The DILI-sim Initiative and DILIsym® Modeling Software Overview

January 2016

Executive Summary

- The DILI-sim Initiative is supporting and guiding the development of DILIsym® modeling software
  - A mechanistic, mathematical model that is being constructed to support risk assessment and decision making
  - DILIsym® is the intersection between PBPK compound distribution and metabolism, mechanisms of hepatotoxicity, and patient variability
  - Input parameters derived from *in vitro* and hepatocellular assays
  - Outputs include standard and emerging biomarkers as well as hepatocyte loss
  - Patient variability (SimPops™) in multiple mechanistic areas included
- DILIsym® can be applied to compound risk assessment throughout the clinical development pipeline
  - DILIsym® was applied to evaluate the clinical risk associated with a novel large molecule as part of a past regulatory submission (*CPT Pharmacometrics Syst Pharmacol 3: e98. 2014.)*
  - Numerous other applications with potential regulatory impact are in progress
- DILI-sim members receive a license to DILIsym® and discounts on modeling and simulation services offered by the DILIsym® development group

Outline

- Overview of the DILI-sim Initiative
- Overview of the DILIsym® Modeling Software
- Example DILIsym® Applications

The DILI-sim Initiative Is a Partnership between DILIsym Services Inc. and Pharmaceutical Companies to Minimize DILI

MITOsym®

DILIsym®

- *Overall Goals*
  - *Improve patient safety*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *16 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $5 million total invested in project*

FDA

DILI-sim Initiative

The DILI-sim Team and the SAB

Goals and Intended Applications of Developing DILIsym® for the DILI-sim Initiative

Near term goals:

- Develop DILIsym® software to better inform safety decisions within drug development
  - *In vitro* to *in vivo*
  - Preclinical to first-in-human
  - Biomarker interpretation

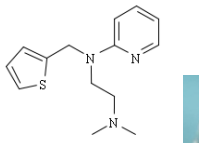
Long term goal:

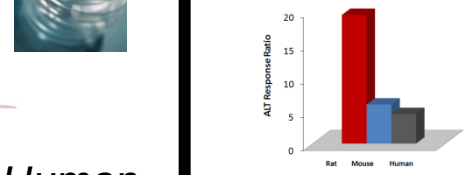
- Use DILIsym® to increase understanding of idiosyncratic DILI

Intended application:

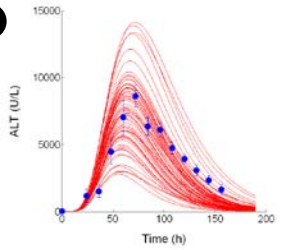
- Simulations of hepatotoxicty for humans and rodents
- *In vitro*, *in vivo*, and/or clinical data as inputs

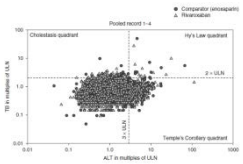
Preclinical

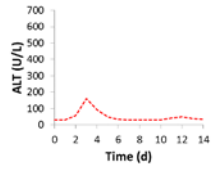




First in Human Clinical Trials





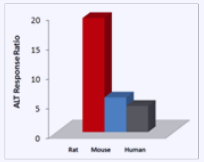


Phase II/III Clinical Trials and Post-Market Surveillance

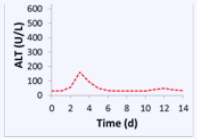
DILI-sim Initiative

DILI-sim Initiative Stage II (2015-2017) Will Focus on Late-Stage Clinical Development

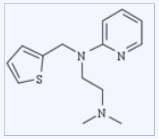
DILI-sim Initiative Stage I Primary Goals

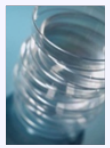


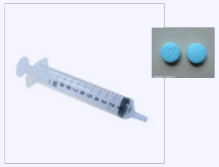
Preclinical



First in Human Clinical Trials



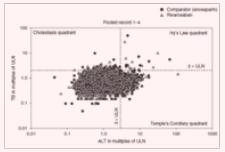


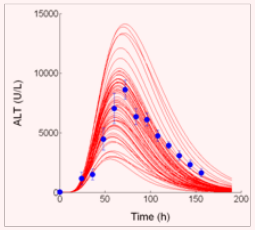


- Dose-related DILI
- Species differences
- Early stage clinical development (FIH)

DILI-sim Initiative Stage II Primary Goals

Phase II/III Clinical Trials and Post-Market Surveillance



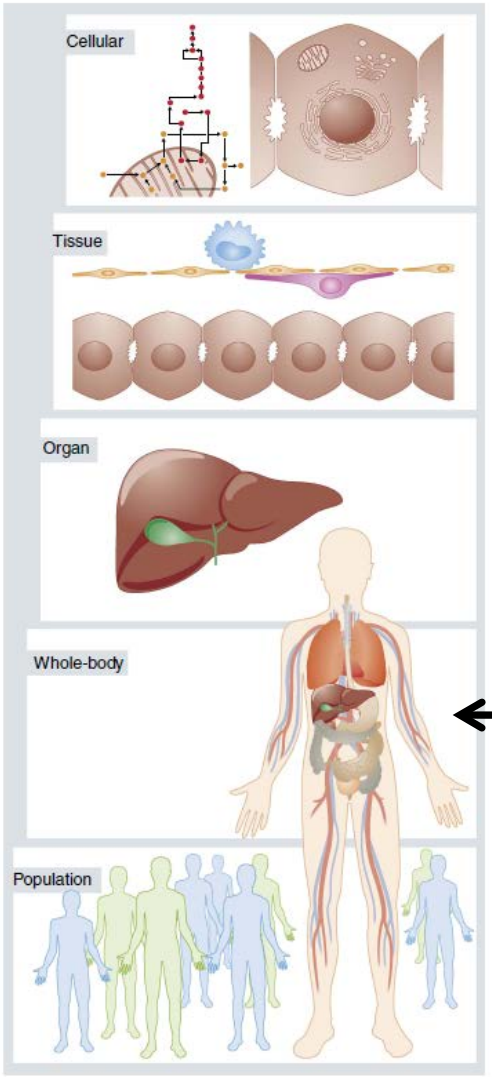


- Less frequent DILI
- Late stage clinical development (phase II, III, IV)

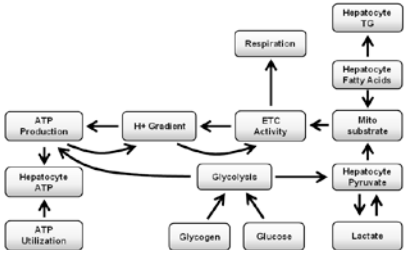
Outline

- Overview of the DILI-sim Initiative
- Overview of the DILIsym® Modeling Software
- Example DILIsym® Applications

