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Using DILIsym, a Quantitative Systems Toxicology (QST) Software Tool of Drug-Induced Liver Injury (DILI), to Assess DILI Risk in Drug Development

August 17, 2018

Brett A. Howell, Ph.D., President, DILIsym Services

DILIsym Talk Agenda

- Mechanistic mathematical modeling within drug development
- Overview of the DILIsym Software
- Example GastroPlus / DILIsym Application

U.S. Department of Health & Human Services

FDA U.S. FOOD & DRUG ADMINISTRATION

FDA Voice by Commissioner Scott Gottlieb, M.D.

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How FDA Plans to Help Consumers Capitalize on Advances in Science

Posted on July 7, 2017 by FDA Voice

To build upon such opportunities, FDA will soon unveil a comprehensive Innovation Initiative. It will be aimed at making sure our regulatory processes are modern and efficient, so that safe and effective new technologies can reach patients in a timely fashion. We need to make sure that our regulatory principles are efficient and informed by the most up to date science. We don't want to present regulatory barriers to beneficial new medical innovations that add to the time, cost, and uncertainty of bringing these technologies forward if they don't add to our understanding of the product's safety and benefits.

Today we announced our detailed work plan for the steps we're taking to implement different aspects of Cures. I want to highlight one example of these steps, which we're investing in, and will be expanding on, as part of our broader Innovation Initiative. It's the use of in silico tools in clinical trials for improving drug development and making regulation more efficient.

FDA's Center for Drug Evaluation and Research (CDER) is currently using modeling and simulation to predict clinical outcomes, inform clinical trial designs, support evidence of effectiveness, optimize dosing, predict product safety, and evaluate potential adverse event mechanisms. We'll be putting out additional, updated guidance on how aspects of these in silico tools can be advanced and incorporated into different aspects of drug development.

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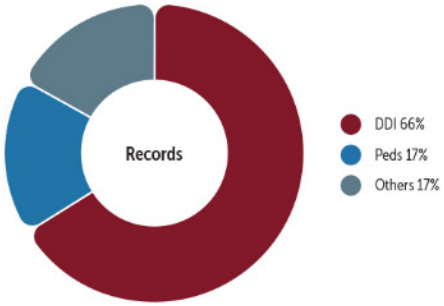
FDA Voice blog: July 7th, 2017

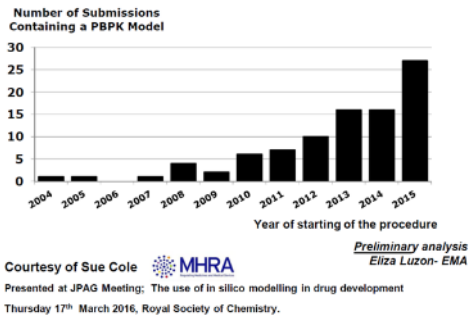
Recent PBPK Modeling Trends: Regulatory Information

- 180 PBPK modeling citations in the FDA's Office of Clinical Pharmacology database (2008-15)
- 60 submissions received by EMA containing PBPK models (2013-15)
- 17 PBPK modeling citations at Japan PMDA (2014-16)

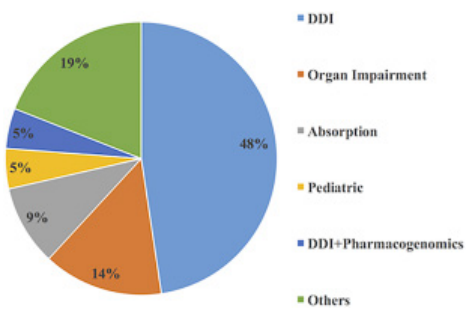
FDA



EMA



PMDA



Mehrotra et al., DMD, 2016
Sato et al., CPT 2017
Cole et al., 2016 JPAG Meeting

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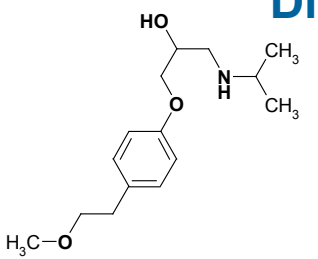
Simulations Plus:
Your end-to-end M&S solutions provider

Discovery



Preclinical

Clinical

ADMET Predictor™ ⟷ **GastroPlus™**

- **MedChem Studio™**
  MedChem Designer™
- **DDDPlus™**
  MembranePlus™
- **PKPlus™**
  DILIsym®
- **KIWI™**
  NAFLDsym®

Consulting Services and Collaborations

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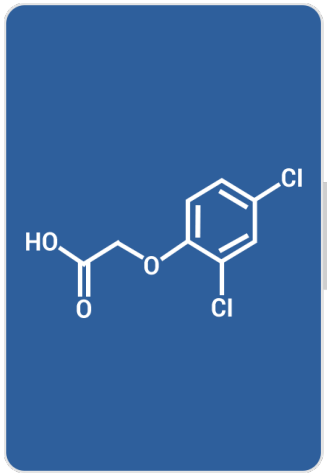
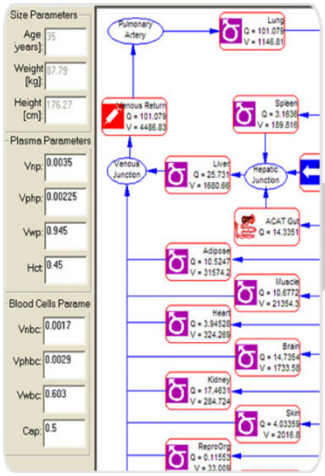
Saying “I do” to the QSAR / PBPK / QST marriage…



Permeability, solubility vs. pH, pKa(s), logD vs. pH, Fup, blood:plasma ratio, tissue Kps, CLint, CLfilt



Local & systemic exposure, drug distribution, parent and metabolite levels, patient variability

Quantitative Structure Activity Relationships (QSAR)

ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™

Quantitative Systems Pharmacology/Toxicology (QSP/QST)

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- Overview of the DILIsym Software
- Example GastroPlus / DILIsym Application

Why is Drug-Induced Liver Injury Important?

FDA Briefing Document

Solithromycin Oral Capsule and Injection

Meeting of the Antimicrobial Drugs Advisory Committee (AMDAC)

November 4, 2016

The committee will discuss new drug applications (NDAs) 209006 and 209007 for solithromycin oral capsule and injection, submitted by Cempra Pharmaceuticals, for the proposed indication of treatment of community acquired bacterial pneumonia.

FDA panel narrowly backs Cempra antibiotic
Posted: 5:17 p.m. Friday, Nov. 4, 2016

The Associated Press
WASHINGTON —

The Food and Drug Administration's outside experts voted 7-6 in favor of the drug, saying its effectiveness outweighed risks of liver toxicity seen in company studies. The vote is nonbinding but the FDA often follows the advice of its panelists.

Reuters News – Thu Dec 29, 2016. 9:05 am EST

"The agency recommended an additional 9,000 patient study to rule out risk".

Cempra lost $1B of valuation in 1 day

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The DILI-sim Initiative is a Partnership between DILIsym Services and Pharmaceutical Companies to Minimize DILI

Select Sample of Current Companies Licensing DILIsym

- *Overall Goals*
  - *Improve patient safety through QST*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *19 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $8 million total invested in project*

FDA

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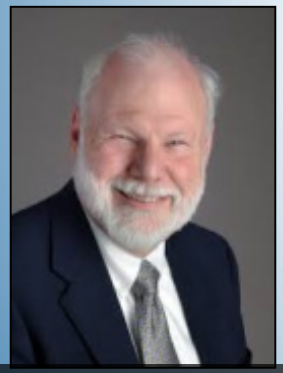
DILI-sim SAB Includes World Class Scientists

Dr. Neil Kaplowitz
Professor of Medicine
USC Thomas H. Brem Chair in Medicine
Chief, Division of Gastroenterology and Liver Diseases

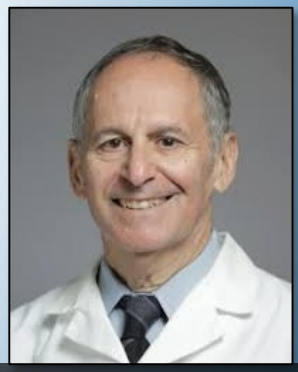
Dr. Paul B. Watkins
DIRECTOR, INSTITUTE FOR DRUG SAFETY SCIENCES
HOWARD Q. FERGUSON DISTINGUISHED PROFESSOR OF MEDICINE
UNC Eshelman School of Pharmacy

Dr. Kevin Park
Head of Institute of Translational Medicine / Director, MRC Centre for Drug Safety Science, University of Liverpool



