An Update on the DILI-sim Initiative and the DILIsym Tool

December 3, 2018

Identifying Right Target, Right Drug, Right Dose and Right Patient – PBSS

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

Talk Summary

- DILIsym is a modeling and simulation tool used for predicting and understanding drug-induced liver injury

- Tools like DILIsym can be used for finding the right dose with respect to safety liabilities; this is already happening in many cases

DILIsym Talk Outline

- Background on the DILIsym software tool

- Three example applications of using DILIsym for finding the “right dose” with respect to liver safety

DILI-sim Stage 3 Participation Includes 10 Current Members

- *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*

FDA

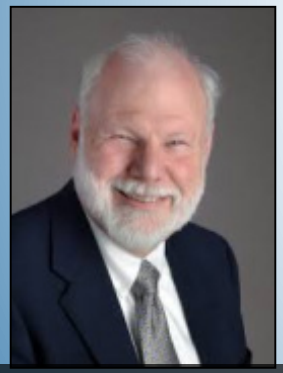
BOSTON PBSS
BOSTON PHARMACEUTICAL & BIOSCIENCE SOCIETY

DILI-sim Scientific Advisory Board

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

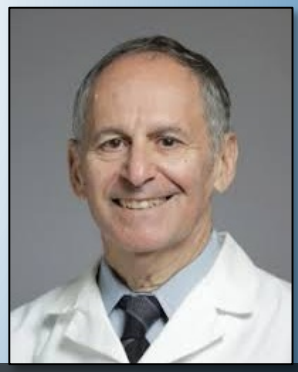
Dr. Paul B. Watkins
DIRECTOR, INSTITUTE FOR DRUG SAFETY SCIENCES
HOWARD Q. FERGUSON DISTINGUISHED PROFESSOR OF MEDICINE
UNC Eshelman School of Pharmacy

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



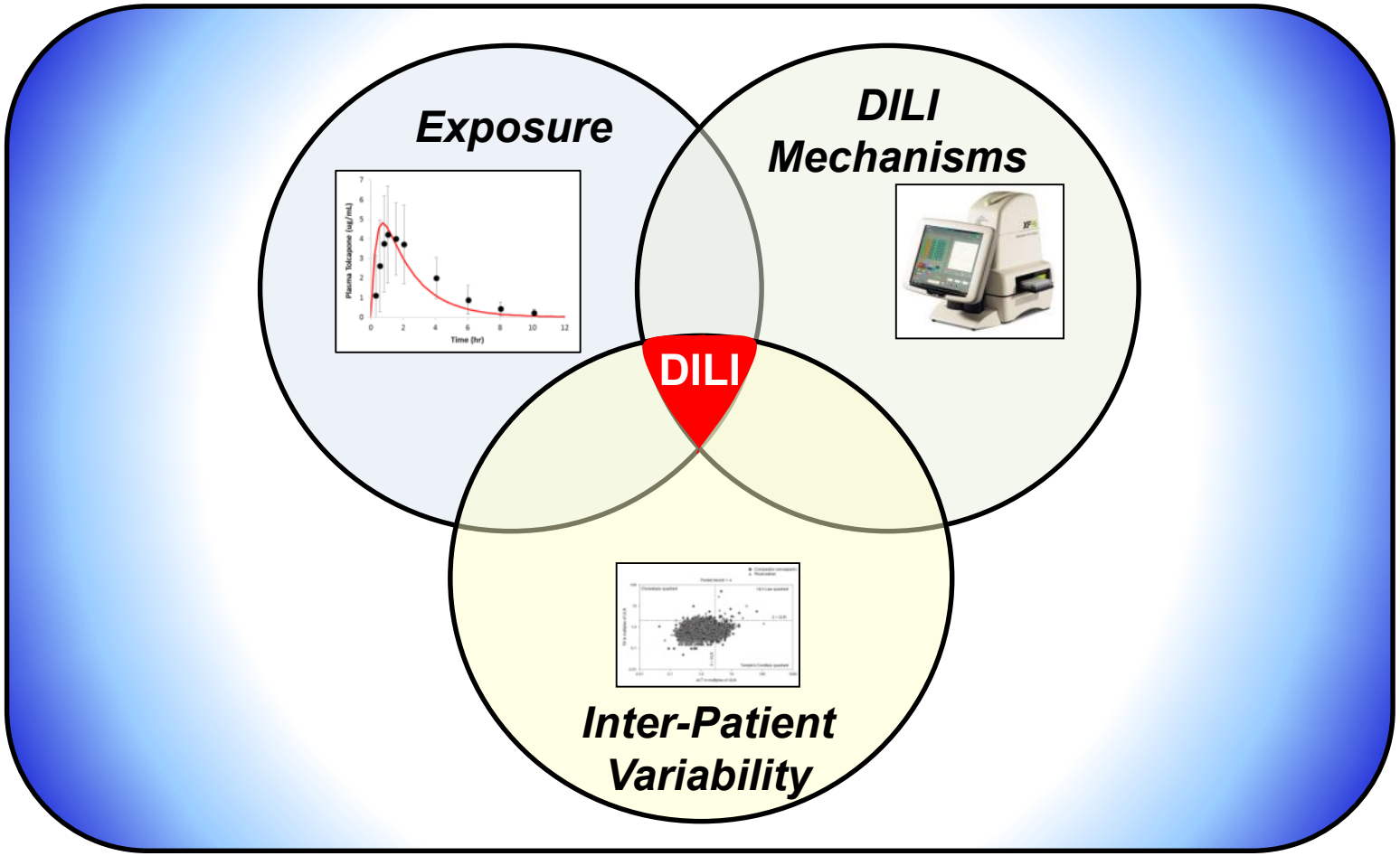
Dr. Jack Uetrecht
Professor, Canada Research Chair in Adverse Drug Reactions
University of Toronto

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

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



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

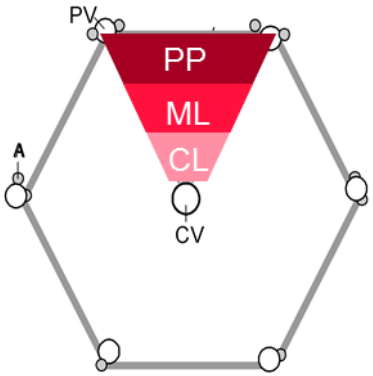
- **Oxidative stress**
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity**
  - *ETC inhibition*
  - *Uncoupling*
- **Bile acid / phospholipid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP, MDR3*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

