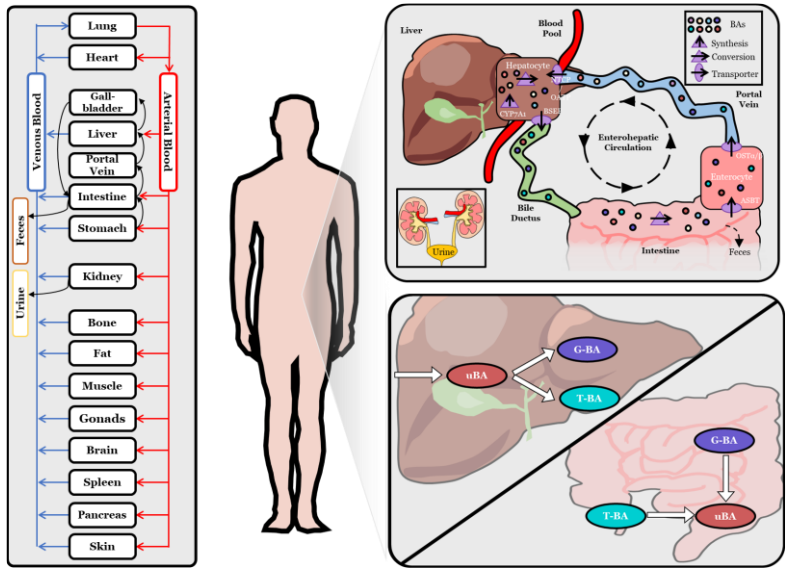
Evaluation 5: Dynamic modelling gives more realistic insights on *in vivo* bile acid perturbations



Kister et al. (2025)



Bile acid model: Kister, B., Blank, L. M., Pollmanns, M., Wirtz, T. H. & Kuepfer, L. A physiologically-based model of bile acid metabolism in humans. *bioRxiv,* 2025.07.19.665677; 10.1101/2025.07.19.665677 (2025).

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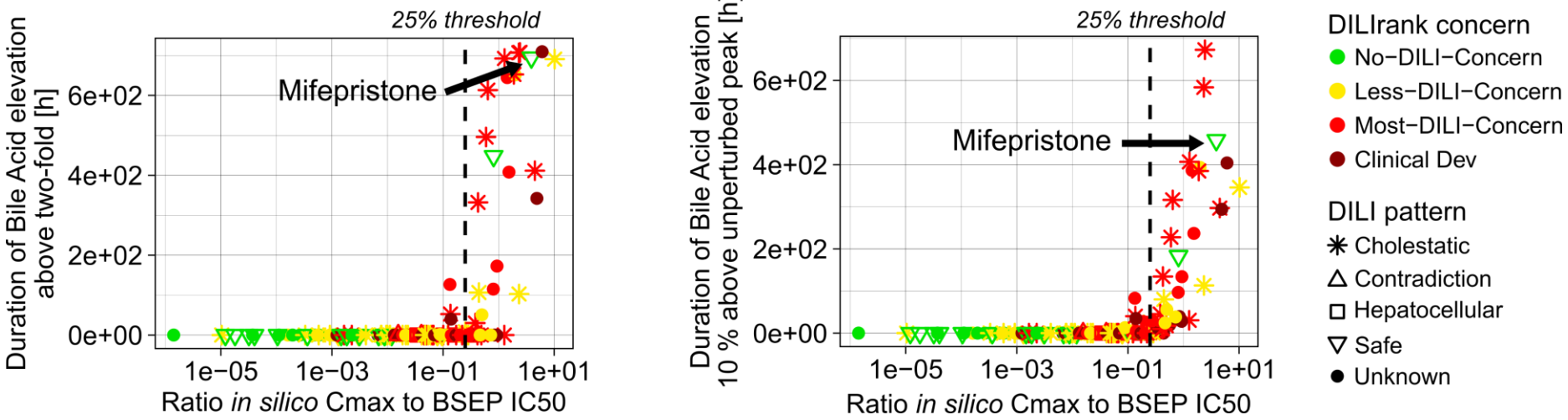