Dr. Jack Uetrecht
Professor, Canada Research Chair in Adverse Drug Reactions
University of Toronto

Dr. Robert Roth
Distinguished Professor of Pharmacology & Toxicology
Director, Graduate Training Program in Environmental and Integrative Toxicological Sciences, Center for Integrative Toxicology
Michigan State University



David Pisetsky
Professor of Medicine
Professor of Immunology
Member of the Duke Cancer Institute
Member of the Duke Human Vaccine Institute

Stage 3 Will Include Key Components Necessary for Predicting Idiosyncratic Liver Injury

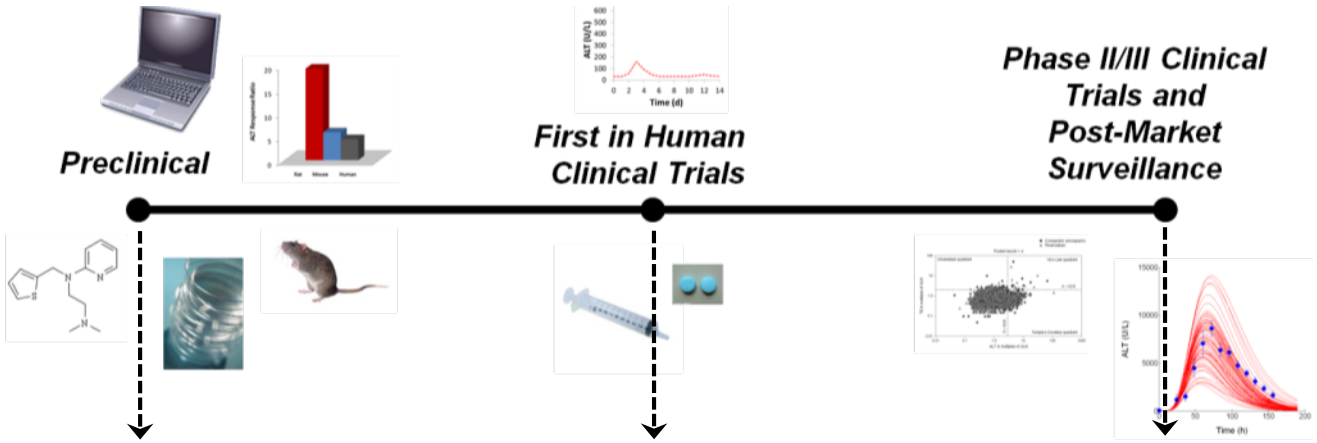
2012 Stage 1	2015 Stage 2	2018 Stage 3
Mechanisms • Reactive metabolites • Oxidative stress • Mitochondrial toxicity • Bile acid toxicity	*Mechanisms* • Lipotoxicity • Innate immunity	*Mechanisms* • Adaptive immunity • Cholestasis • Improve *in vitro* assay systems
Patients and animals • Rats, mice, dogs • Healthy volunteers	*Patients and animals* • Healthy volunteers • Disease area patients	*Patients and animals* • Larger more robust SimPops and biomarkers • Disease area patients
Compounds • Exemplars for optimization	*Compounds* • Exemplars for optimization • Exemplars for validation	*Compounds* • Exemplars for optimization • Exemplars for validation

SLP Software Advances

Application of DILIsym in Drug Development

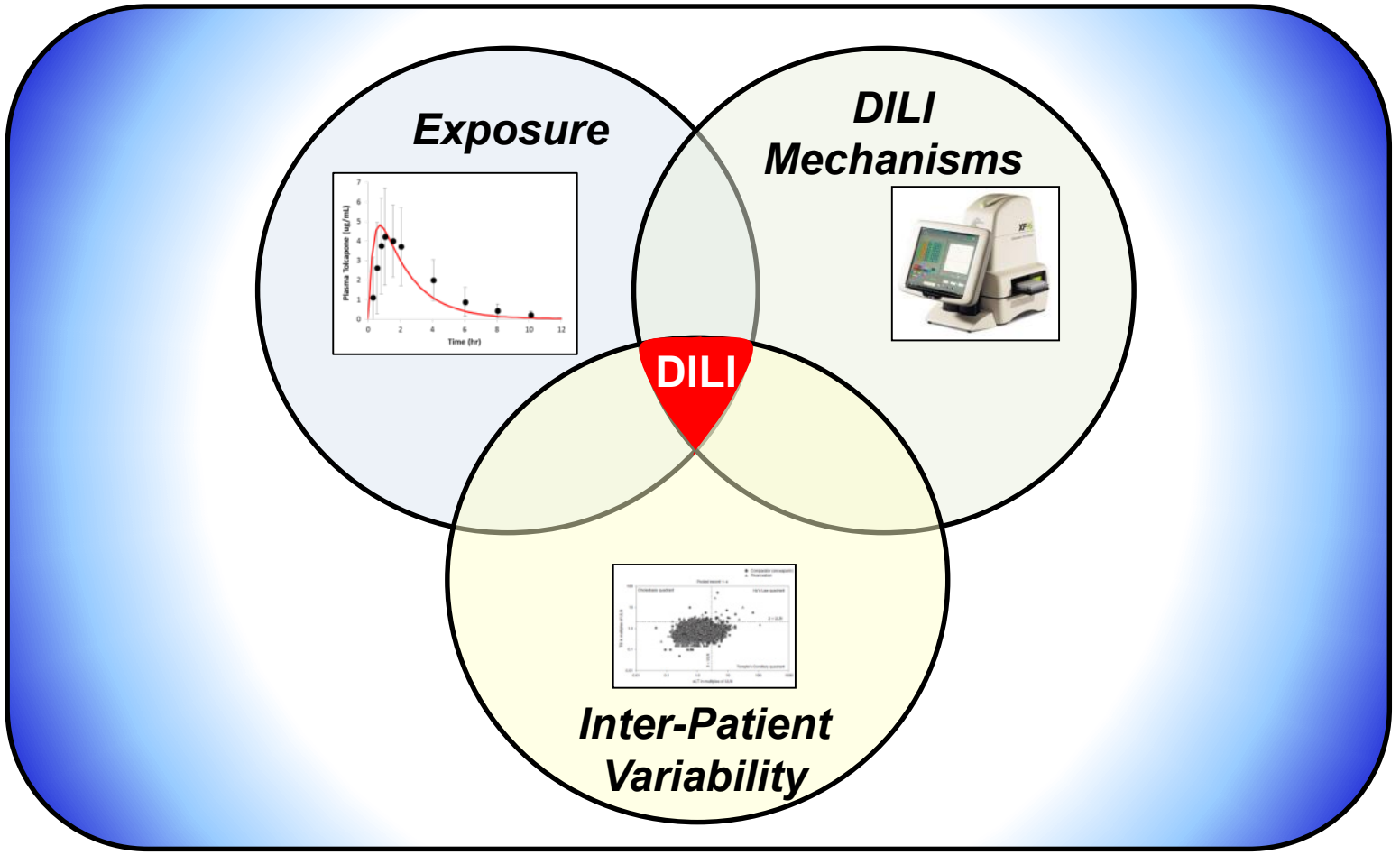
Applications of DILIsym Along the Drug Development Pipeline

Predictions of hepatotoxicity for humans and preclinical animal models



• Mechanism exploration • Rank candidates for DILI potential • Extrapolation from animal and *in vitro* findings to humans	• Dose optimization (risk versus presumed benefit) • Infer magnitude of injury based on measured biomarkers • Extrapolation from healthy volunteers to patient groups • Guide incorporation of emerging biomarker measurements in clinical trials • Analysis of mechanisms underlying observed liver signals	• Inform choice and timing of biomarker measurement • Aid identification of risk factors leading to personalized medicine approaches • Analysis of mechanisms underlying observed liver signals