Clinical Data

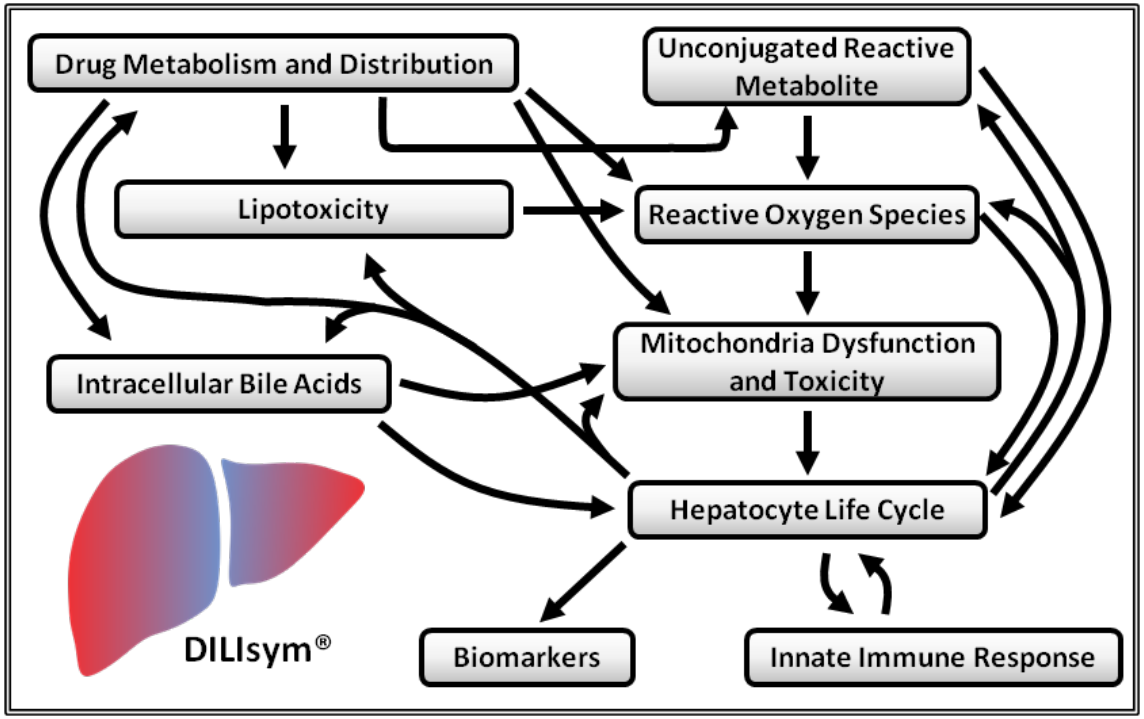
- **Dosing Protocols, fasting/fed state, meal times**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

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
  - Cholangeocyte apoptosis
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers



- **Over 60 detailed representations of optimization or validation compounds**
- **Single and combination drug therapies**



Biomarkers of Hepatocellular Function and Death Are Outputs of DILIsym

- Biomarkers are outputs of DILIsym
  - Used for validation
  - Used for comparison with clinical and preclinical data
  - Functional, necrotic, and apoptotic indicators
- More biomarkers being added as data are becoming available
  - GLDH
- Additional DILIsym outputs include:
  - Fraction of viable hepatocytes
  - Liver ATP
  - Liver glutathione
  - Circulating, liver, and excreted drug and metabolites

Marker	Category
Alanine aminotransferase (ALT)[1,2,3,4,5]	Necrosis
Bilirubin (total)[1,2,5]	Function/Cholestasis
Aspartate aminotransferase (AST)[1,2,3,4,5]	Necrosis
Prothrombin time [1,2]	Function
High mobility group box protein 1 (HMGB1)[1,10]	Necrosis/Apoptosis
Full length cytokeratin-18[1]	Necrosis
Cleaved cytokeratin-18[1]	Apoptosis
Sorbitol dehydrogenase (SDH)[1,6]	Necrosis
Arginase-1[9]	Necrosis
Liver derived mRNA[7] and miRNA[8] (miR122)	Necrosis

[1]Antoine *Xenobiotica* 2009; [2]Giannini *CMAJ* 2005; [3]Horn *Am J Clin Pathol* 1999; [4]Ozer J *Toxicology* 2008; [5]Hy's Law: Temple R *Pharmacoepidemiol Drug Saf* 2006; [6]Ozer *Toxicology* 2008; [7]Wetmore *Hepatology* 2010, , [8]Yang *Tox Sci* 2012, [9]Murayama *Clin Chimica Acta 2008*, [10]Harrill *Clin Pharmacol Ther* 2011, [11]Church *Exp Biol Med* 2017, [12]Yang *Clin Pharmacol Ther* 2017

Support of the DILI-sim Initiative Has Led to Significant Research Achievements

- **27** accepted manuscripts and **5+** more in final preparation focused on DILIsym content
  - Many of these are co-publications between DILIsym Services and a member or non-member pharma company
- DILIsym related publications have been cited **444** times as of September 2018
- Academic and government licenses issued for teaching and research, including to FDA across multiple divisions
- Seven versions of DILIsym released, including DILIsym v7A in Jan 2018
- At least **18** applications of DILIsym directly related to regulatory submissions for drug development (that we are aware of)
- More than **35** pharmaceutical companies have utilized DILIsym via consulting contracts for projects related to regulatory issues or applications, internal validation, or DILIsym use help internally
  - Insights go directly back into software for members
- **80%** of the simulation scenarios evaluated within DILIsym have generally been predicted well (of the 66 cases and 59 compounds simulated)

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 DILIsym Services
4	BARDA*	Simulation results presented to sponsor group at BARDA	Sponsor responding to concerns over liver safety signals	Mechanistic liver injury (predictive)	DILIsym Services and Sponsor
5	FDA and PMDA	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 DILIsym Services
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 and EMA	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 other regulators globally	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver safety signals	Hepatocyte loss (biomarker fitting) and 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
14	FDA	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver safety signals	Mechanistic liver injury (predictive)	Sponsor
15	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
16	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
17	EMA	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver safety signals	Mechanistic liver injury (predictive)	Sponsor
18	FDA	Agency reviewed results publicly available during evaluation	Agency addressing concerns over liver safety signals	Mechanistic liver injury (predictive)	Publicly available materials

*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

DILIsym Talk Outline

- Background on the DILIsym software tool

- Three example applications of using DILIsym for finding the “right dose” with respect to liver safety

Project Example Introduction

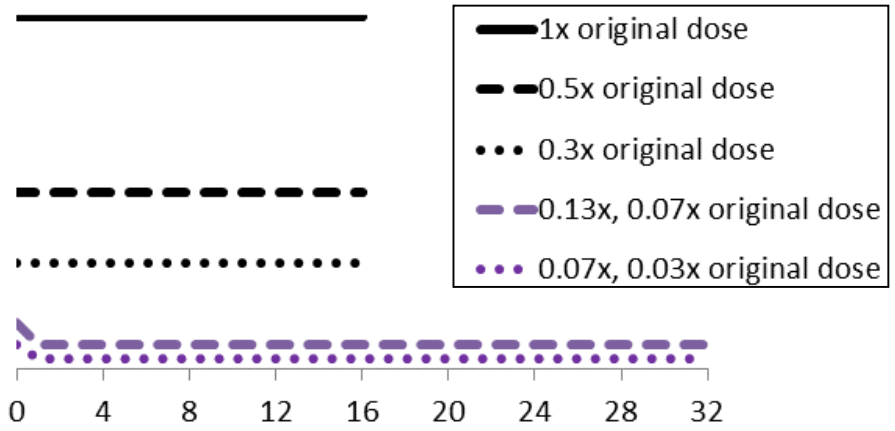
DILIsym Validation Using Clinical Data for Compound X