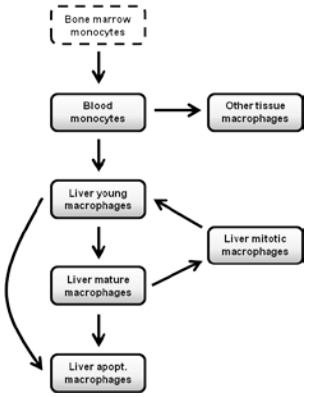
DILIsym®: 'Middle Out' and Multi-Scale



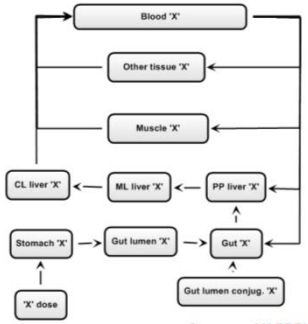
Mitochondrial dysfunction



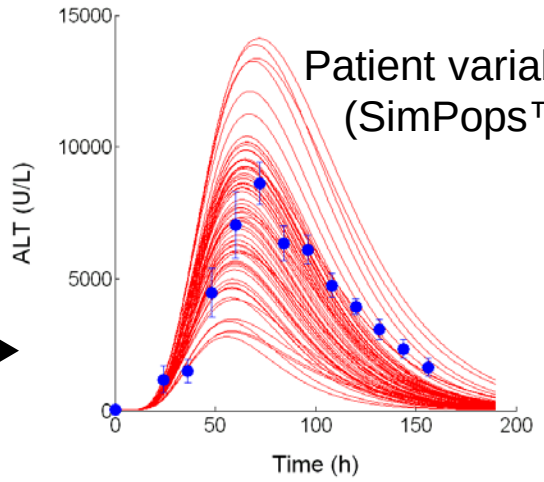
Cellular life-cycle



Drug distribution & metabolism



Compound X PBPK



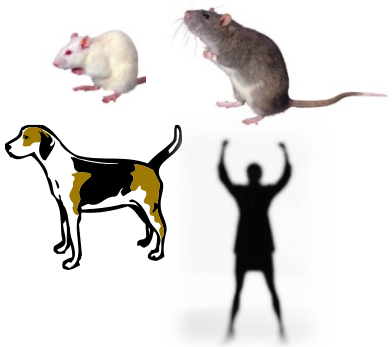
Patient variability (SimPops™)

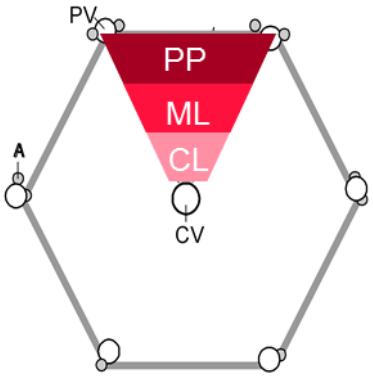
Kuepfer 2010, Molecular Systems Biology

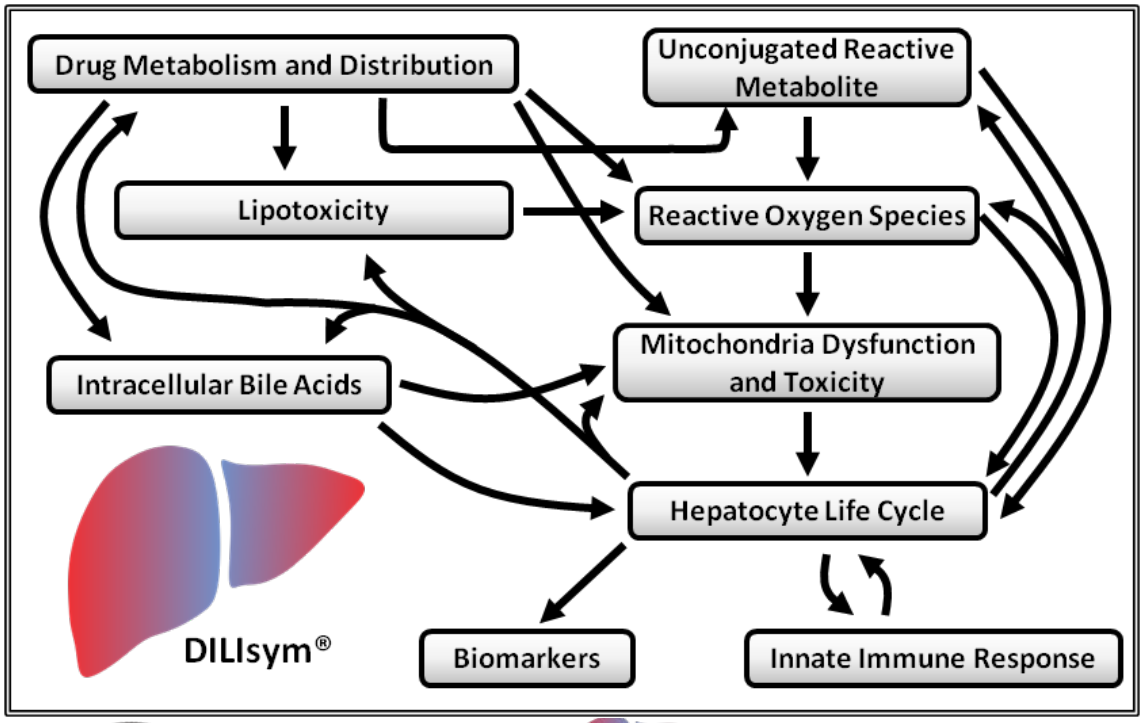
DILI-sim Initiative

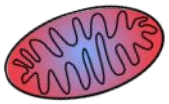
DILIsym® Overview

- **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
  - 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

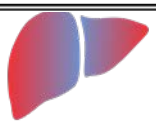








DILI-sim Initiative



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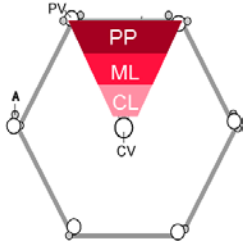
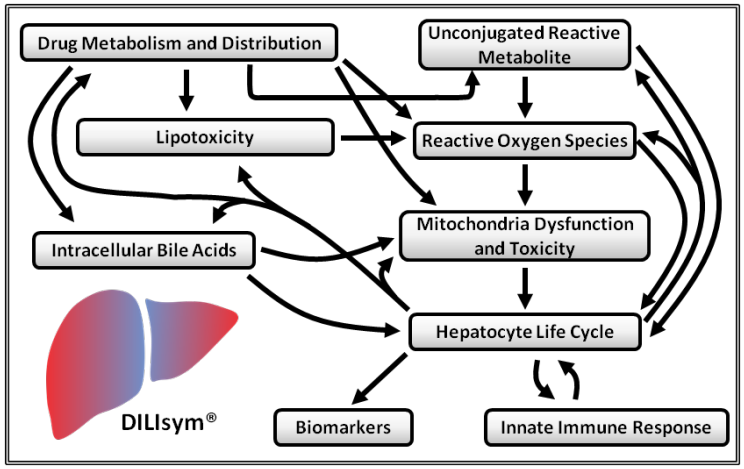
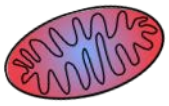




- **Compartment-based modeling**
  - >500 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - Code or GUI functionality

- **Hepatotoxicity exemplars**
  - Reactive metabolite mediated
    - Acetaminophen
    - Methapyrilene
    - Furosemide
    - Aflatoxin B1
    - Carbon tetrachloride
  - Mitochondrial dysfunction
    - Etomoxir
    - Buprenorphine
    - Tolcapone
    - Entacapone
    - CP-724714
  - Bile acid transporter inhibition
    - Glibenclamide
    - CP-724714
    - Bosentan
    - Telmisartan
    - Tolcapone
    - Troglitazone
    - Pioglitazone
    - AMG009
  - Single, multiple dose protocols
  - Single, combination drug protocols