DILIsym Predicts DILI via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



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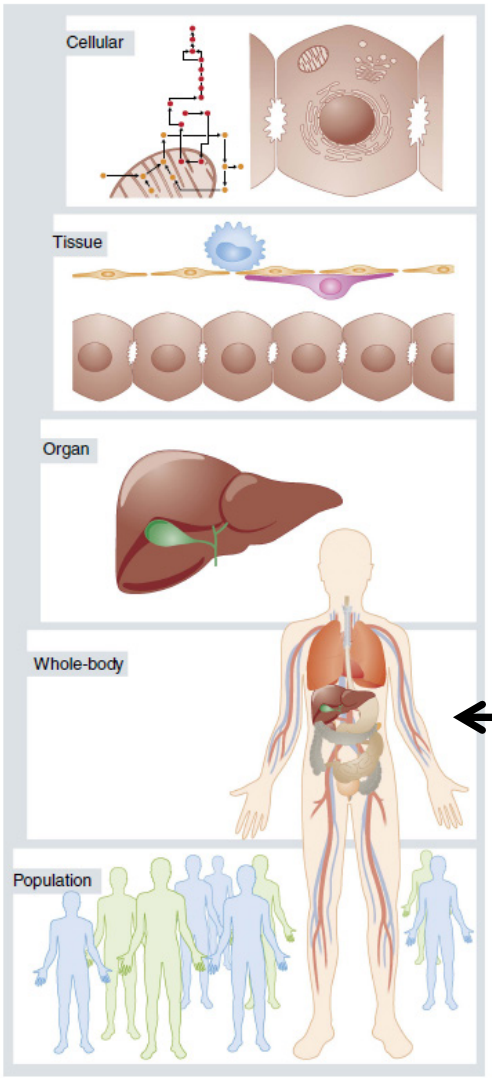
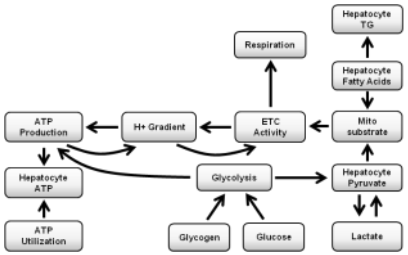
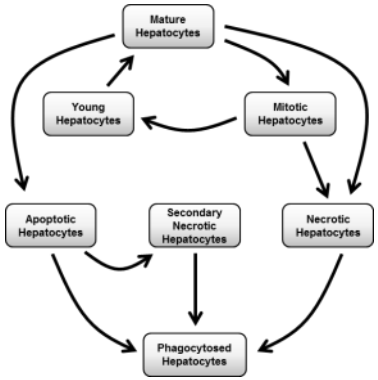
DILIsym: Quantitative Systems Toxicology



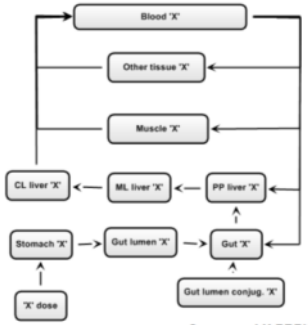
Mitochondrial dysfunction



Cellular life-cycle

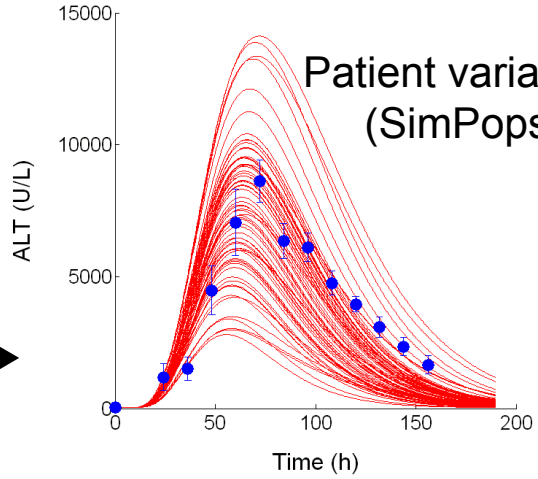


Drug distribution & metabolism



Compound X PBPK

Patient variability (SimPops)



Kuepfer 2010, Molecular Systems Biology

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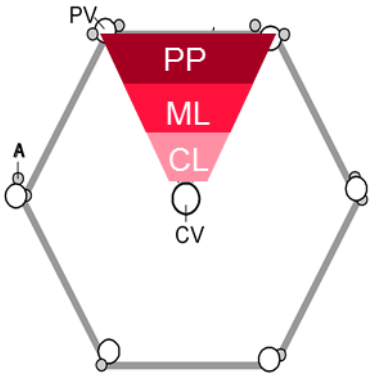
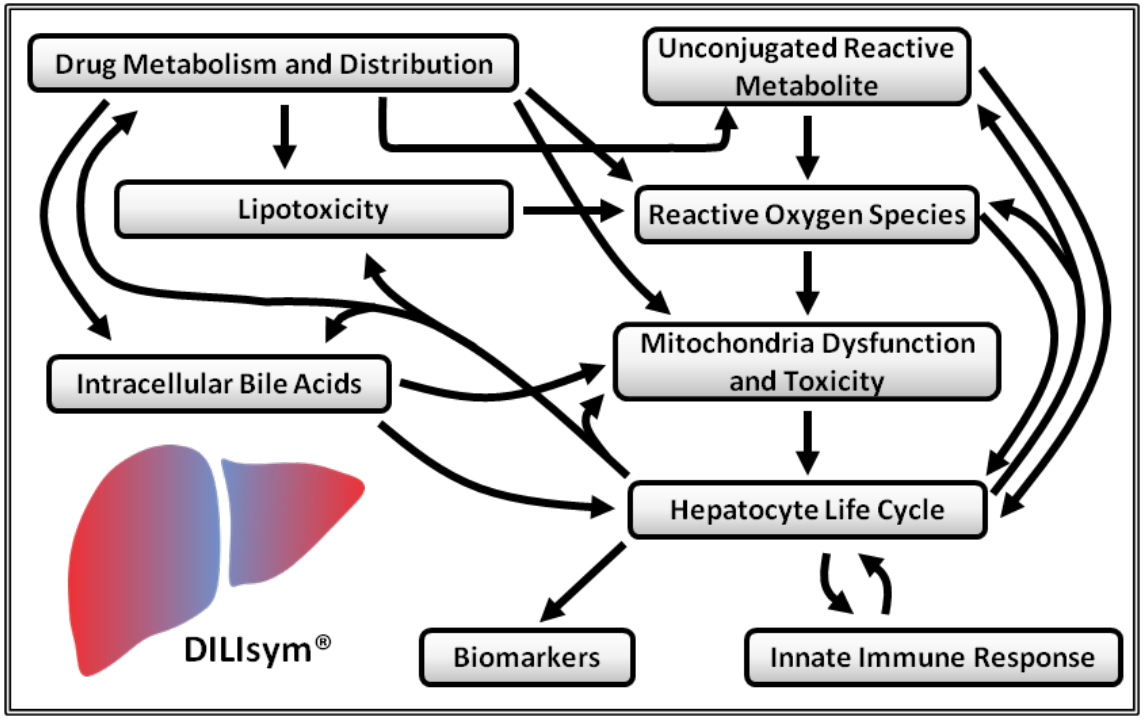
DILIsym Overview

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential cellular processes represented to multiple scales in interacting sub-models**
  - Pharmacokinetics
  - Dosing (IP, IV, Oral)
  - Transporter Inhibition
  - Drug metabolism
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Bile acid mediated toxicity
  - Steatosis and lipotoxicity
  - Cellular energy balance
  - Hepatocyte apoptosis and necrosis, and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers



- **Over 30 detailed representations of optimization or validation compounds**
- **Single and combination drug therapies**



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DILIsym Utilizes Various Data Types to Inform Decisions

Exposure Data

PBPK Modeling

- **Compound Properties**
  - Tissue partition coefficients
- **Tissue penetration studies**
  - *Liver to blood ratio*
- **Pharmacokinetic data**
  - *Absorption, extra-hepatic clearance, metabolites*
- ***in vitro* data**
  - *Metabolite synthesis, active uptake*

Modeling & Simulation

DILIsym®

Simulations and Assays inform:

- **Prediction of DILI risk**
- **Participating DILI mechanisms**
- **Characteristics of patients at risk for DILI**
- **Drug dosing paradigms**
- **DILI monitoring strategies**

In vitro Mechanistic DILI Data

Assays performed to determine quantitative aspects of DILI mechanisms

- **Oxidative stress**
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity**
  - *ETC inhibition*
  - *Uncoupling*
- **Bile acid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

Clinical Data

- **Dosing Protocols, fasting/fed state, meal times**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

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DILIsym Performance Review – Level 1

- Key Question: would the weight of evidence from the drug case and from the DILIsym results have led to the same overall conclusion regarding the presence or absence of a possible drug-induced liver injury liability for the compound?
  - Secondary question: was the general magnitude of injury over-predicted (O), under-predicted (U), or correctly predicted (C), based on severity and frequency of injury?