- ALT elevations were predicted in DILIsym simulations of previous Compound X clinical protocols where liver injury occurred clinically
  - Predicted delayed ALT elevations due to accumulation of a Compound X metabolite over time within DILIsym
  - Compound X metabolite-mediated mitochondrial electron transport chain (ETC) inhibition and oxidative stress (ROS) were responsible for predicted ALT signals

Prospective Compound X Development using DILIsym

- Optimal, prospective (much lower) dosing protocols were simulated within DILIsym to assess efficacy (exposure) and safety

Compound X Clinical Protocols for DILIsym Hepatotoxicity and Exposure Simulations



Past Clinical Studies

- 0.3X mg Compound X, 16 weeks
- 0.5X mg Compound X, 16 weeks
- 1X mg Compound X, 16 weeks

Prospective Studies

- 0.13X Compound X loading dose / 0.07 Compound X steady state dose, 32 weeks total
- 0.07X Compound X loading dose / 0.03 Compound X steady state dose, 32 weeks total

Final DILIsym Input Parameters For Compound X and Compound X Metabolite

Compound	Mechanism	Parameter	Unit	Value*
Compound X	Mitochondrial Dysfunction	Coefficient for ETC Inhibition 1	µM	3.5×10^{6}
Compound X Metabolite	Oxidative Stress	RNS/ROS production rate constant 1	mL/mol/hr	3×10^{-5}
	Mitochondrial Dysfunction	Coefficient for ETC Inhibition 2	µM	2000
		Coefficient for ETC Inhibition 3	µM	50
		Max inhibitory effect for ETC inhibition 3	Dimensionless	0.4

* 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

No Hepatotoxicity Predicted for Prospective Clinical Protocols

- Compound X effects simulated in SimPops (n=285) that represent variability in toxicity mechanisms and PK
- DILIsym predicted delayed hepatotoxicity with varying grades for previous clinical protocols
- **No ALT elevations predicted for prospective clinical protocols**

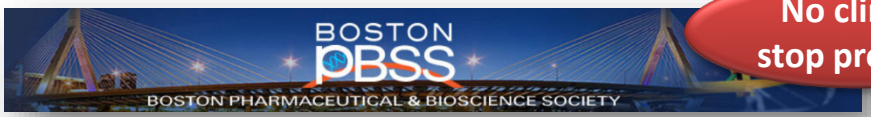
	Comp X Protocol	Grade 1 (ALT 1-2.5X ULN*)		Grade 2 and above (ALT > 2.5X ULN)		
		Observed	Simulated†	Observed	Simulated†	
Prospective	0.07X load / 0.03X steady, 32 weeks‡	-	0% (0/285)	-	0% (0/285)	Prospective
Prospective	0.13X load / 0. 07X steady, 32 weeks‡	-	0% (0/285)	-	0% (0/285)	Prospective
Previous	0.3X, 16 weeks	25% (13/52)	0.35% (1/285)	3.8% (2/52)	0.35% (1/285)	Previous
Previous	0.5X, 16 weeks	14% (1/7)	8.4% (24/285)	0% (0/7)	22.5% (64/285)	Previous
Previous	1X, 16 weeks	20% (1/5)	4.9% (14/285)	0% (0/5)	37.5% (107/285)	Previous

*upper limit of normal (ULN) in DILIsym is 40 U/L.

†SimPops™ Human_ROS_apop_mito_BA_v4A_1 (n=285) combined with Compound X PK variability used.

‡PROSPECTIVE clinical protocols

Clinical Data and Simulation Results

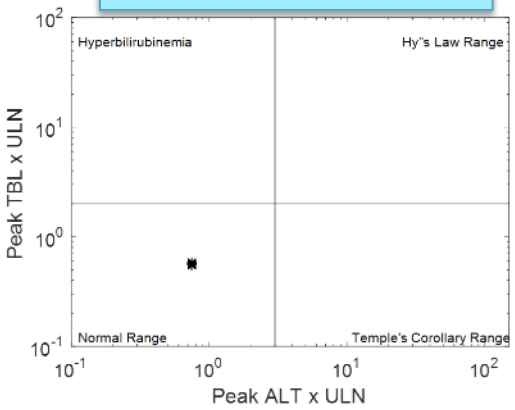


No clinical stop protocol

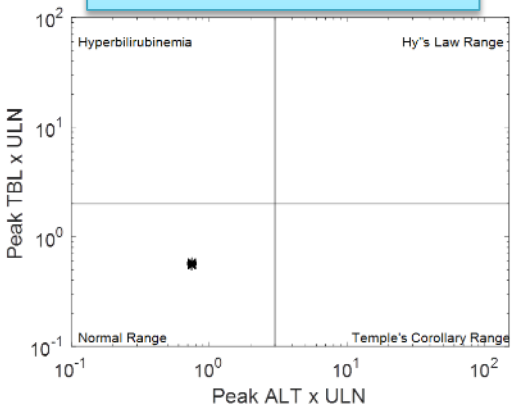
No Hepatotoxicity Predicted for Additional Prospective Clinical Protocols

- Dose dependent DILI frequency and severity correctly predicted for Compound X
- Prospective dose levels predicted to be safe from DILI
- Severity of response not appropriate to consider
  - Clinical stop protocol not included in simulations
  - Simulations may not have included some adaptation mechanisms