DILI-sim Initiative

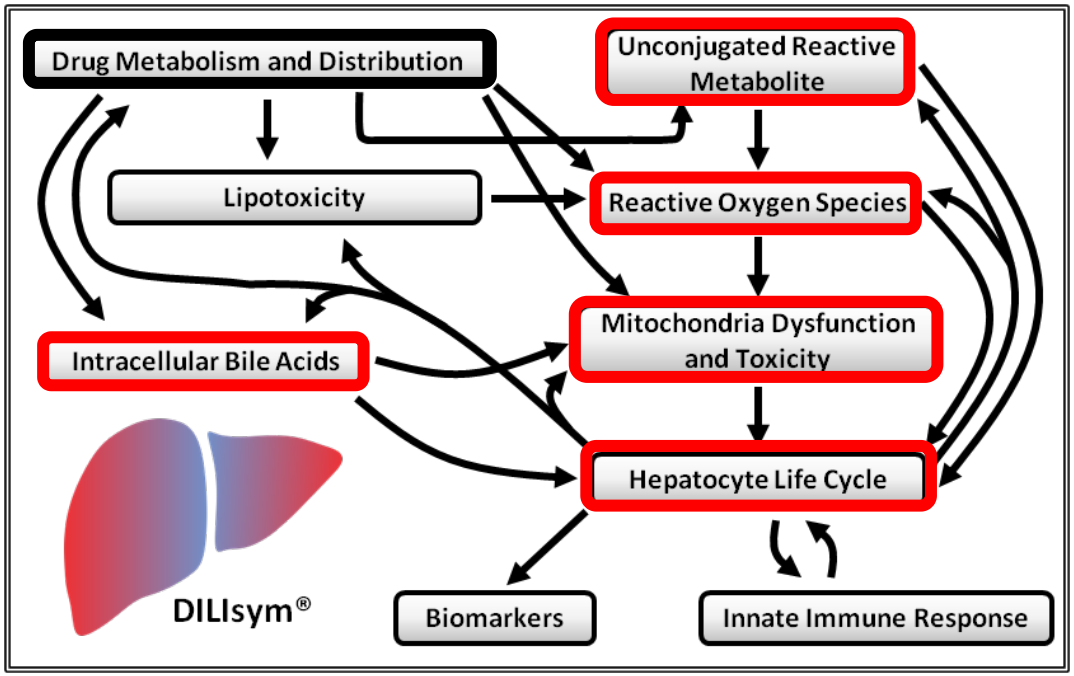
Key Areas for DILIsym® Data Inputs and Simulation Results Comparators

Drug Absorption and Distribution

Drug Metabolism

Proposed Hepatotoxicity Mechanism

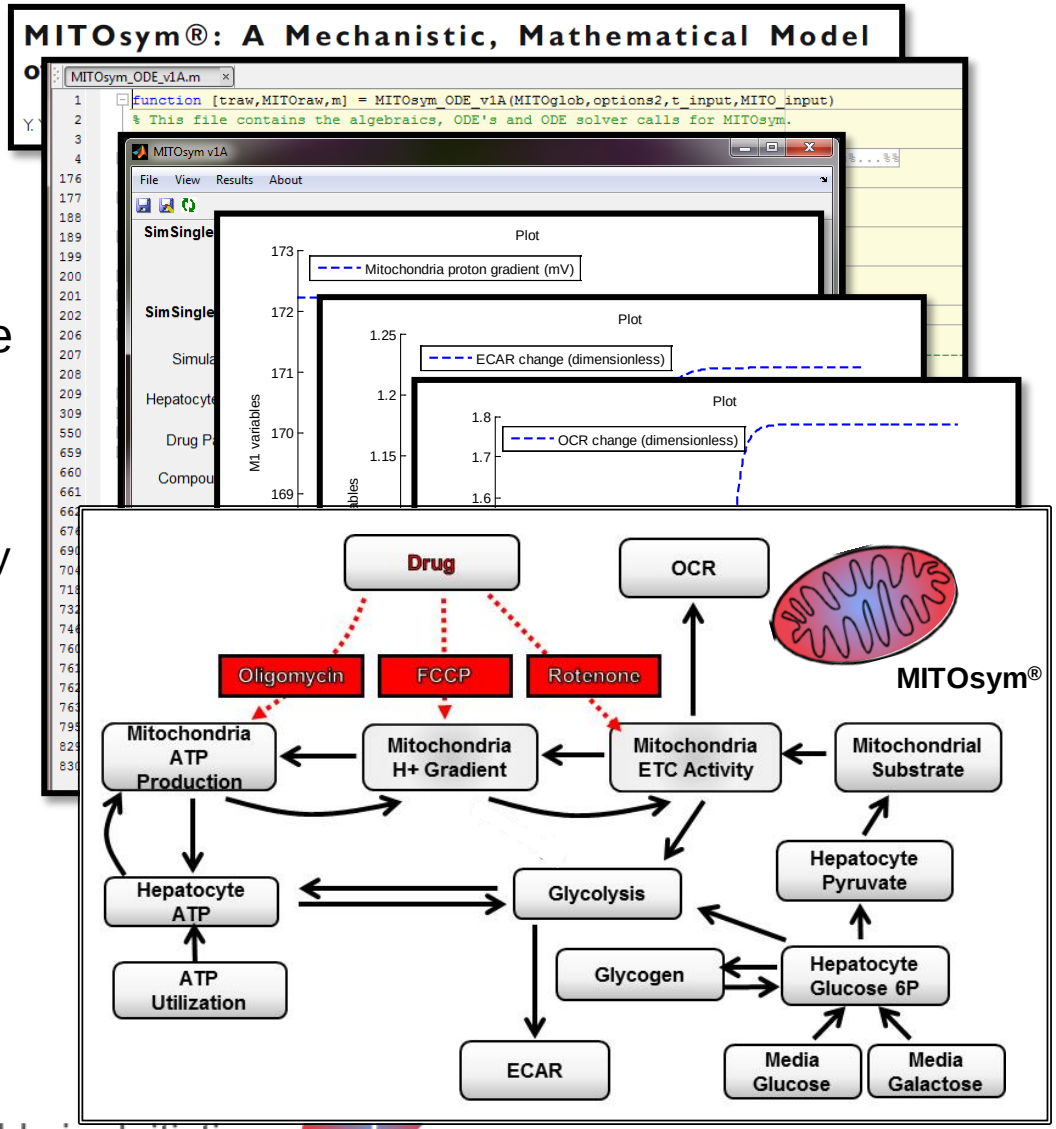
Biomarkers



- *BSEP, NTCP, MRP Ki*
- *OCR, $\Delta\Psi m$*
- *ROS/RNS increases*
- *GSH depletion, adduct formation*
- *ATP depletion*
- *Apoptosis vs necrosis*

MITOsym® Is Designed to Support IVIVE DILI Predictions and Mechanistic Data Interpretation

- MITOsym® is a standalone model of hepatocyte bioenergetics
  - Yang et al. 2015
- MITOsym® can be used to facilitate predictions of hepatotoxicity based on *in vitro* cellular respiration data
  - Combine with DILIsym® model
  - Multiple cell types: HepG2, primary human hepatocytes, primary rat hepatocytes
- MITOsym® can be used to develop and explore hypotheses of the mechanisms underlying observed changes in respiration and glycolysis in hepatocytes
  - Comparison with exemplar drugs