Drug	Human	Rat	Mouse	Dog
Entacapone (Clean)	C	--	--	--
Tolcapone (DILI)	C	--	--	--
Methapyrilene (Clean)	C	C	C	--
Troglitazone (DILI)	O	C	--	--
Pioglitazone (Clean)	C	--	--	--
AMG009 (DILI)	C	C	--	--
Compound A (DILI)	U	--	--	--
Bosentan (DILI)	O	C	--	--
Telmisartan (Clean)	C	--	--	--
Tolvaptan (DILI)	O	--	--	--
Compound B (DILI)	O	--	--	--
Compound C (DILI)	O	C	--	--
Compound E (DILI)	U	--	--	--
AMAP (N/A)	--	--	U	--
Compound F (DILI)	C	--	--	--
AMG 853 (Clean)	C	--	--	--
Compound G (DILI)	O	--	--	--
Solithromycin (DILI)	C	--	--	--
CKA (Clean/Some DILI)	U	U	--	--
Compound H (Clean)	C	--	--	--
Erythromycin (DILI)	C	--	--	--
Clarithromycin (DILI)	C	--	--	--
Compound N (DILI)	O	--	--	--
Compound O (DILI)	C	--	--	--
Compound P (DILI)	U	--	--	--
Telithromycin (DILI)	U	--	--	--
Azithromycin (DILI)	U	--	--	--
MK-0536 (DILI)	U	--	--	--
TAK-875 (DILI)	C	--	--	--
Metformin (clean)	C	--	--	--
Phenformin (lactic acidosis)	C	--	--	--
Compound Q sc. 1 (DILI)	U	--	--	--
Compound Q sc. 2 (DILI)	U	--	--	--
Lixivaptan (clean)	C	--	--	--
Compound R (DILI)	U	--	--	--
Compound S (DILI)	O	--	--	--
Sitaxsentan (DILI)	C	--	--	--
Ambrisentan (clean)	C	--	--	--
Didanosine (clean)	C	--	--	--
Compound L (DILI)	U	--	--	--

Color Key – Accuracy of DILIsym	
Good	
Bad	

83% (33/40) generally predicted well

HUMAN **MICE** **RATS** **DOGS**

Clinical, Preclinical Data and Simulation Results

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Known DILIsym Applications Submitted to or Intended for Regulatory Agencies

N	Agency	Context	Scenario	Simulation Type	Presented/ Submitted By
1	FDA	Simulation results included in formal, written correspondence to agency	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
2	FDA	Simulation results included in formal, written correspondence to agency	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
3	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor and DSS
4	BARDA*	Simulation results presented to sponsor group at BARDA	Sponsor responding to concerns over liver safety signals	Mechanistic liver injury (predictive)	DSS and Sponsor
5	FDA and Japanese FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor addressing concerns over liver safety in NDA submission	Mechanistic liver injury (predictive)	Sponsor and DSS
6	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor repurposing compound that failed due to hepatotoxicity in IND submission	Mechanistic liver injury (predictive)	Sponsor and DILIsym Services
7	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor addressing concerns over liver signals from other drug in same class with same indication	Mechanistic liver injury (predictive)	Sponsor
8	FDA	Simulation results included in formal, written correspondence to agency	Sponsor addressing concerns over liver safety in NDA submission	Mechanistic liver injury (predictive)	Sponsor
9	FDA	Simulation results included in formal, written correspondence to agency and discussed during call with FDA	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
10	FDA and global regulators	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver safety signals	Hepatocyte loss (biomarker fitting) Mechanistic liver injury (predictive)	Sponsor
11	FDA	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver signals from other drug in same class with same indication	Mechanistic liver injury (predictive)	Sponsor
12	FDA	Sponsor intended to submit simulation results	Sponsor reformulating existing compound on the market	Mechanistic liver injury (predictive)	Sponsor
13	FDA	Sponsor intended to submit simulation results and present at meeting	Sponsor addressing concerns over liver safety signals	Mechanistic bilirubin (predictive)	Sponsor

*Not a direct regulatory agency, but affiliated closely with NIH and FDA

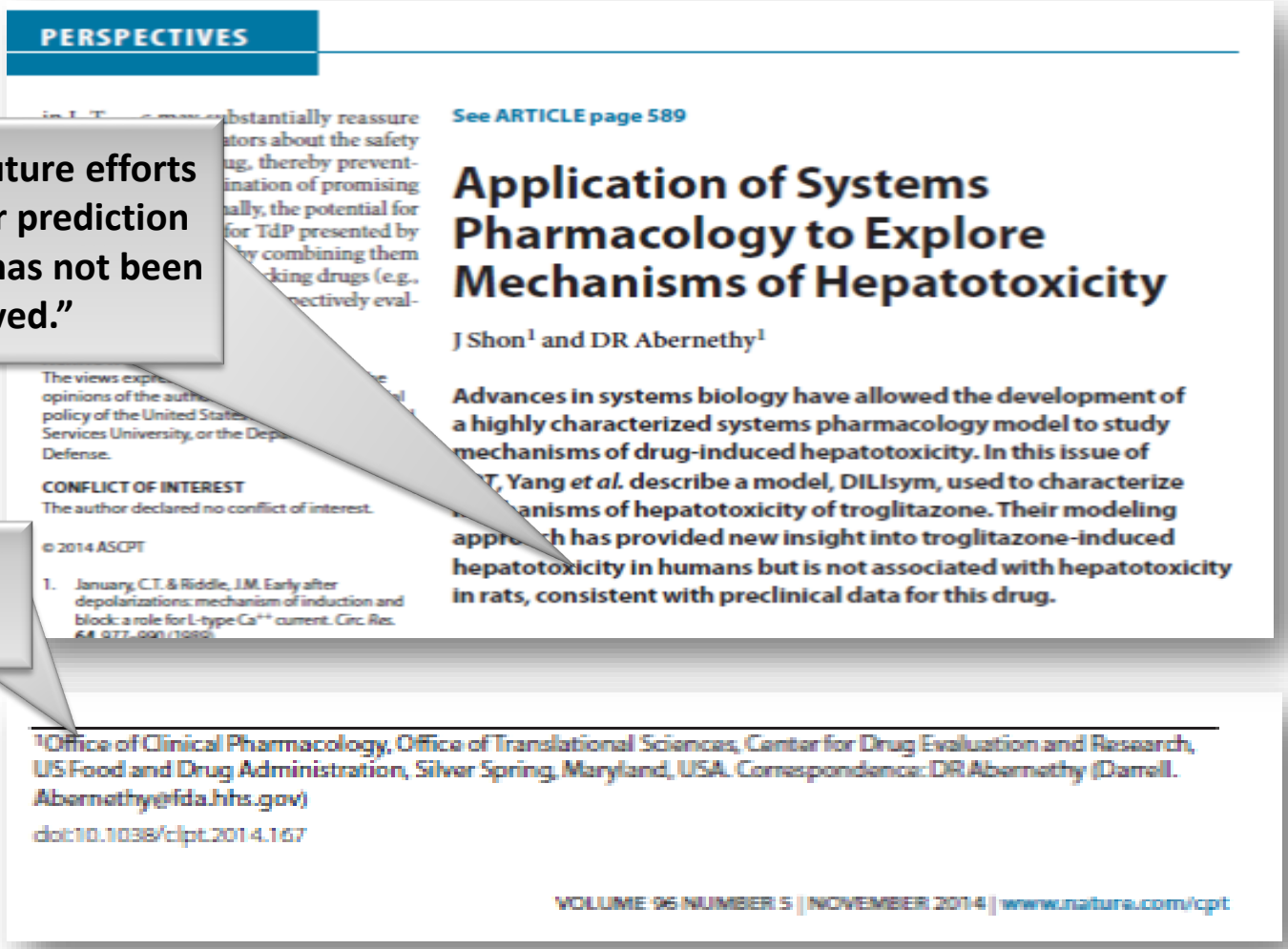
**Several additional sponsors have declared intent to include results in regulatory communications in the future

***Additional drug development teams have implied that regulators have informally requested or recommended DILIsym simulations

Scientists at the FDA Have Expressed a Strong Interest in DILIsym Results

"We look forward to future efforts to apply this model for prediction of hepatotoxicity that has not been clinically observed."

FDA Office of Clinical Pharmacology



PERSPECTIVES

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The views expr
opinions of the auth
policy of the United State
Services University, or the Dep
Defense.

CONFLICT OF INTEREST
The author declared no conflict of interest.

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1. January, C.T. & Riddle, J.M. Early after depolarizations: mechanism of induction and block: a role for L-type Ca^{++} current. Circ. Res. 64, 977–990 (1989).

See ARTICLE page 589

Application of Systems Pharmacology to Explore Mechanisms of Hepatotoxicity

J Shon[1] and DR Abernethy[1]

Advances in systems biology have allowed the development of a highly characterized systems pharmacology model to study mechanisms of drug-induced hepatotoxicity. In this issue of CPT, Yang *et al.* describe a model, DILIsym, used to characterize mechanisms of hepatotoxicity of troglitazone. Their modeling approach has provided new insight into troglitazone-induced hepatotoxicity in humans but is not associated with hepatotoxicity in rats, consistent with preclinical data for this drug.