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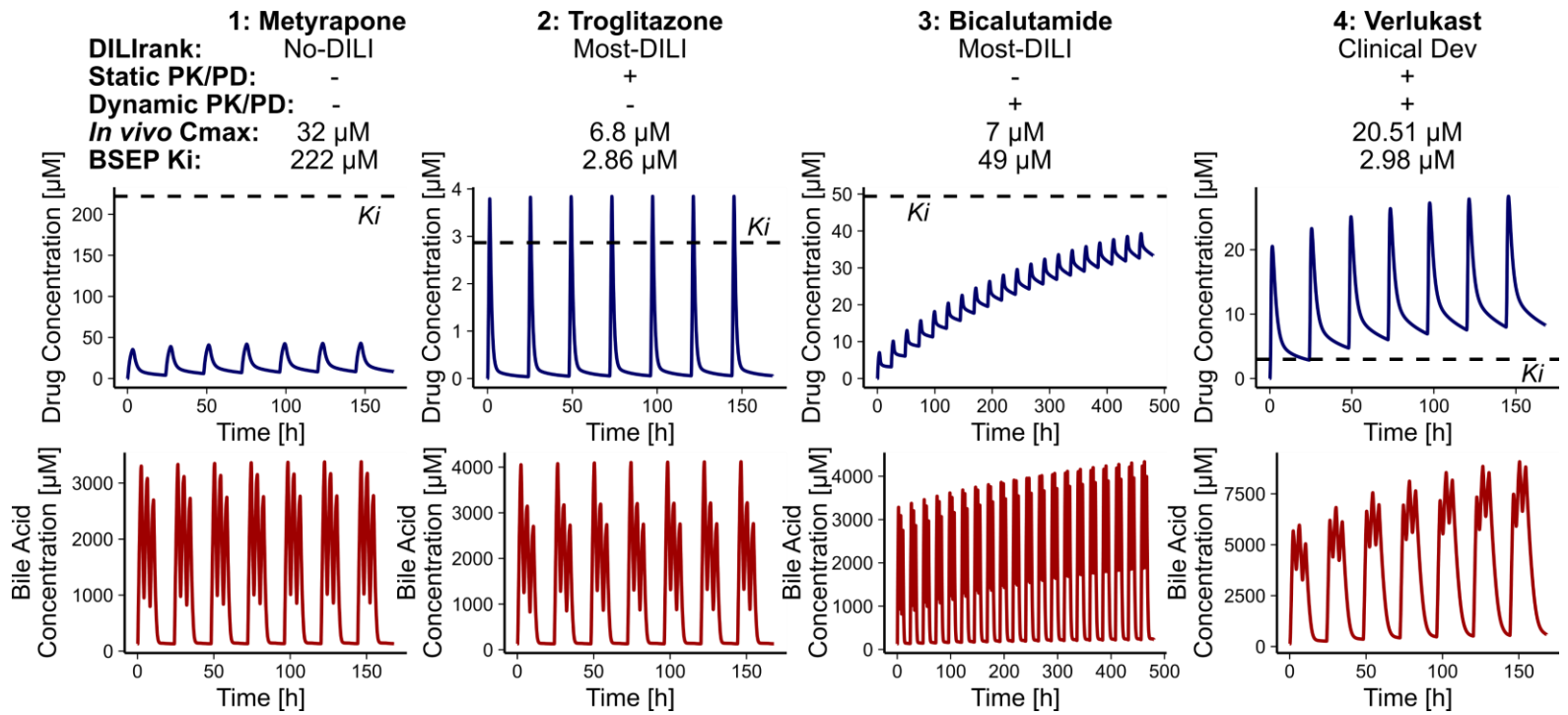
Evaluation 5: Dynamic modelling gives more realistic insights on *in vivo* bile acid perturbations



Limitations

- Heterogeneous literature data with incomplete endpoint coverage per drug
- Immune-mediated, metabolite-driven, and gene-regulatory mechanisms are underrepresented
- Retrospective sources lack formulation details and dosing schedules
- Key confounders (co-medications, comorbidities, genetics) are largely unavailable
- Ideal thresholds are probably endpoint-specific