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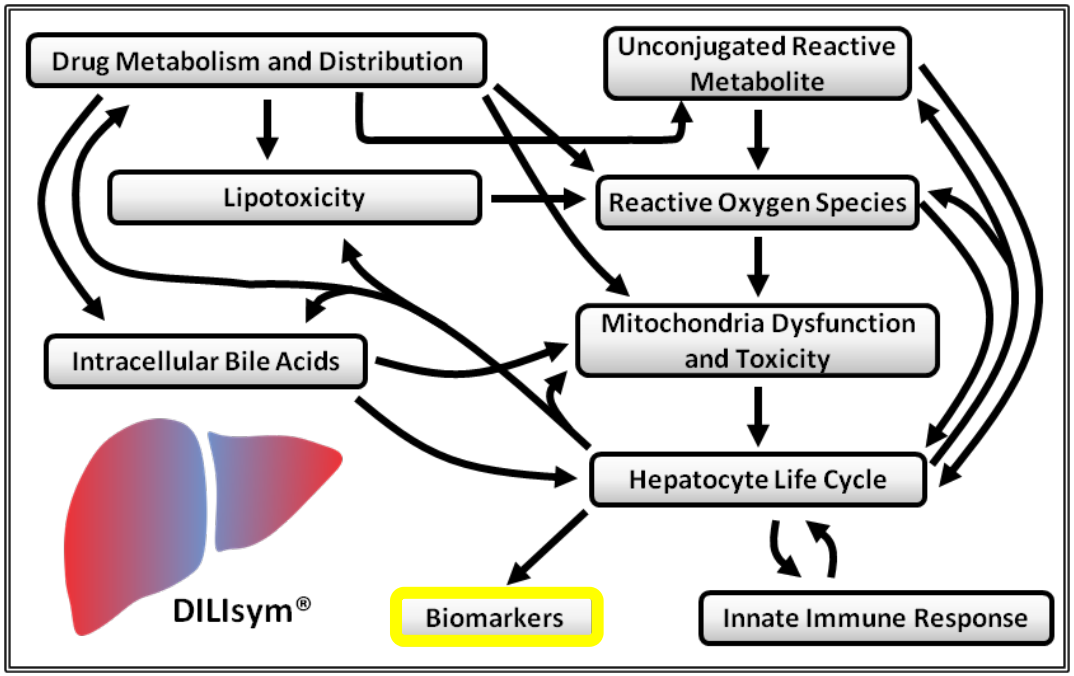

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DILI-sim Initiative

Biomarkers of Hepatocellular Function and Death Are Outputs of DILIsym®

- Biomarkers are outputs of model
  - Used for validation of DILIsym® model
  - Used for comparison with clinical and preclinical data
  - Functional, necrotic, and apoptotic indicators
- More biomarkers being added as data are becoming available
  - Cleaved cytokeratin-18 is recent example
  - Various forms of HMGB1 recently added (oxidized, reduced, and acetylated)
- Additional DILIsym® model outputs include:
  - Fraction of viable hepatocytes
  - Liver ATP
  - Liver glutathione
  - Circulating, liver, and excreted drug and metabolites

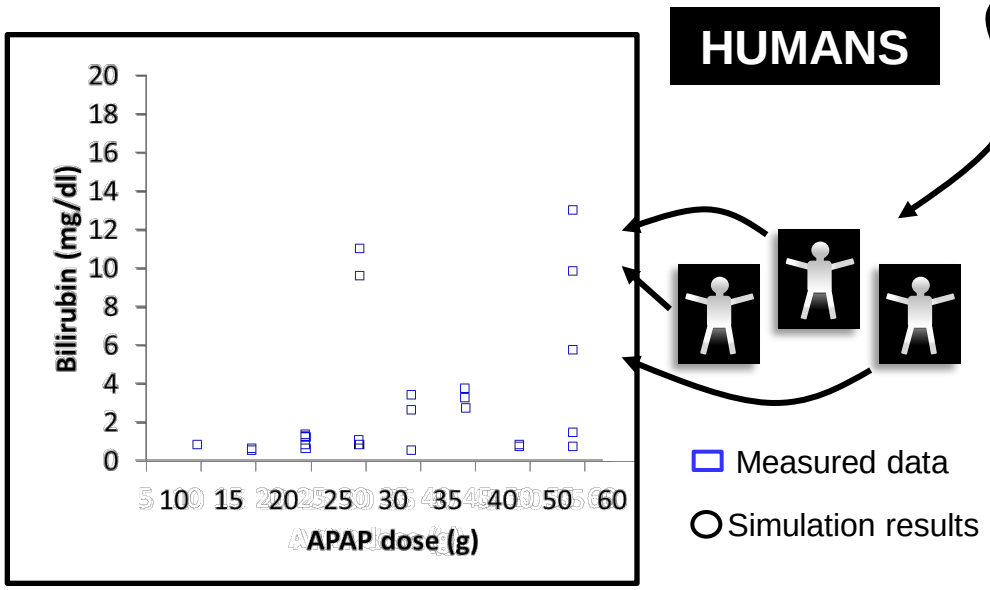
Marker	Category
Alanine aminotransferase (ALT)[1,2,3,4,5]	Necrosis
Bilirubin (total)[1,2,5]	Function/Cholestasis
Aspartate aminotransferase (AST)[1,2,3,4,5]	Necrosis
Prothrombin time [1,2]	Function
High mobility group box protein 1 (HMGB1)[1,10]	Necrosis/Apoptosis
Full length cytokeratin-18[1]	Necrosis
Cleaved cytokeratin-18[1]	Apoptosis
Sorbitol dehydrogenase (SDH)[1,6]	Necrosis
Arginase-1[9]	Necrosis
Liver derived mRNA[7] and miRNA[8]	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, [9]Murayama *Clin Chimica Acta 2008*, [8]Yang *Tox Sci* 2012, [10]Harrill *Clin Pharmacol Ther* 2011

Range of Hepatotoxic Responses in SimPops™ Due to Variability in Underlying Biochemistry

- SimPops™ are population samples with variability in hepatotoxic drug responses
- Several parameters are varied to produce diverse simulated patients
- Response data (e.g., APAP overdose) are also used to construct the SimPops™

Variables Used to Construct the SimPops™ Population Sample
Body weight
Baseline GSH
GSH precursor transport (V_{max})
RM creation of RNS-ROS (V_{max})
RNS-ROS effect on ATP (V_{max})
NRF adaptation (V_{max})
Hepatocyte proliferation rate
Necrotic flux effect on propagation (K_m)