[1]Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA. Correspondence: DR Abernethy (Darrell.Abernethy@fda.hhs.gov)

doi:10.1038/clpt.2014.167

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DILIsym Talk Agenda

- Mechanistic mathematical modeling within drug development
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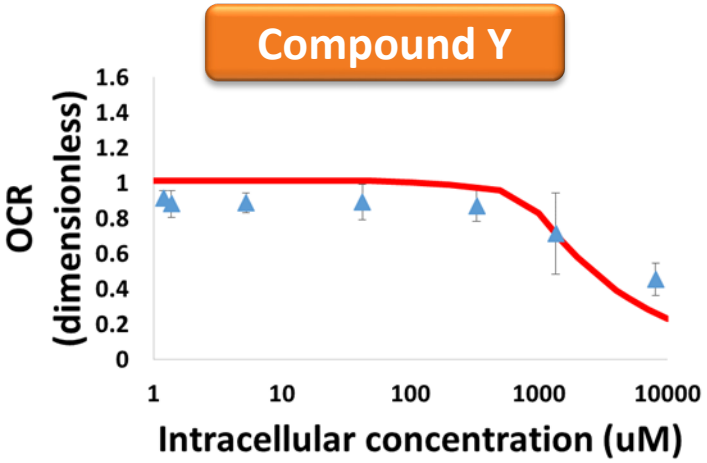
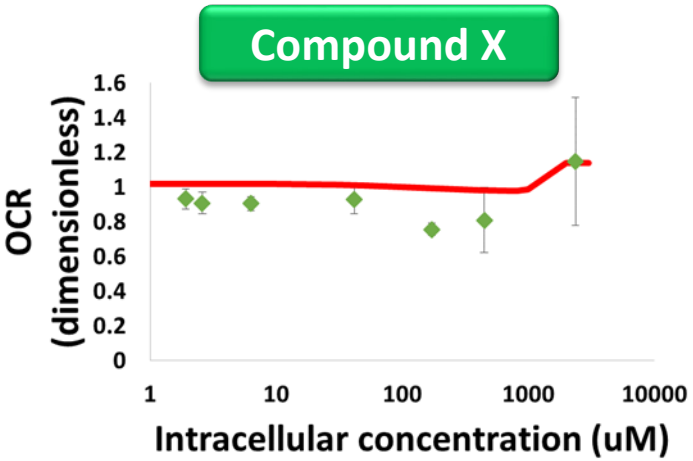
Example Project Goal – Assess Compound X and Compound Y

- The primary goal of this simulation work within the DILIsym software was to:
  - quantitatively and mechanistically assess the liver toxicity potential of Compound X and Compound Y combining clinical and mechanistic *in vitro* data with DILIsym and GastroPlus software simulations of previous or prospective clinical dosing paradigms.

Mitochondrial Toxicity Parameters Determined for Compound Y and Compound X

- Parameter values were fit to mitochondrial data for Compound Y and Compound X
  - Electron transport chain inhibition for Compound Y
  - Both electron transport chain inhibition and uncoupling for Compound X
  - 24 hour data used
- MITOsym and DILIsym used to parameterize both compounds





DILIsym Parameter	Compound Y Value	Compound X Value	Units
Coefficient for ETC inhibition 1	38,000	Not used	µM
Coefficient for ETC Inhibition 3	0.1	4,200	µM
Max inhibitory effect for ETC inhibition 3	0.2	0.4 (max effect)	dimensionless
Uncoupler 1 effect Km	No effect	15,000	µM
Uncoupler 1 effect Vmax	No effect	22	dimensionless
Uncoupler 1 effect Hill	No effect	4	dimensionless

Preclinical Data and Simulation Results

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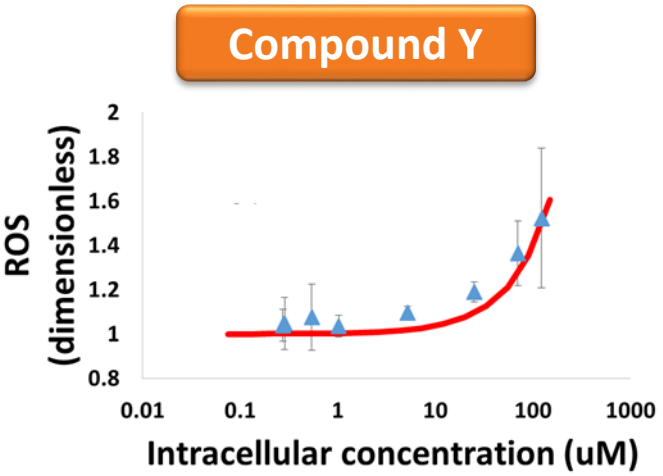
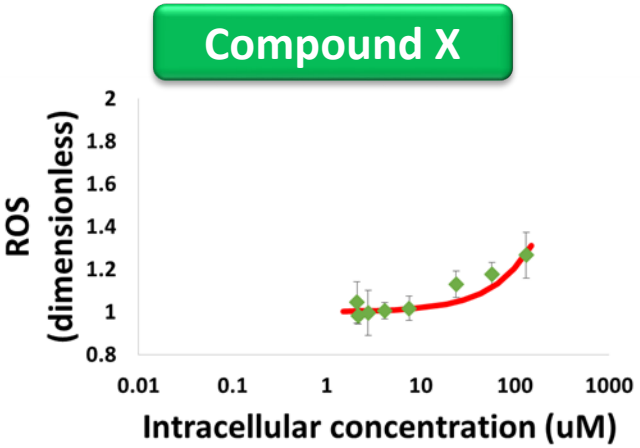
Oxidative Stress Parameters Determined for Compound Y and Compound X

- Parameter values were fit to 24-hour ROS data for Compound Y and Compound X
- DILIsym representation of *in vitro* environment used to parameterize both compounds
- Saturable model explored but did not lead to better fit



DILIsym Parameter	Compound Y Value	Compound X Value	Units
RNS/ROS production rate constant 1	3.4×10^{-4}	1.7×10^{-4}	mL/nmol/hr



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Preclinical Data and Simulation Results

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Compound Y Weakly Inhibits BSEP; Compound X Does Not

- Compound Y is a weak but noncompetitive/uncompetitive inhibitor of BSEP
- Compound X does not inhibit BSEP
  - No changes to V_{max} or K_m of transporters observed over course of assay

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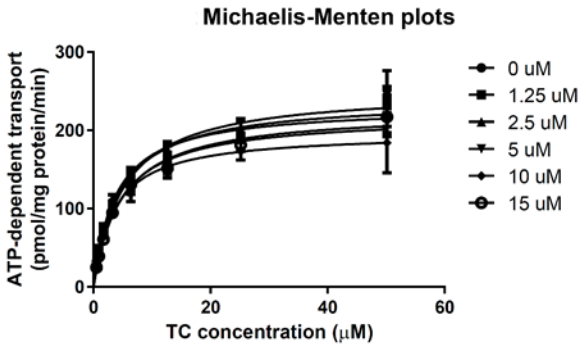
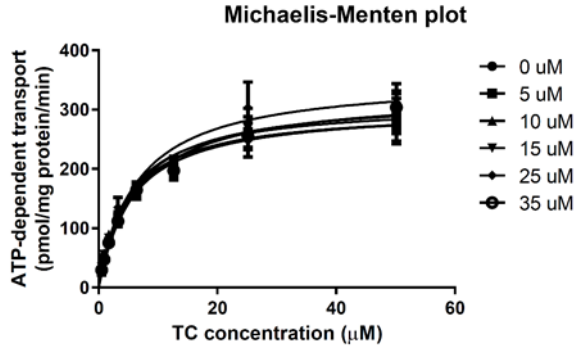
Compound Y; K_i = 140 μM, α = 0.6



Compound X; no inhibition



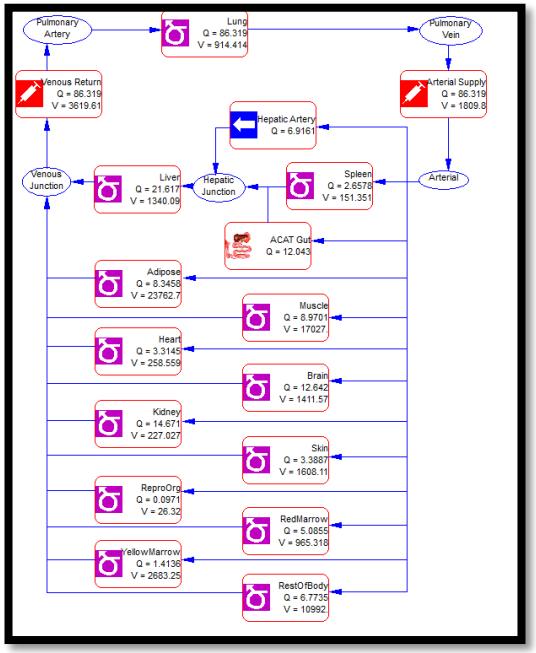
DILIsym Toxicity Parameters for Compound Y and X