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Conclusions

1. **Available *in vitro* toxicity assays are already very useful for predicting DILI**

… even better predictivity when more toxicity mechanisms are covered

2. **Exposure (Cmax) is key**

… high-throughput PBK modelling allows prospective predictions before any clinical studies have been performed

3. **BSEP inhibition is an important mechanism of DILI**
4. Dynamic models capture time-dependent effects and yield more realistic *in vivo* insights than static metrics, although those are good first approximations

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In vitro toxicity alone is a poor predictor

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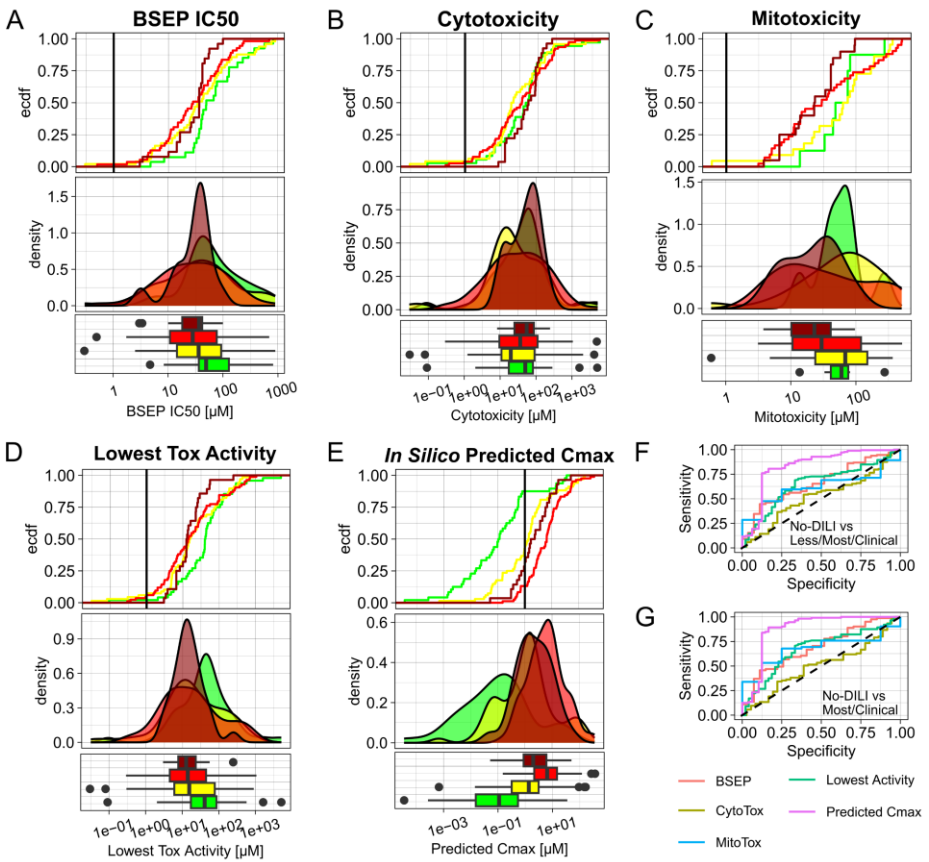
Per assay ratios