Davis 1976

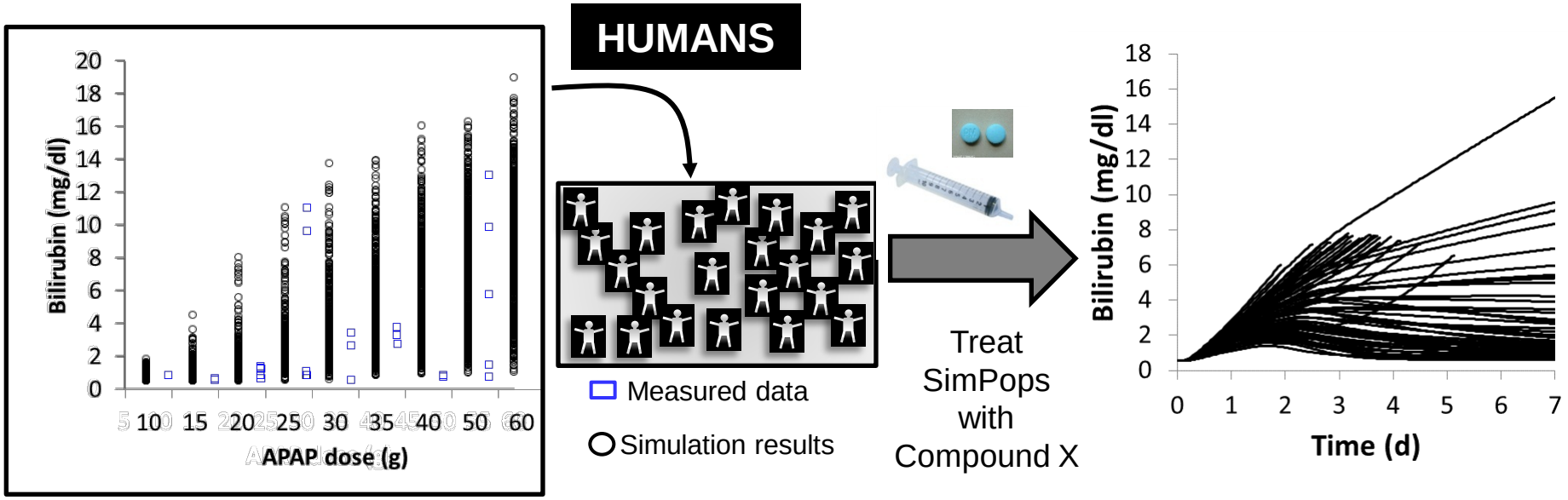
Simulation Results and Clinical Data

DILI-sim Initiative

Modeling Approach and Simulation Design for Predicting Patient Variability

- Numerous simulated patients are generated, consistent with range of observed response data
- These simulated patients are aggregated to form a SimPops™
- SimPops™ are subsequently used to predict responses to a different compound

Novel predictions of hepatotoxicity that incorporate variability



Davis 1976

Simulation Results and Clinical Data

DILI-sim Initiative

Examples of DILIsym® Applications involving Bile Acid Transporter Inhibition

IVIVE

J Pharmacokinet Phar 39(5): 527-541. 2012.

Rank compounds by risk

Toxicol Lett 226(2): 163-172. 2014.

Preclinical biomarker study design

Preclinical

in vitro

in vivo

Single Ascending Dose

Phase II/III/IV

Clinical

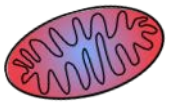
DILI Dose Response Estimation

Clinical biomarker analysis

CPT Pharmacometrics Syst Pharmacol 3: e98. 2014.

Predicting variability in response

J Pharmacol Exp Ther 342(2): 529-540. 2012.

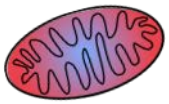


DILI-sim Initiative

Systems Pharmacology Modeling Predicts Delayed Presentation and Species Differences in Bile Acid–Mediated Troglitazone Hepatotoxicity

K Yang[1], JL Woodhead[2], PB Watkins[1,2], BA Howell[2] and KLR Brouwer[1,3]

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 96 NUMBER 5 | NOVEMBER 2014

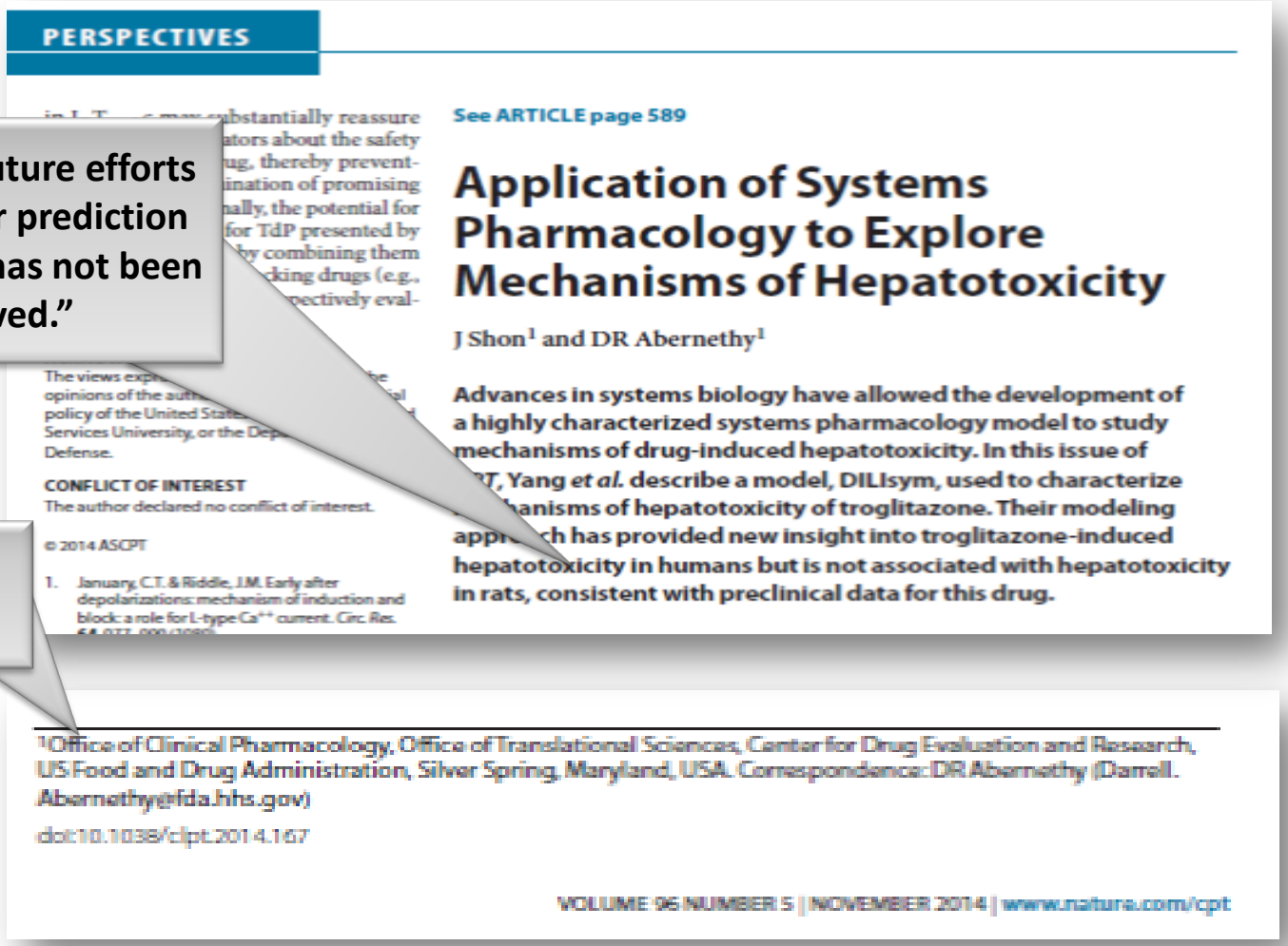


DILI-sim Initiative

Scientists at the FDA Authored a Positive Commentary on the DILIsym® Troglitazone Work

"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

substantially reassure
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for TdP presented by
by combining them
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pectively eval-

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

CONFLICT OF INTEREST
The author declared no conflict of interest.