Mechanism	Parameter	Unit	DILIsym Parameter Value*	
			Compound Y	Compound X
Mitochondrial Dysfunction	Coefficient for ETC inhibition 1	µM	38,000	Not used
	Coefficient for ETC Inhibition 3	µM	0.1	4,200
	Max inhibitory effect for ETC inhibition 3	dimensionless	0.2	0.4
	Uncoupler 1 effect Km	µM	No effect	15,000
	Uncoupler 1 effect Vmax	dimensionless	No effect	22
	Uncoupler 1 effect Hill	dimensionless	No effect	4
Oxidative Stress	RNS/ROS production rate constant 1	mL/nmol/hr	3.4×10^{-4}	1.7×10^{-4}
Bile Acid Transporter Inhibition	BSEP inhibition constant	µM	140	No inhibition
	BSEP inhibition alpha value	dimensionless	0.6	No inhibition
	NTCP inhibition constant	µM	No inhibition	No inhibition
	MRP4 inhibition constant	µM	40	75

*Values shown in the table for DILIsym input parameters should not be interpreted in isolation with respect to clinical implications, but rather, should be combined with exposure in DILIsym to produce simulations that have predictive and insightful value

GastroPlus PBPK Model Used to Predict Liver Exposure of Compound Y and Compound X

- Data on Compound Y and Compound X pharmacokinetics not available in the literature
  - No plasma time courses available; no *in vitro* or animal studies available either
  - Data on T_{max}, Compound Y $f_{u,plasma}$ available
  - *In vitro* data on liver distribution available from intracellular data collected for this project
- Structure of each compound available online
  - QSAR modeling using ADMET Predictor and GastroPlus provided the best possible estimate of Compound Y and Compound X distribution and pharmacokinetics



- Plasma time course was estimated in GastroPlus and translated into DILIsym using "specified data" option
  - Liver:plasma partition coefficient was calculated from the cell:media ratio in the *in vitro* data and used as input into GastroPlus; the remainder of the parameters were calculated by ADMET Predictor
- Both compounds distribute significantly into the liver
  - Compound Y average cell:media was 18; Compound X average cell:media was 9

Compound Y

Compound X

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Compound Y PBPK Representation Calculated at Clinical Dose

- GastroPlus predictions for liver and plasma at clinical dose shown at right
  - PBPK model specific predictions shown below
  - Dose escalation was simulated

Scale Pediatric Fup & Rbp

Blood/plasma Conc Ratio: 0.72

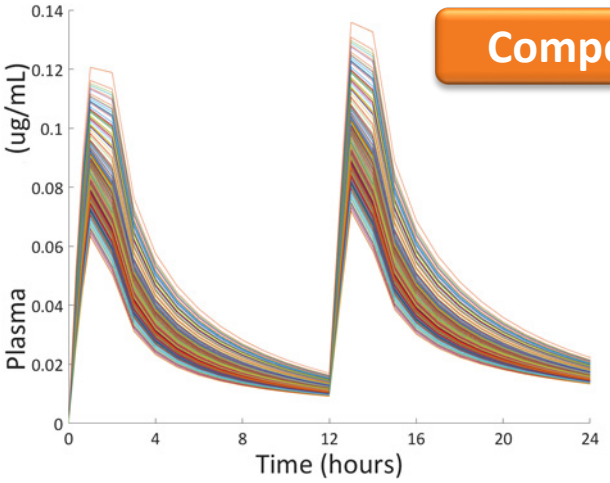
Use Exp Plasma Fup [%]: 4.3

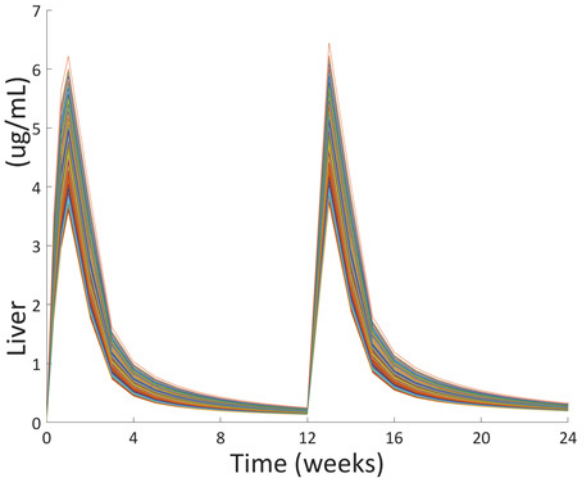
Use Adj Plasma Fup [%]: 2.6893

PBPK Summary

Tissue	Kp	CL	CLint	Fut/FuInt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.51	0.000	0.000	0.053
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	5.33	0.000	0.000	0.005
Muscle	1.66	0.000	0.000	0.016
Liver	18.30	0.000	0.000	0.001
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	1.69	0.000	0.000	0.016
Heart	1.89	0.000	0.000	0.014
Brain	4.24	0.000	0.000	0.006
Kidney	1.69	0.318	0.000	0.016
Skin	2.17	0.000	0.000	0.012
ReproOrg	1.70	0.000	0.000	0.016
RedMarrow	4.70	0.000	0.000	0.006
YellowMarrow	5.33	0.000	0.000	0.005
RestOfBody	1.71	0.000	0.000	0.016

Compound Y





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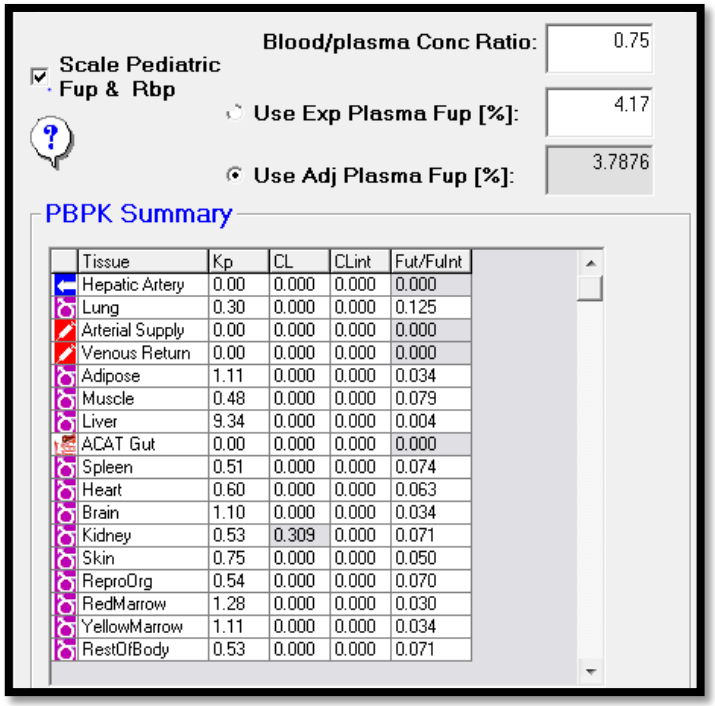
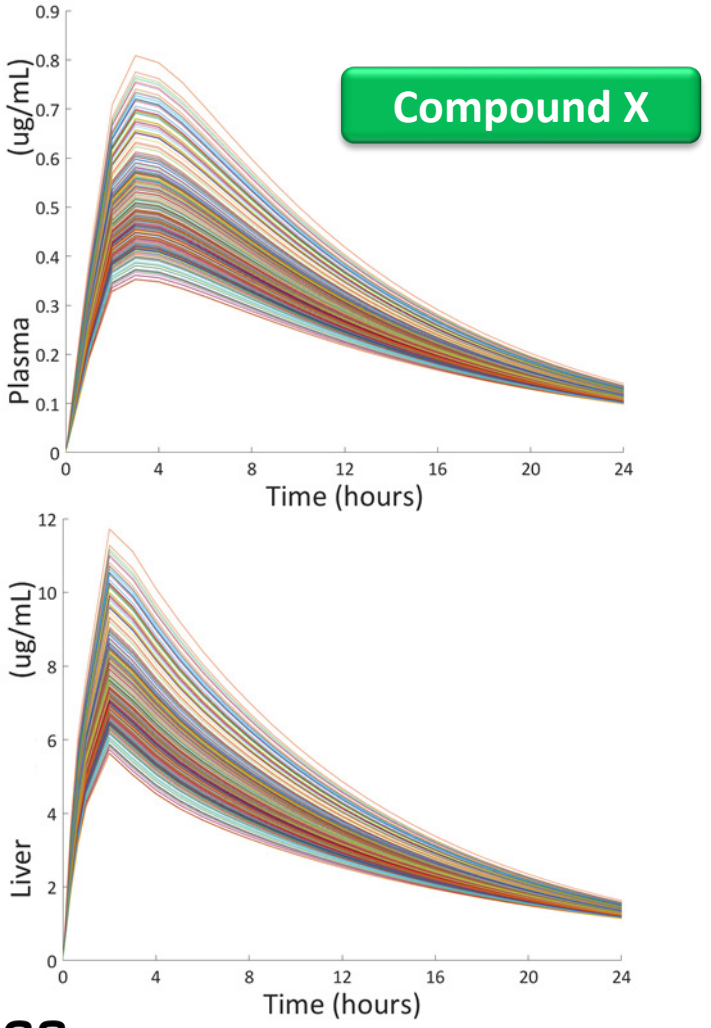
Compound X PBPK Representation Calculated at Clinical Dose