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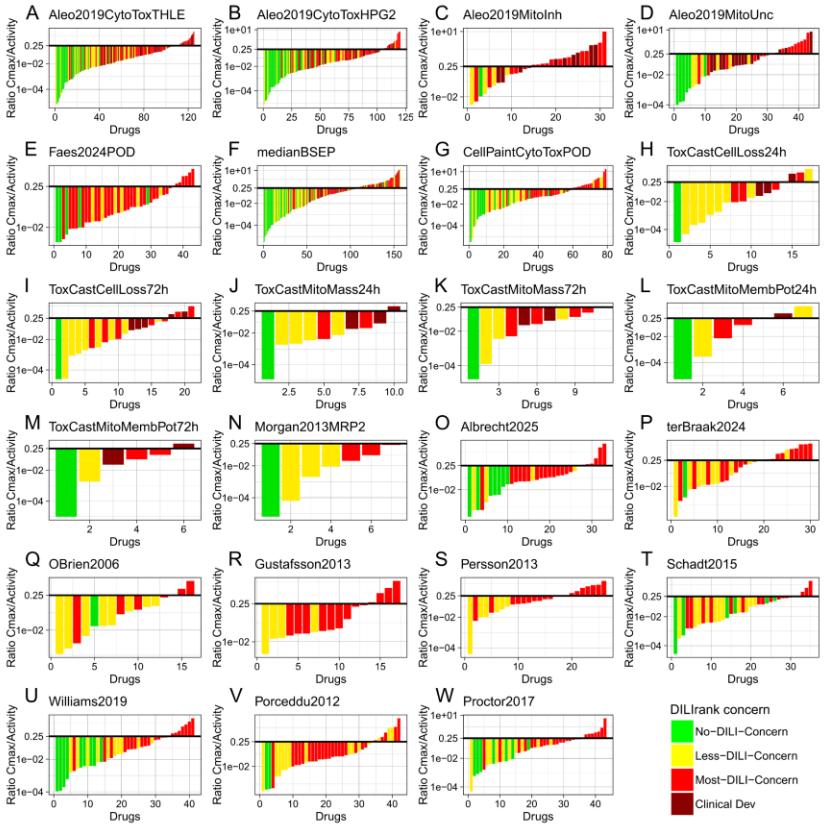
Per assay ratios



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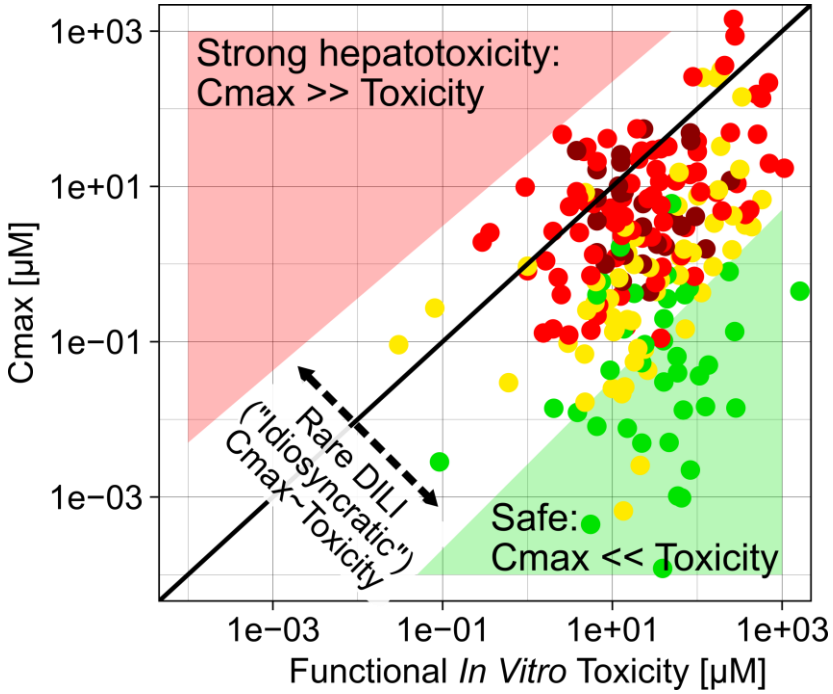
Dose & Idiosyncratic DILI