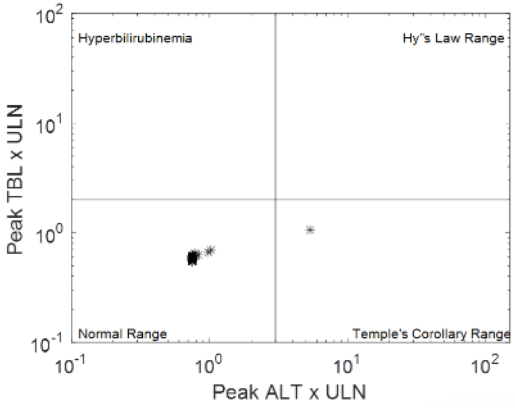
0.07X/0.03X Compound X Dosing



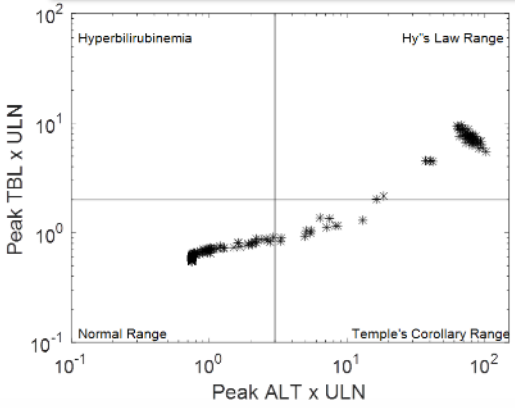
0.13X/0.07 Compound X Dosing



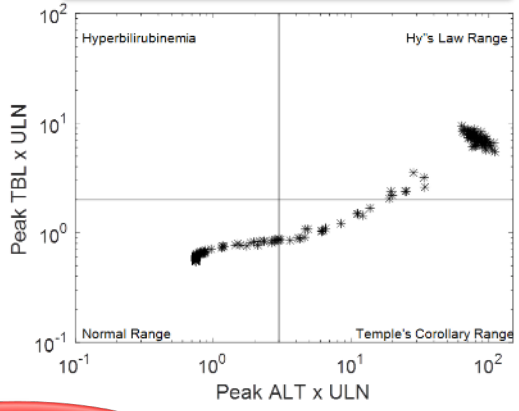
0.3X Compound X Dosing



0.5X Compound X Dosing



1X Compound X Dosing



Simulation Results

No clinical stop protocol

Project Example Executive Summary

DILIsym Validation Using Clinical Data for Compound X

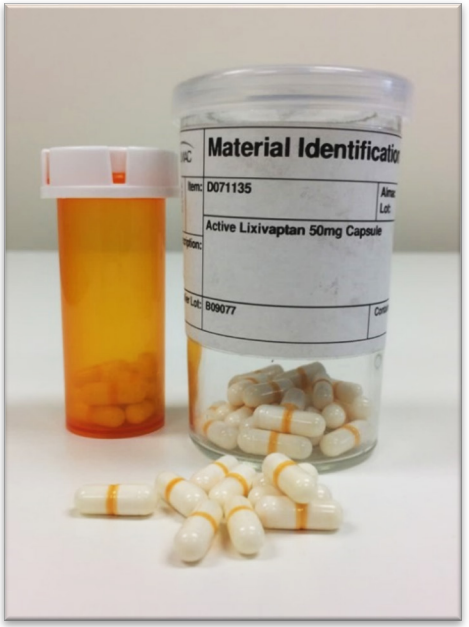
- ALT elevations were predicted in DILIsym simulations of previous Compound X clinical protocols where liver injury occurred clinically
  - Predicted delayed ALT elevations due to accumulation of a Compound X metabolite over time within DILIsym
  - Compound X metabolite-mediated mitochondrial electron transport chain (ETC) inhibition and oxidative stress (ROS) were responsible for predicted ALT signals

Prospective Compound X Development using DILIsym

- Optimal, prospective (much lower) dosing protocols were identified to achieve maximum drug efficacy using the DILIsym software and a custom SimPops with Compound X PK variability included
- ALT elevations were not predicted to occur in DILIsym simulations of Compound X dosing at the optimal, prospective clinical dose levels identified from the exposure simulations
- **The Company is finalizing their IND approval communications with FDA and will likely be able to move forward with clinical studies; they will use DILIsym iteratively after each cohort to predict the effects of the next dose selected**

Lixivaptan Background

- Lixivaptan is Palladio Bio's selective, competitive vasopressin V2 receptor antagonist
- Lixivaptan was originally developed by others for the treatment of hyponatremia associated with heart failure and syndrome of inappropriate antidiuretic hormone secretion (SIADH)
- An NDA for lixivaptan was filed in 2011; development was terminated following receipt of a CRL in 2012
- Palladio Biosciences acquired lixivaptan and intends to reposition lixivaptan for the treatment of Autosomal-Dominant Polycystic Kidney Disease (ADPKD)



Lixivaptan DILIsym Project

DILI Background

- An approved compound in the same class had no DILI signals in hyponatremia, but signals were observed in ADPKD patients
- Lixivaptan has had no DILI signals in hyponatremia

Question

- Will lixivaptan experience similar DILI liability as the competitor in ADPKD patients?

Approach

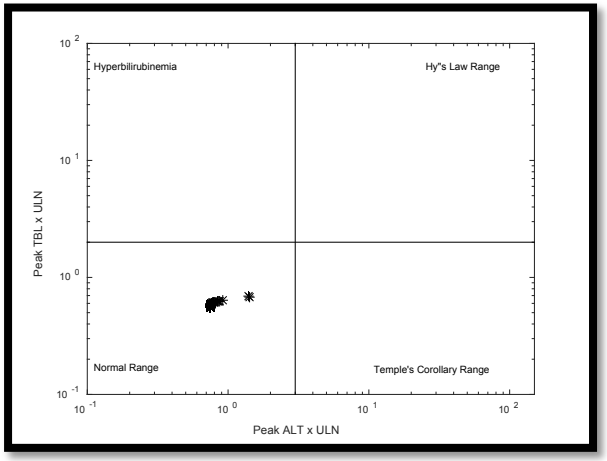
- Develop a mechanistic representation of lixivaptan in DILIsym, a QST model of drug-induced liver injury (DILI), to assess the potential for liver toxicity with the intended dosing for lixivaptan

Lixivaptan Project Executive Summary

- Simulations of lixivaptan dosing in custom SimPops of 285 simulated individuals with exposure variability show no ALT elevations (0/285 >2X ULN) at 200/100 mg BID dosing

- The DILIsym results suggest that lixivaptan is likely safer than the competitor
  - Competitor had significant ALT elevations at its clinical dose (simulated and clinically observed); lixivaptan simulations predict none

Simulated 200/100 mg dosing over 12 weeks in Custom SimPops of 285 with PK variability



Lixivaptan Simulations Predict Minimal ALT Elevations at 200/100 mg BID

- Lixivaptan simulated in the custom n=285 individual SimPops including PK variability
- No ALT elevations simulated in 100 mg BID 60-day simulation
  - Consistent with observed clinical similarity to placebo
- 7/285 (2.46%) of simulated individuals had ALT elevations with 400 mg BID for 7 days
  - Simulations more conservative than clinical data from a safety standpoint
- No ALT elevations simulated in 200/100 split daily dosing scenario for 12 weeks
  - Maximum intended clinical dosing for ADPKD
  - Highest simulated ALT = 57 U/L
- Dose escalation simulations suggest possible ALT elevations at doses beyond the intended maximum clinical dose (not shown)

Dose and Duration	Parameter Settings	Clinical ALT > 3x ULN	Simulated ALT >3X ULN*
100 mg BID for 60 days	Default measured#	On treatment similar to placebo**	0/285
400 mg BID for 7 days	Default measured#	0/67	7/285
200 / 100 mg for 12 weeks	Default measured#	Study not yet conducted	0/285

*Upper limit of normal (ULN) in DILIsym is 40 U/L

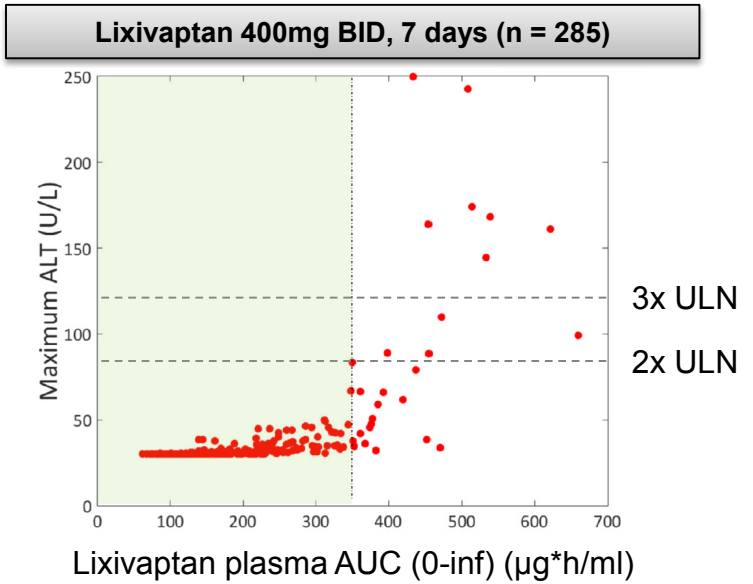
**In study CK-LX3401, 8/315 individuals in the treatment group had ALT > 200 U/L, compared to 6/319 in the placebo group; this was judged to not be a statistically significant increase in AEs due to lixivaptan treatment.