- GastroPlus predictions for liver and plasma at clinical dose for 25 days shown at right
  - PBPK model specific predictions below
  - Dose escalation and alternate protocols were also simulated

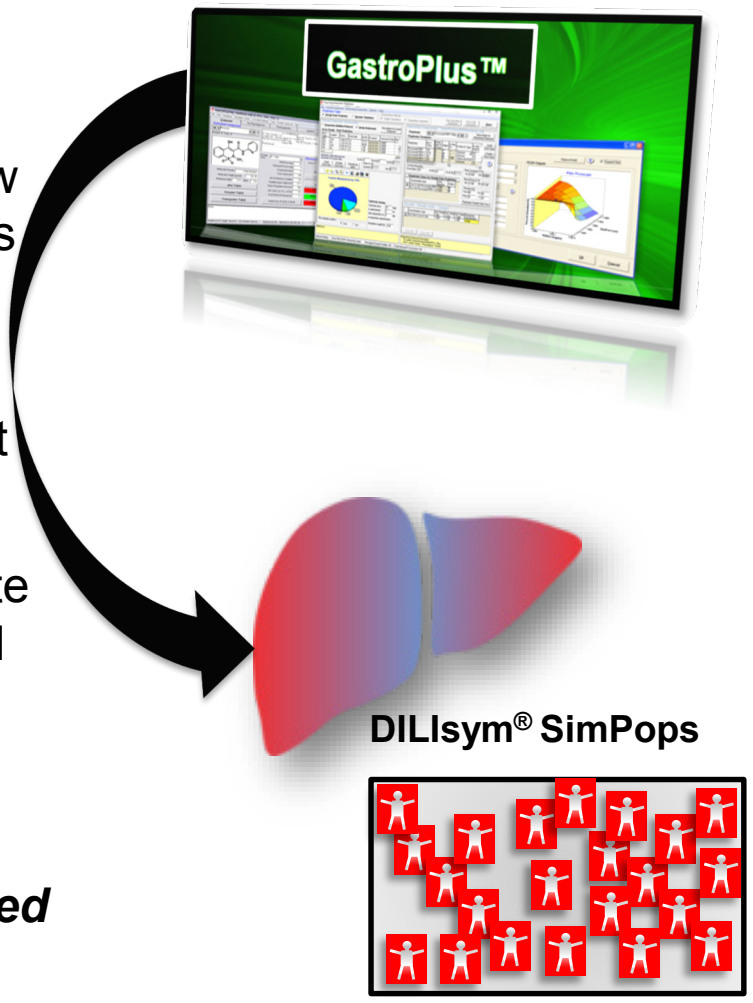


Tissue	Kp	CL	CLint	Fut/Fulnt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.30	0.000	0.000	0.125
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	1.11	0.000	0.000	0.034
Muscle	0.48	0.000	0.000	0.079
Liver	9.34	0.000	0.000	0.004
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	0.51	0.000	0.000	0.074
Heart	0.60	0.000	0.000	0.063
Brain	1.10	0.000	0.000	0.034
Kidney	0.53	0.309	0.000	0.071
Skin	0.75	0.000	0.000	0.050
ReproOrg	0.54	0.000	0.000	0.070
RedMarrow	1.28	0.000	0.000	0.030
YellowMarrow	1.11	0.000	0.000	0.034
RestOfBody	0.53	0.000	0.000	0.071



GastroPlus 9.6 Allows for Efficient Use of GastroPlus PBPK Models in Combination with DILIsym SimPops

- GastroPlus users build PBPK models within GastroPlus
- The “DILIsym” simulation mode in v9.6 will allow users to select a mapping of GastroPlus outputs to DILIsym PK inputs
- All DILIsym SimPops and SimCohorts are embedded within GastroPlus so user can select option of their choice
- Exported DILIsym Specified Data Excel template will be seamlessly compatible with DILIsym and contain PK outputs for **the right number of body-weight matched** rats, dogs, mice or humans
- ***This makes the manual creation of a Specified Data template unnecessary***



SimPops Results Show Compound X and Compound Y to be Safe at Clinical Doses; ALT Elevations Occur at Higher Doses for Both Compounds

Compound Y | Compound X

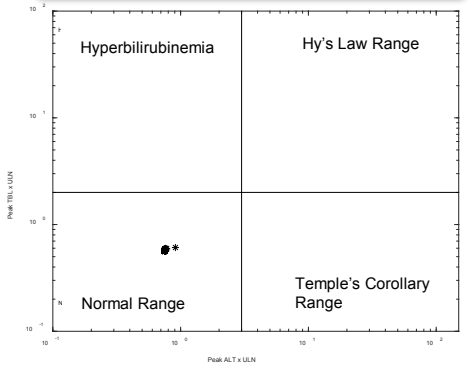
- Neither Compound Y nor Compound X are predicted to cause toxicity at the highest clinical dose
  - Some exposure variability included in these predictions due to GastroPlus population generation
- Both Compound Y and Compound X are predicted to cause mild ALT elevations at supratherapeutic doses
  - No bilirubin elevations or Hy's Law cases occurred in simulations with Compound X
  - 2 Hy's Law cases occurred at 10x clinical dose simulations with Compound Y

	Compound	Dosing Protocol	Simulated* ALT > 3X ULN**
Compound Y	Compound Y	1X Dose, 12 weeks	0% (0/285)
		2X Dose, 12 weeks	0% (0/285)
		5X Dose, 12 weeks	0.3% (1/285)
		10X Dose, 12 weeks	10.2% (29/285)
Compound X	Compound X	1X Dose, 15 days	0% (0/285)
		2X Dose, 15 days	0% (0/285)
		5X Dose, 15 days	1.1% (3/285)
		10X Dose, 15 days	11.6% (33/285)

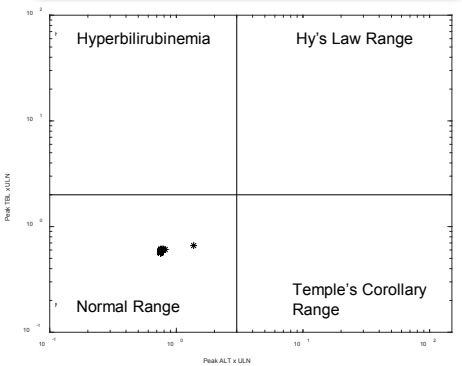
*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used
**Upper limit of normal (ULN) in DILIsym is 40 U/L

SimPops Results Show Lack of Severe Liver Injury for Both Compound Y and Compound X at Clinical Doses