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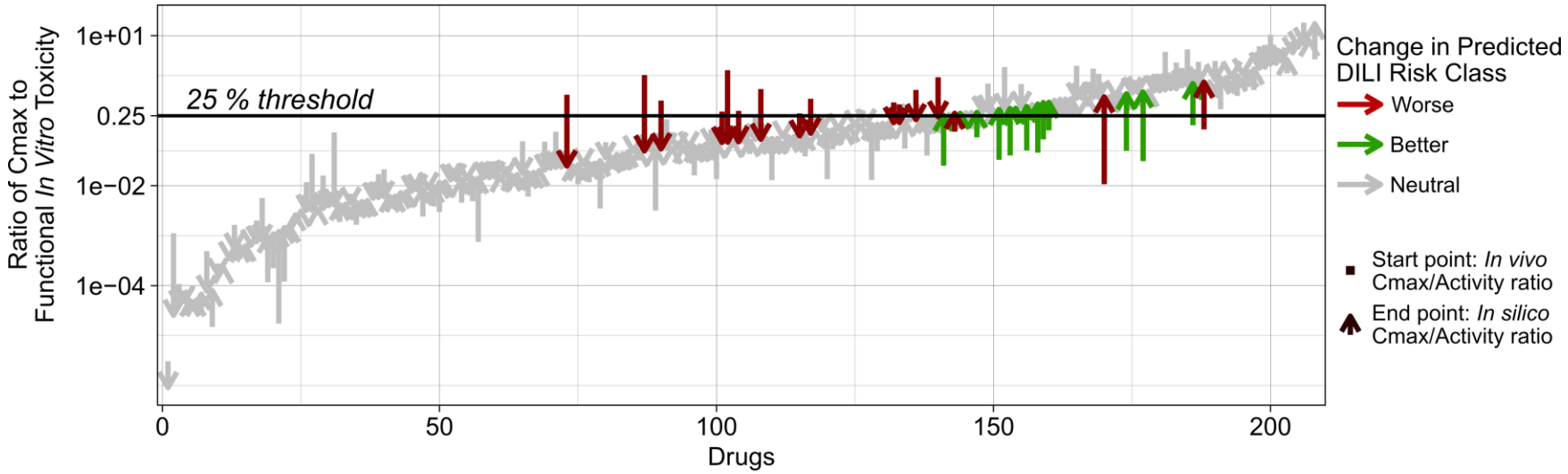
In silico predicted Cmax enables prospective DILI evaluation



14% of drug DILI risk classifications changed when using *in silico* predicted Cmax instead of *in vivo* observed values

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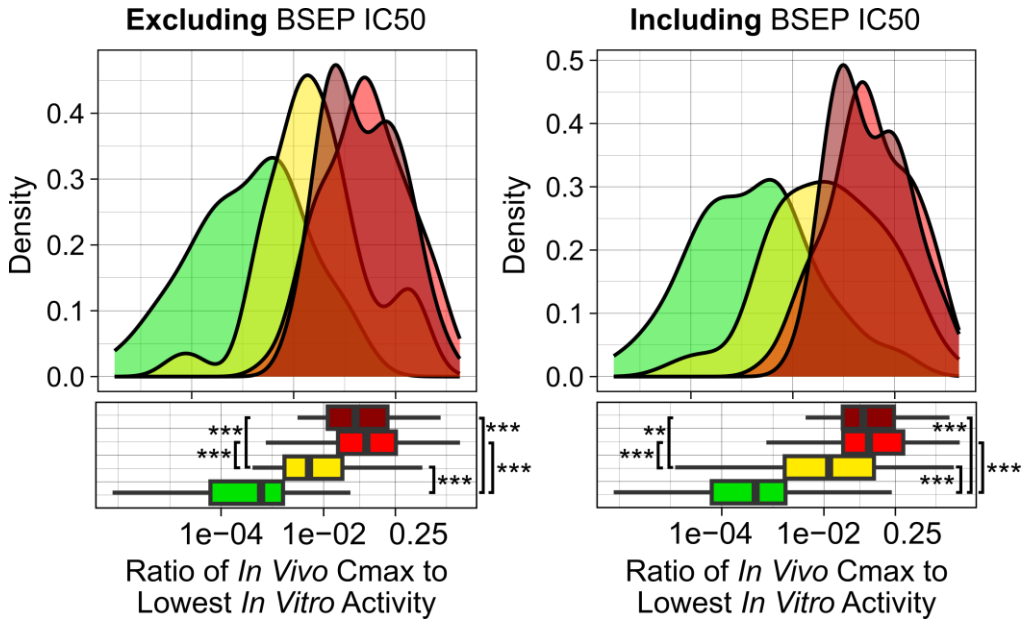
BSEP inhibition seems associated with “Less DILI” classification



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