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

See ARTICLE page 589

Application of Systems Pharmacology to Explore Mechanisms of Hepatotoxicity

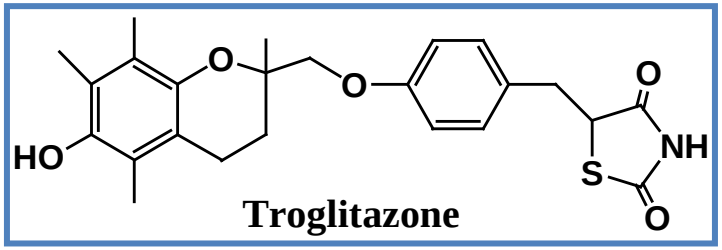
J Shon[1] and DR Abernethy[1]

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 ...T, Yang *et al.* describe a model, DILIsym, used to characterize ...hanisms of hepatotoxicity of troglitazone. Their modeling appr...ch 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

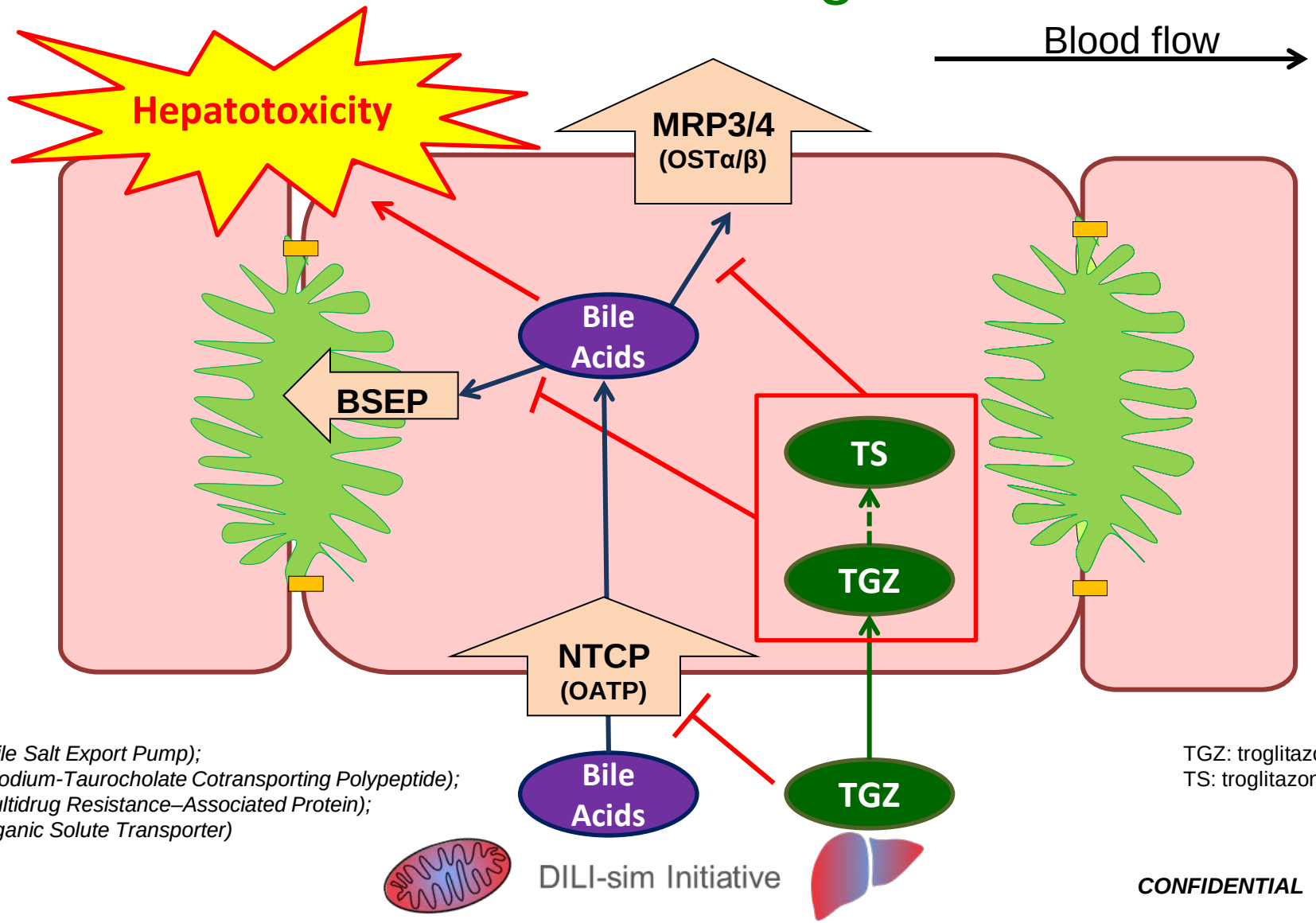
Troglitazone (TGZ)



- First in thiazolidinedione class; PPARγ agonist
  - Reduces hepatic and peripheral insulin resistance
  - Approved for the treatment of type II diabetes

- Hepatotoxicity
  - Hepatotoxicity was not detected in preclinical studies
  - 2% of patients developed ALT elevations >3X ULN in clinical trials
  - Withdrawn from the market due to idiosyncratic hepatotoxicity

Mechanisms of DILI: Transport Protein-Mediated Bile Acid-Drug Interaction

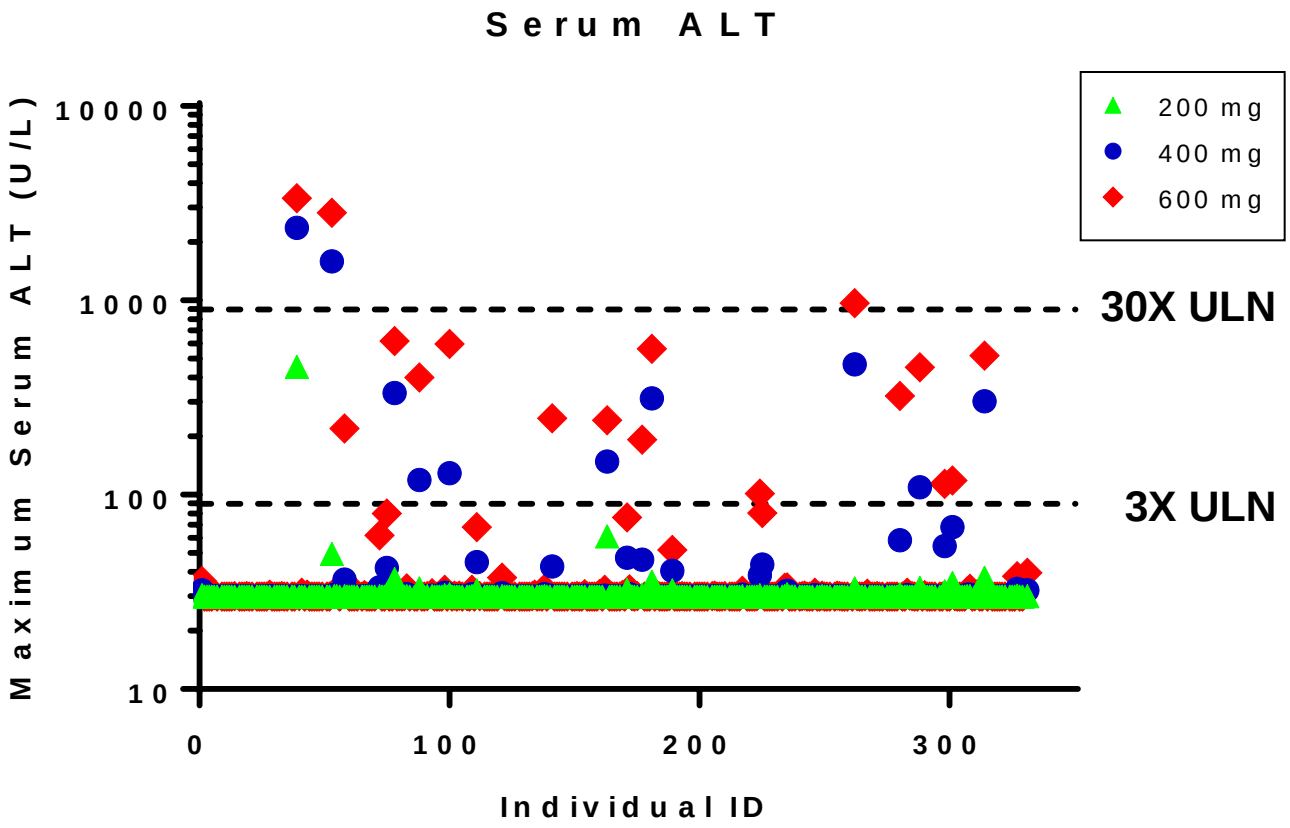


BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)