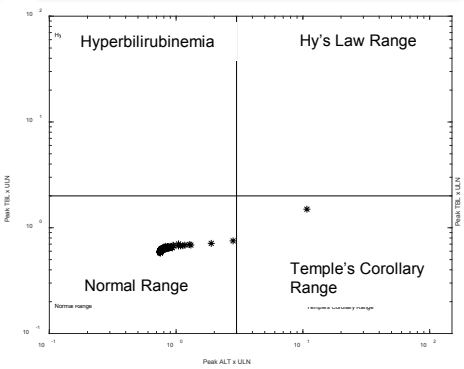
Compound Y; 1X Dose, 12 weeks



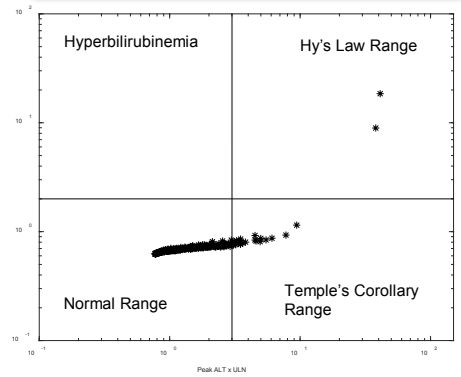
Compound Y; 2X Dose, 12 weeks



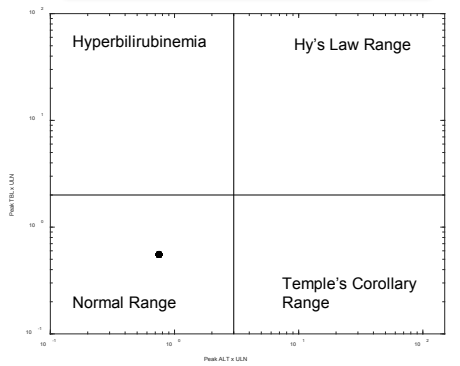
Compound Y; 5X Dose, 12 weeks



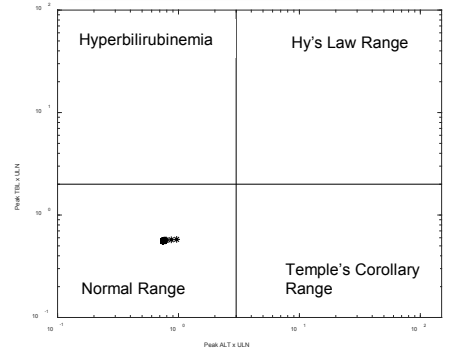
Compound Y; 10X Dose, 12 weeks



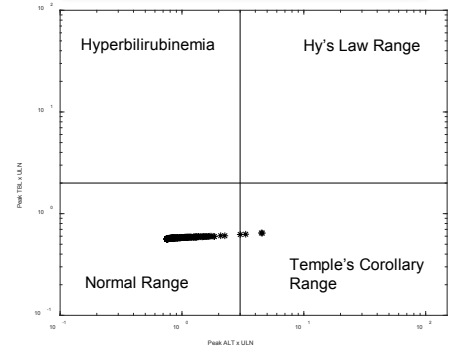
Compound X; 1X Dose



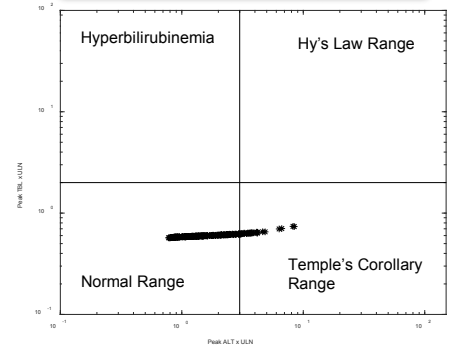
Compound X; 2X Dose



Compound X; 5X Dose



Compound X; 10X Dose



*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used
**Upper limit of normal (ULN) in DILIsym is 40 U/L

Simulation Results

DILIsym S

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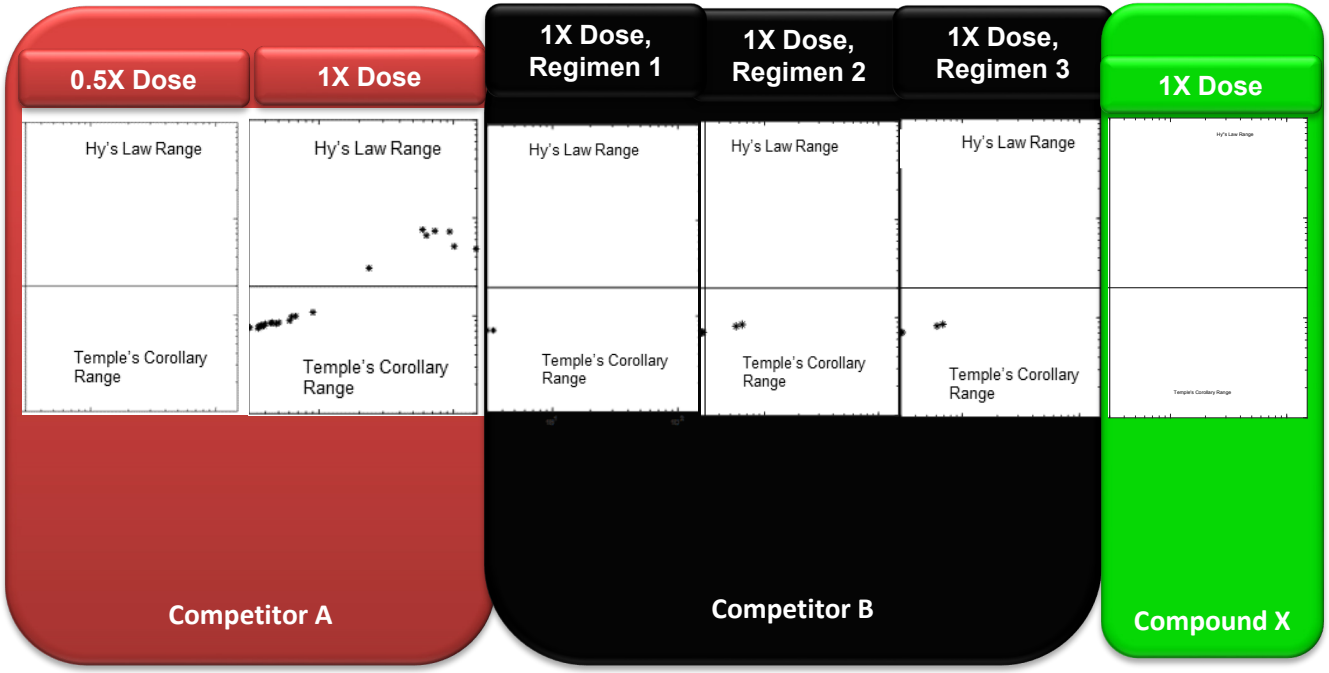
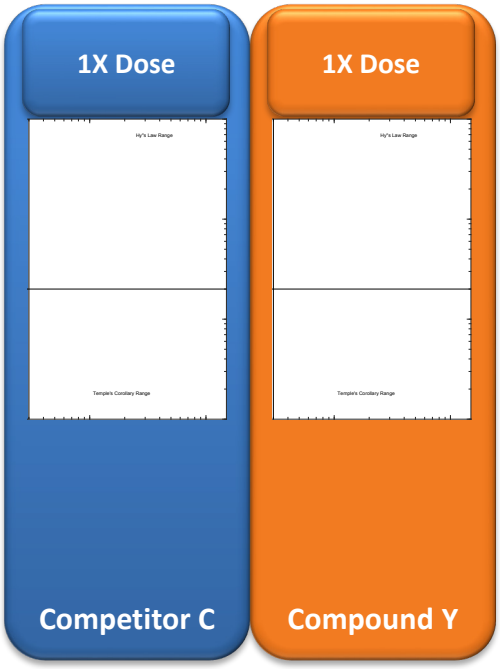
Focus on Hy's Law Side of eDISH Plot – Comparison of Competitors and Compound X at Clinical Doses (285 Simulated Individuals in All Cases)



Focus on Hy's Law Side of eDISH Plot – Comparison of Competitor and Compound Y at Predicted Clinical Doses (285 Simulated Individuals in All Cases)



Example Project Summary

- GastroPlus™ software, along with *in vitro* data, was used to construct PBPK representations to predict liver exposures for both compounds
- DILIsym parameters were successfully calculated from *in vitro* data for both compounds
- SimPops results show Compound X and Compound Y to be safe at projected clinical doses
- ALT elevations predicted within DILIsym at higher doses for both compounds
- SimPops results suggest that neither compound is likely to cause severe liver injury
- ***Phase IIb / III clinical trial results have subsequently confirmed the predictions for Compound Y***

Presentation Summary

- A combination of multiple mechanistic, *in silico* modeling approaches can facilitate drug discovery (QSAR, PBPK, QSP and QST)
- DILIsym is a mechanistic, mathematical model that has been constructed to support pharmaceutical risk assessment and decision making
- DILIsym simulation results have been included in numerous communications with regulatory agencies
- DILIsym has been applied to support decisions related to compound DILI risk throughout the clinical development pipeline
  - Evaluated and interpret clinical biomarker signals in clinical trials
  - Optimized clinical trial design (dose selection, monitoring, inclusion/exclusion criteria)
  - Translated preclinical safety risk to first in human clinical trials
  - Ranked compounds by risk

Acknowledgements - The DSS Team

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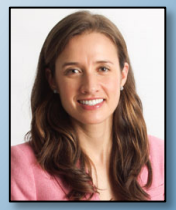

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