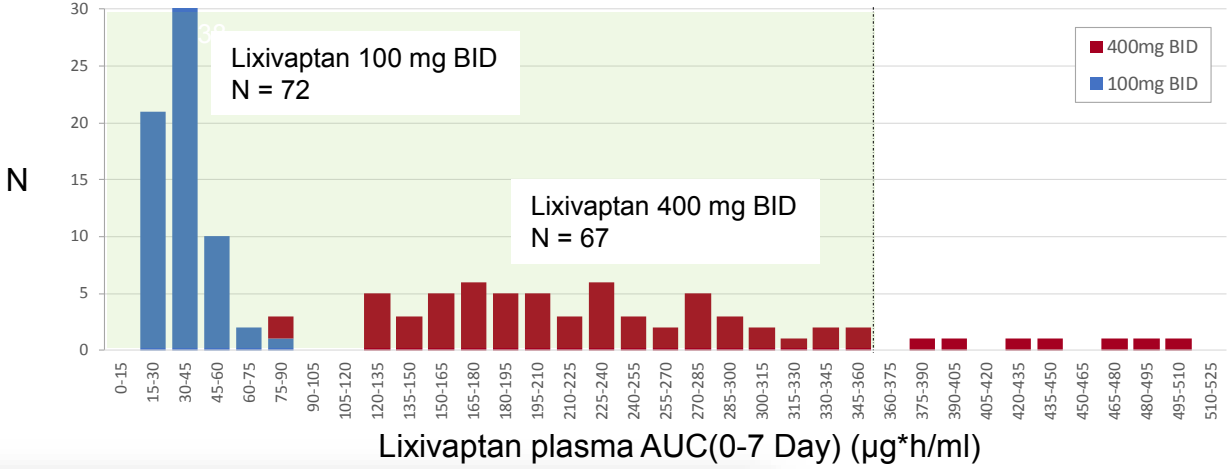
#Default assumption for BA inhibition is mixed inhibition type with $\alpha = 5$ in the absence of K_i studies, based on the experience of the DSS team.

Clinical Application – Dose Selection

- ALT elevations are correlated with total lixivaptan exposure
- Project established exposure threshold below which lixivaptan is safe ($AUC_{0\text{-}7\ days}$< 350 µg*h/ml)

- Existing data indicate lixivaptan exposure rarely exceeds the exposure threshold
- Intended clinical dose not expected to exceed threshold





Clinical Data and Simulation Results

Next Steps for Lixivaptan Development

- Palladio and DILIsym Services to publish results
- Update simulation with PK profile of lixivaptan in ADPKD patients, if necessary, once data are available
- Discuss results with FDA

Palladio Biosciences Website

me | Polycystic Kidney Disease | Pipeline | Clinical Trials | Information Resources | About Us | N

Palladio Biosciences Receives FDA IND Clearance to Begin the ELiSA Study, a Phase 2 Clinical Trial with Lixivaptan in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

- Results from a state-of-the-art, predictive modeling tool based on in vitro inputs, physiologically based pharmacokinetic modeling and *in silico* simulations suggested that lixivaptan may have a differentiated safety profile compared to tolvaptan with respect to the potential to cause liver injury.

  Among other key findings, this investigation indicated that lixivaptan may have lower liver exposure than tolvaptan; that lixivaptan does not affect bile acid homeostasis and mitochondrial function, two key mechanisms of liver injury that may contribute to tolvaptan liver toxicity; and that lixivaptan, unlike tolvaptan, may not cause ALT elevations. These findings need to be confirmed in the clinics.

- In April 2018, the FDA granted IND (investigational new drug) clearance for the ELISA Study (Evaluation of Lixivaptan In Subjects with ADPKD), a Phase 2 clinical trial that will evaluate the safety, pharmacokinetics and pharmacodynamics of multiple doses of lixivaptan in patients with ADPKD with relatively preserved kidney function (chronic kidney disease stages CKD1 and CKD2) and moderately impaired renal function (CKD3). The study is expected to enroll 32 patients beginning the end of June 2018.

PALLADIO BIOSCIENCES
Working to bring new hope to patients with orphan diseases of the kidney

LIFE SCIENCES PA
CONNECT - ADVOCATE - ADVANCE

Bio Member

Please note that lixivaptan is for investigational use only.

Palladio Biosciences
12 Penns Trail
Unit A

ALS Project Example Introduction

- Riluzole is used to slow the progression of ALS
  - Approved in 1995
  - Largely taken orally, which is difficult for late-stage disease patients
  - Associated with liver enzyme elevations in portion of patients

- Biohaven Pharma is developing an alternative, sublingual formulation (BHV-0223) with the following goals:
  - Improved delivery, compliance, and general ease-of-use for ALS patients, who often have trouble swallowing
  - Improved liver safety profile?

Clinical Incidence of ALT Elevations in ALS Patients ~10-15%

- *Bensimon 2004* – Riluzole in ALS patients
  - ALT elevations >3x ULN in 10-15% of patients
  - Elevations are dose-related
  - Median time to onset < 3 months
- No data on ALT elevations in healthy volunteers

Table 2. Frequency of hepatic enzyme abnormalities in patients receiving riluzole (100 mg/day, 50 mg b.i.d.) or placebo.*

Enzyme		Riluzole (n = 395)	Placebo (n = 406)
ALT	3 – 5 x ULN	35 (9)	12 (3)
	> 5 x ULN	15 (4)	7 (2)
AST	3 – 5 x ULN	18 (5)	3 (1)
	> 5 x ULN	4 (1)	3 (1)

*Data [8,9] are given as numbers of patients % total.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; n: Number of patients; ULN: Upper limit of normal.

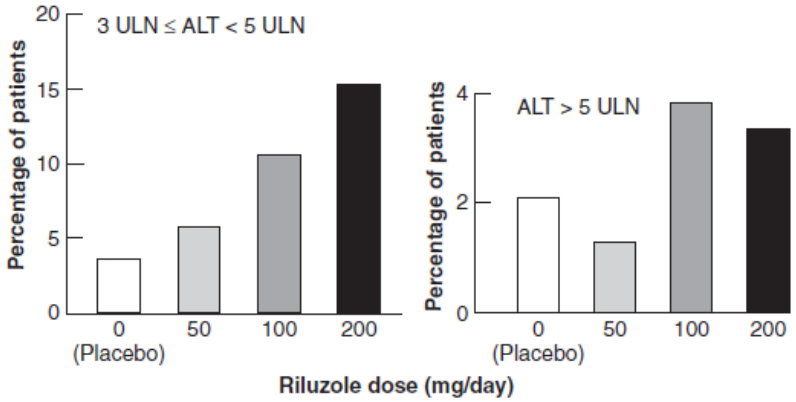


Figure 2. Dose-incidence relationship for transaminase elevations occurring with riluzole. Data are taken from [7]
ALT: Alanine aminotransferase; ULN: Upper limit of normal.