TGZ: troglitazone
TS: troglitazone sulfate

DILI-sim Initiative

Bile Acid Transport Inhibition Alone Predicted TGZ Hepatotoxicity in Human SimPops™



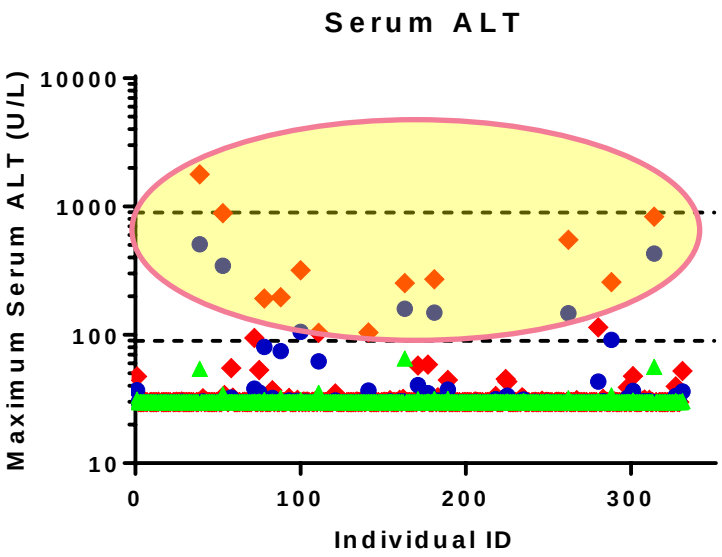
Simulated DILI responses in human SimPop™ (n=331) administered 200, 400, or 600 mg/day TGZ for 6 months

HUMANS

Simulation Results

DILI-sim Initiative

Bile Acid Transport Inhibition Alone Predicted TGZ Hepatotoxicity in Human SimPops™



17 individuals with ALT>3X in simulation of 600 mg TGZ

	Simulations			Clinical Trials
	TGZ 200 mg (n=331)	TGZ 400 mg (n=331)	TGZ 600 mg (n=331)	TGZ 200–600 mg (n=2510)
ALT > 3X ULN (%)*	**0.3**	**3.0**	**5.1**	**1.9**
ALT > 5X ULN (%)*	0.3	1.8	4.2	1.7
ALT > 8X ULN (%)*	0.3	1.8	3.6	0.9
ALT > 30X ULN (%)*	0	0.6	0.9	0.2
Bili > 2X (%)	**0.3**	**1.8**	**3.6**	N/A
Jaundice (%)	N/A	N/A	N/A	**0.08**
Hy's law (%)	**0.3**	**1.8**	**3.6**	N/A

*ULN = 34 in the clinical trials
N/A, not available

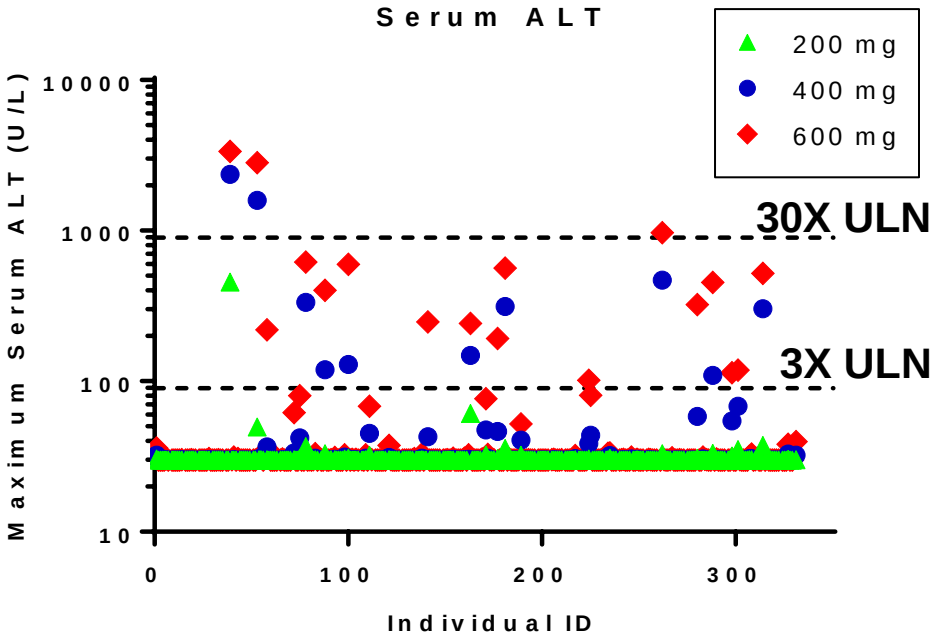
Simulated DILI responses in human SimPop™ (n=331) administered 200, 400, or 600 mg/day TGZ for 6 months

Watkins and Whitcomb 1998; Yang 2014

HUMANS

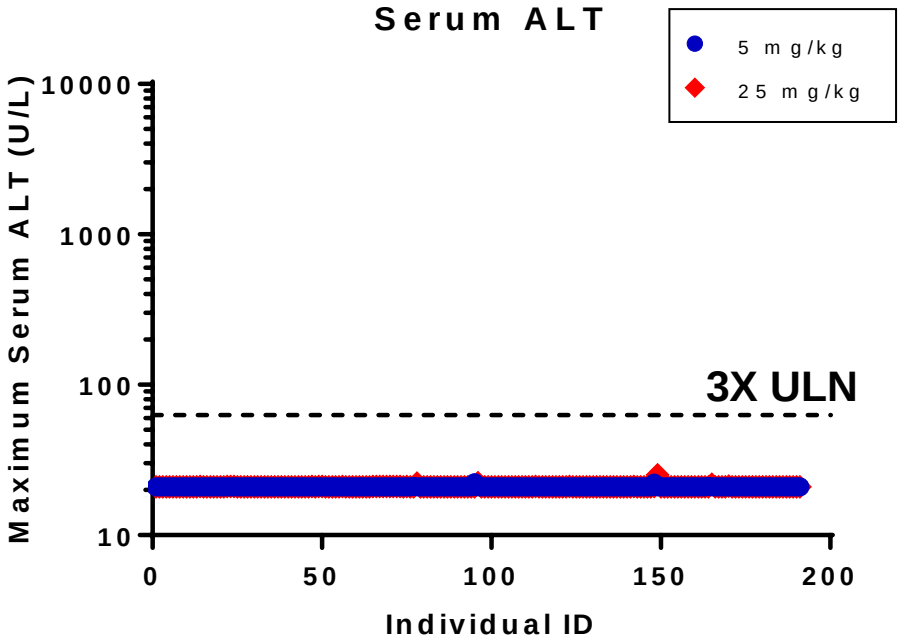
Species Difference in TGZ Hepatotoxicity Predicted