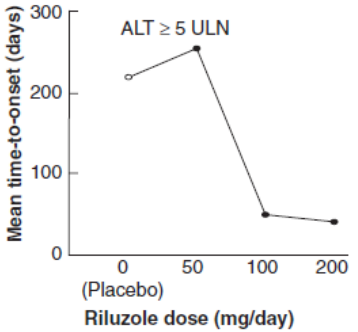


Figure 3. Relationship between riluzole dose and median time to ALT elevations. Data are taken from [16].

Clinical Data and Simulation Results

Summary of DILIsym Toxicity Parameter Values For Riluzole

Mechanism	DILIsym Parameter	Unit	Value***
Mitochondrial Dysfunction	Coefficient for ETC inhibition	µM	382
Oxidative Stress	RNS/ROS production rate constant	mL/nmol/hr	6×10^{-4}
Bile Acid Transporter Inhibition	BSEP inhibition constant	µM	200*
	NTCP inhibition constant	µM	NA
	Basolateral inhibition constant**	µM	125*

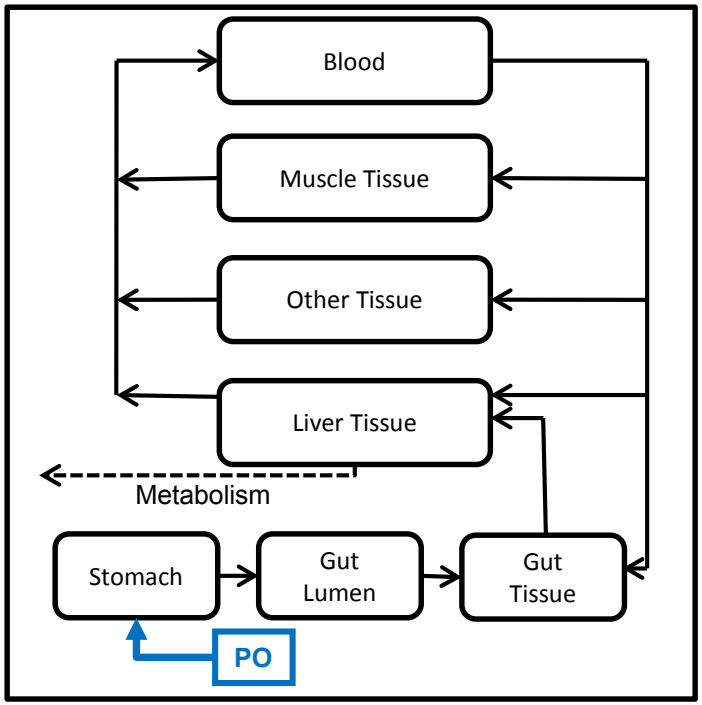
*IC_{50} values; default assumption is mixed inhibition type with α = 5, based on the experience of the DSS team

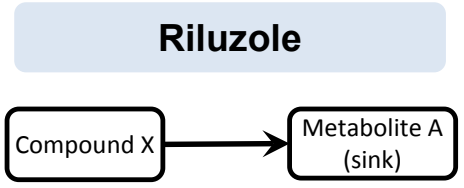
**Basolateral inhibition constant represents the lowest IC_{50} of the experimentally derived MRP3 and MRP4 IC_{50} values

***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

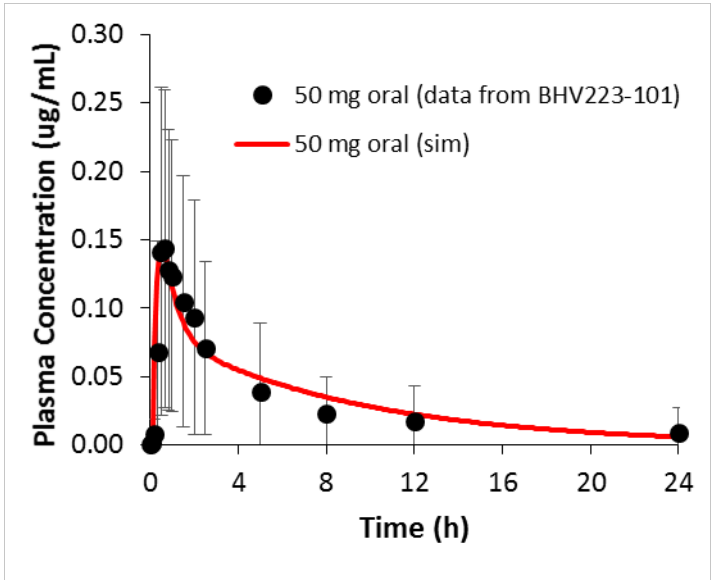
DILIsym PBPK Framework for Oral Riluzole (Rilutek)

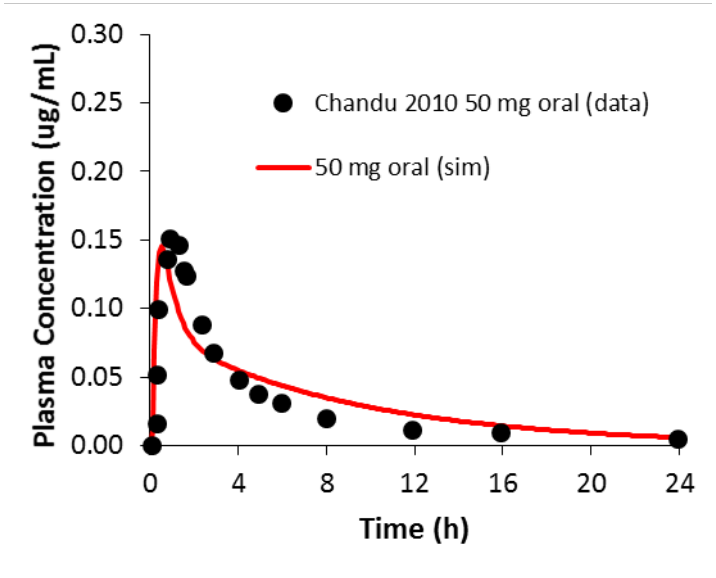
- PBPK sub-model in DILIsym was used to represent riluzole disposition
  - Consists of blood, liver, gut, muscle, and other tissue compartments (Compound X scaffold)
- Riluzole metabolism represented by one metabolic pathway
  - Sink pathway (Metabolite A) represents aggregate of all riluzole metabolic pathways
  - Metabolites (Metabolite A) will not contribute to toxicity
- Riluzole is predominantly eliminated via hepatic metabolism
  - Excreted predominantly via urine in form of metabolites
  - Low urinary excretion of unchanged parent





Oral Riluzole PBPK Representation in DILIsym Validated with Clinical PK Data





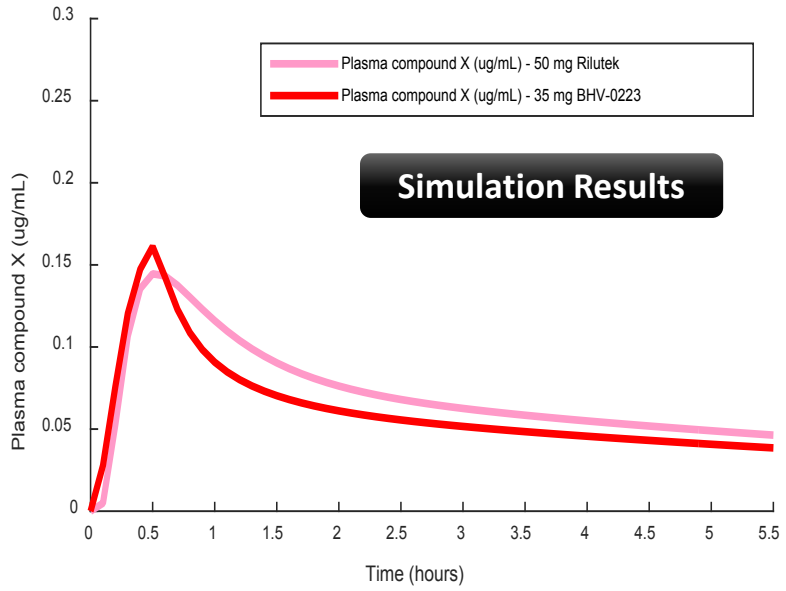
Dose	Disease Status	Reference	Sim/Obs	
			Cmax	AUC
50 mg oral	Volunteer	BHV223-101	0.9	1.1

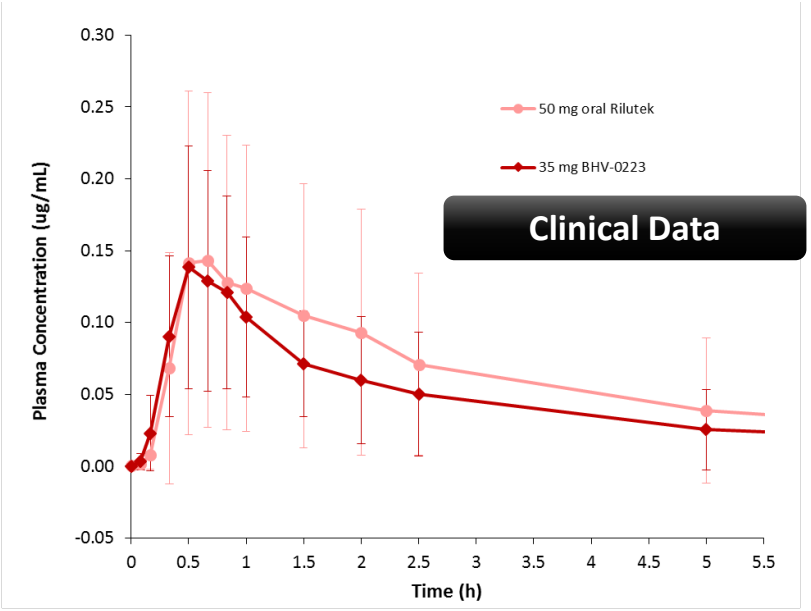
Dose	Disease Status	Reference	Sim/Obs	
			Cmax	AUC
50 mg oral	Volunteer	Chandu 2010	1.0	1.2

- Simulations reasonably capture plasma profiles of riluzole that were not used in optimization (pure validation results)

Clinical Data and Simulation Results

Plasma Profiles Simulated in DILIsym Comparable for 35 mg Sublingual Riluzole (BHV-0223) and 50 mg Oral Riluzole (Rilutek)





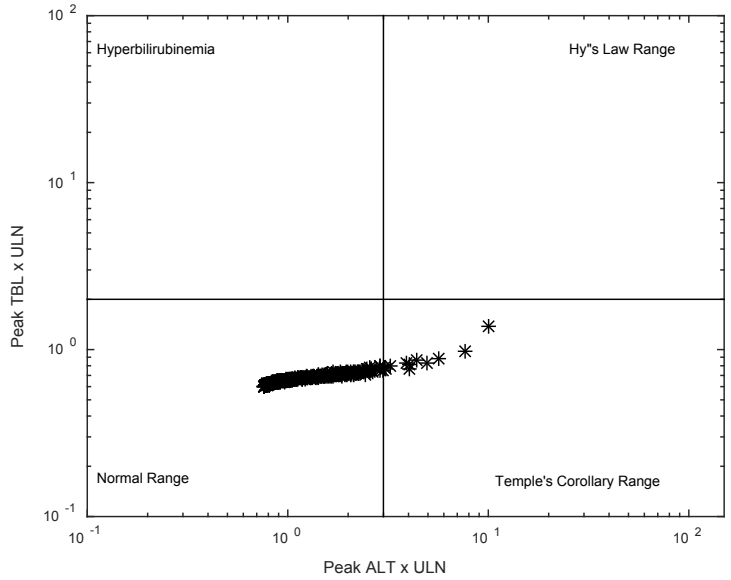
- Plasma Cmax and AUC for 35 mg BHV-0223 are comparable to plasma Cmax and AUC for 50 mg Rilutek
- Clearance dynamics also line up well with observed differences

Clinical Data and Simulation Results

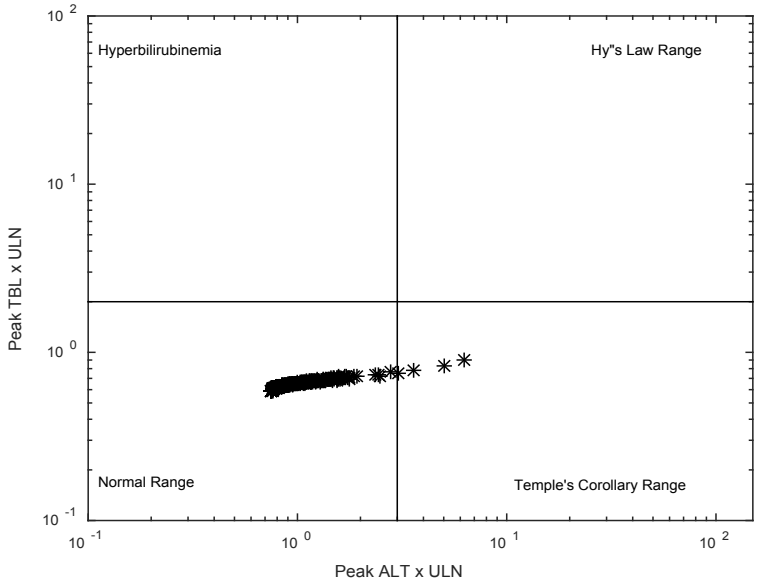
Riluzole Simulations in Normal Healthy Volunteer SimPops Show ALT Elevation Differences Between Oral and Sublingual Dosing with Certain Assumptions

Simulated eDISH Plots

Oral 50 mg BID 12 weeks
High PK, Liver Kb 10
(11/285 > 3x ULN*)



Sublingual 40 mg BID 12 weeks
High PK, Liver Kb 10
(4/285 > 3x ULN*)



*Upper limit of normal (ULN) in DILIsym is 40 U/L

Multi16 SimCohort Simulations Reveal Oxidative Stress as the Driver of Liver Injury in the DILIsym Riluzole Simulations

- Multi16 SimCohort includes 13 sensitive individuals and 2 insensitive individuals from the full n=285 SimPops and includes the baseline human
- **Liver injury predicted to be predominantly due to oxidative stress**
  - Oxidative stress is required for simulated ALT elevations
  - Mitochondrial toxicity is not required for simulated ALT elevations
  - Bile acid transport inhibition is not required for simulated ALT elevations

	Riluzole Dose and Duration	Parameter Settings	Mechanisms	ALT > 3X ULN*	ALT > 5X ULN*
Oral	Oral 50 mg BID for 12 weeks	High PK, Liver Kb 10	All	3/16	1/16
			No ROS	0/16	0/16
			No Mitochondrial Toxicity	3/16	1/16
			No BA Transport Inhibition	3/16	1/16
Sublingual	Sublingual 40 mg BID for 12 weeks	High PK, Liver Kb 10	All	1/16	1/16
			No ROS	0/16	0/16

*Upper limit of normal (ULN) in DILIsym is 40 U/L

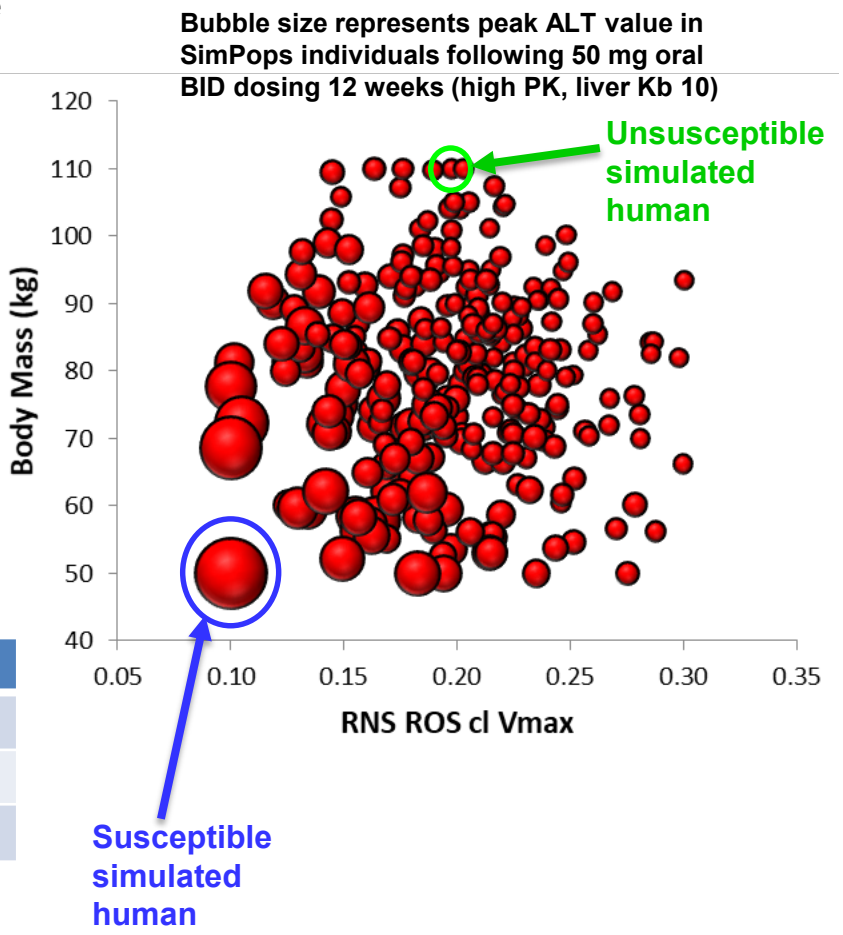
**Multi16 SimCohorts used is group of 16 individuals (n=16) among the full v4A_1 SimPops (n=285); 13 of the simulated individuals are sensitive to DILI mechanisms or combinations, 2 are insensitive, and 1 is the baseline (average) human

Multiple Factors Responsible for Differences in the Hepatotoxicity Response to Riluzole Among Simulated Individuals

- Differences in the hepatotoxicity response for the **unsusceptible individual** and the **susceptible individual** due to multiple factors (primarily the combined effect of differences in body mass and RNS/ROS clearance)
- Covariate analysis of the SimPops simulation results revealed 3 SimPops parameters that showed a statistically significant correlation with ALT elevations:

Parameter	Parameter Description	P-value
RNS ROS cl Vmax	Liver RNS/ROS baseline clearance Vmax	7.6 E-39
Body mass	Body mass	8.3E-24
CAS apop scale	Caspase-mediated apoptosis scaling constant	1.4E-10

Regression analysis performed with peak ALT (oral BID dosing, high PK and liver Kb 10 assumptions) as the dependent variable and the 34 SimPops parameters as independent variables



Simulation Results

ALS Project Example Summary

- Riluzole is used to slow the progression of ALS
  - Approved in 1995
  - Largely taken orally, which is difficult for late-stage disease patients
  - Associated with liver enzyme elevations in portion of patients
- Biohaven Pharma is developing an alternative, sublingual formulation (BHV-0223) with the following goals:
  - Improved delivery, compliance, and general ease-of-use for ALS patients, who often have trouble swallowing
  - Improved liver safety profile?

Primary Project Outcomes

- DILIsym was used to compare the liver safety profile for both formulations
  - The analysis showed that the sublingual formulation is likely to produce less ALT elevations than the oral formulation
  - The benefit is largely derived from the reduced dose needed for sublingual dosing and reduced liver exposure (less first pass metabolism)
  - A detailed exposure-response analysis helped to define possible safe exposure cut-offs and identified patient susceptibility factors for ALT elevations

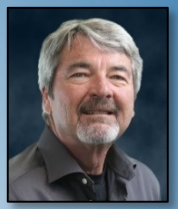
Acknowledgements: The DILIsym Services Team

Paul B. Watkins
DILI-sim Initiative
Scientific Advisory Board Chair
RTP, NC

Scott Q Siler
Chief Scientific Officer
Bay Area, CA

Brett Howell
President
RTP, NC

Shawn O'Connor
CEO, Simulations Plus Inc.
Lancaster, CA



Grant Generaux
Scientist II
Philadelphia, PA

Jeff Woodhead
Scientist II
RTP, NC

DILIsym Services

A SIMULATIONS PLUS COMPANY

Lisl Shoda
Principal Scientist
Director of Immunology
Bay Area, CA

Kyunghee Yang
Scientist II
Middleton, DE

Vinal Lakhani
Scientist I
RTP, NC

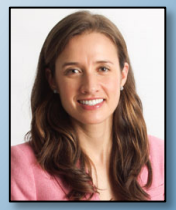
Guncha Taneja
Postdoctoral Fellow
RTP, NC

Shailendra Tallapaka
Postdoctoral Fellow
RTP, NC

Christina Battista
Scientist I
Buffalo, NY

Zack Kenz
Scientist I
RTP, NC

Diane Longo
Scientist II
Arlington, VA



Yeshi Gebremichael
Scientist II
RTP, NC

Corey Berry
Senior Software Engineer
RTP, NC

Bud Nelson
Director of Operations
RTP, NC

Patti Steele
Executive Assistant
RTP, NC

BOSTON PHARMACEUTICAL & BIOSCIENCE SOCIETY

Thank You: Questions?