HUMAN



Simulated DILI responses in human SimPops™ (n=331) administered 200, 400, or 600 mg/day TGZ for 6 months

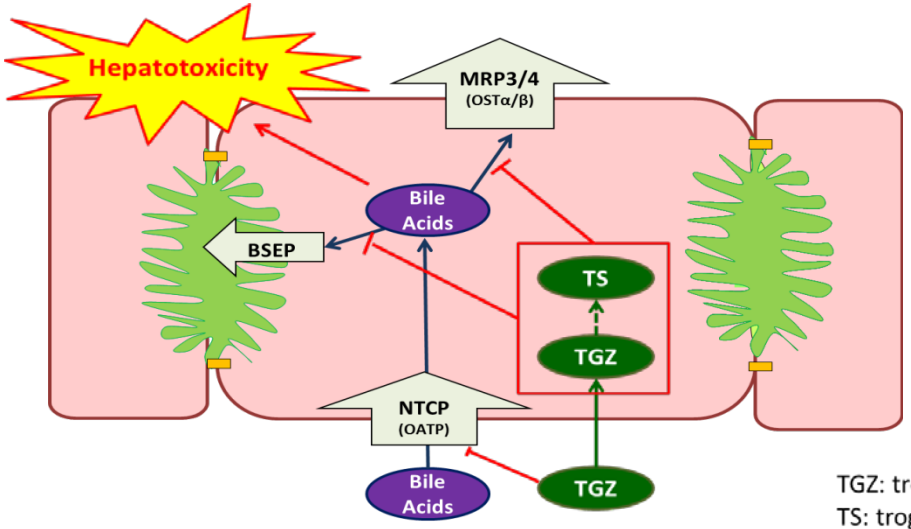
RAT



Simulated DILI responses in rat SimPops™ (n=192) administered 5 or 25 mg/kg/day TGZ for 6 months

Yang et al. CPT

Inhibition Data for Multiple Bile Acid Transporters Provides More Reliable Prediction



TGZ: troglitazone
TS: troglitazone sulfate

Transporter	Simulation I	Simulation II	Simulation III	Simulation IV
BSEP inhibition	Yes	No	Yes	Yes
MRP4 inhibition	Yes	Yes	No	Yes
NTCP inhibition	Yes	Yes	Yes	No
ALT > 3X	**3.6% (12/331)**	**0% (0/331)**	**0.3% (1/331)**	**5.1% (17/331)**
Bilirubin > 2X	**0.6% (2/331)**	**0% (0/331)**	**0% (0/331)**	**1.2% (4/331)**
Death	**0.3% (1/331)**	**0% (0/331)**	**0% (0/331)**	**0.3% (1/331)**

Simulation I – IV: 600mg/day troglitazone for 1 month

TGZ Project Outcomes

- Incidence and delayed presentation of TGZ hepatotoxicity was predicted in humans by TGZ-mediated bile acid transport inhibition alone
- Mechanistic modeling incorporating species-specific bile acid and TGZ disposition correctly predicted species differences in TGZ hepatotoxicity
- Analysis of TGZ SimPops™ results identified susceptibility factors for TGZ hepatotoxicity

Examples of DILIsym® Applications

IVIVE

J Pharmacokinet Phar 39(5): 527-541. 2012.

Rank compounds by risk

Toxicol Lett 226(2): 163-172. 2014.

Preclinical biomarker study design

Preclinical

in vitro

in vivo

Single Ascending Dose

Phase II/III/IV

Clinical

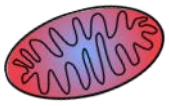
DILI Dose Response Estimation

Clinical biomarker analysis

CPT Pharmacometrics Syst Pharmacol 3: e98. 2014.

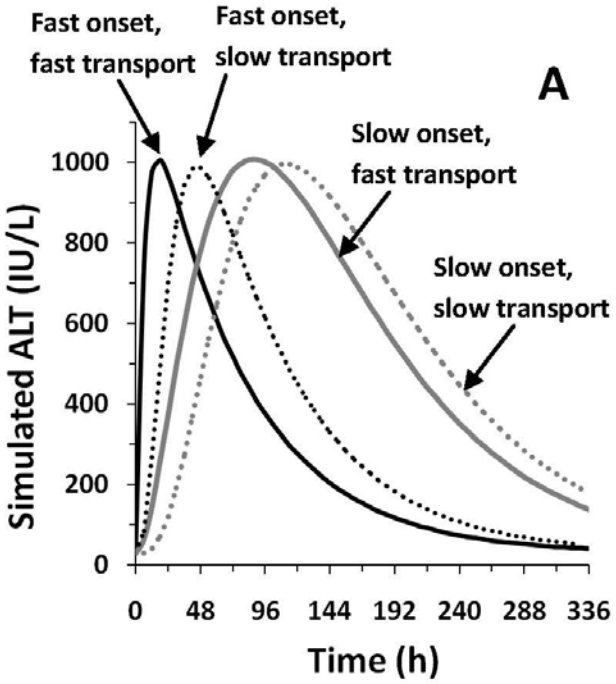
Predicting variability in response

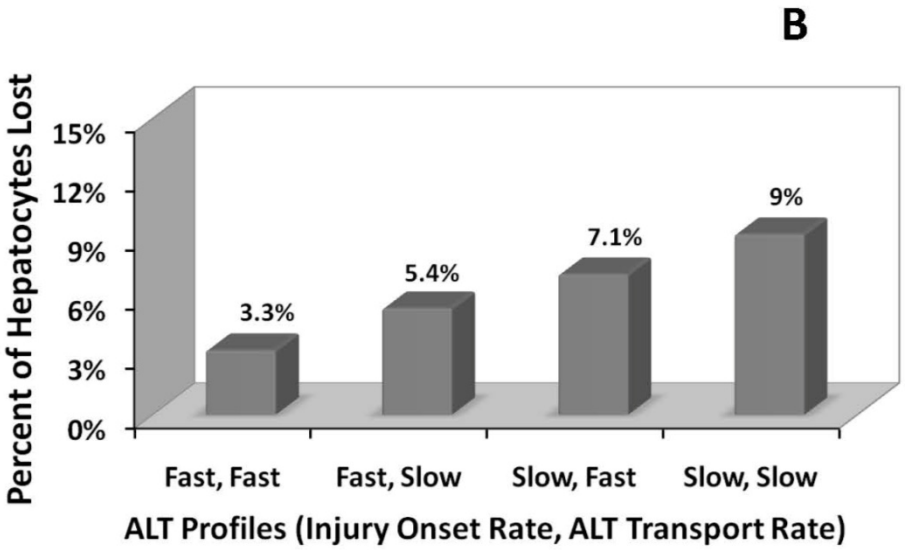
J Pharmacol Exp Ther 342(2): 529-540. 2012.



DILI-sim Initiative

The Kinetics of Liver Enzyme Profiles are Critical for Assessment of Injury





- Various ALT profiles shown with different kinetics, same peak
- Rapid rises and early peaks in ALT lead to less predicted hepatocyte loss
- The detailed time course of liver enzymes is important

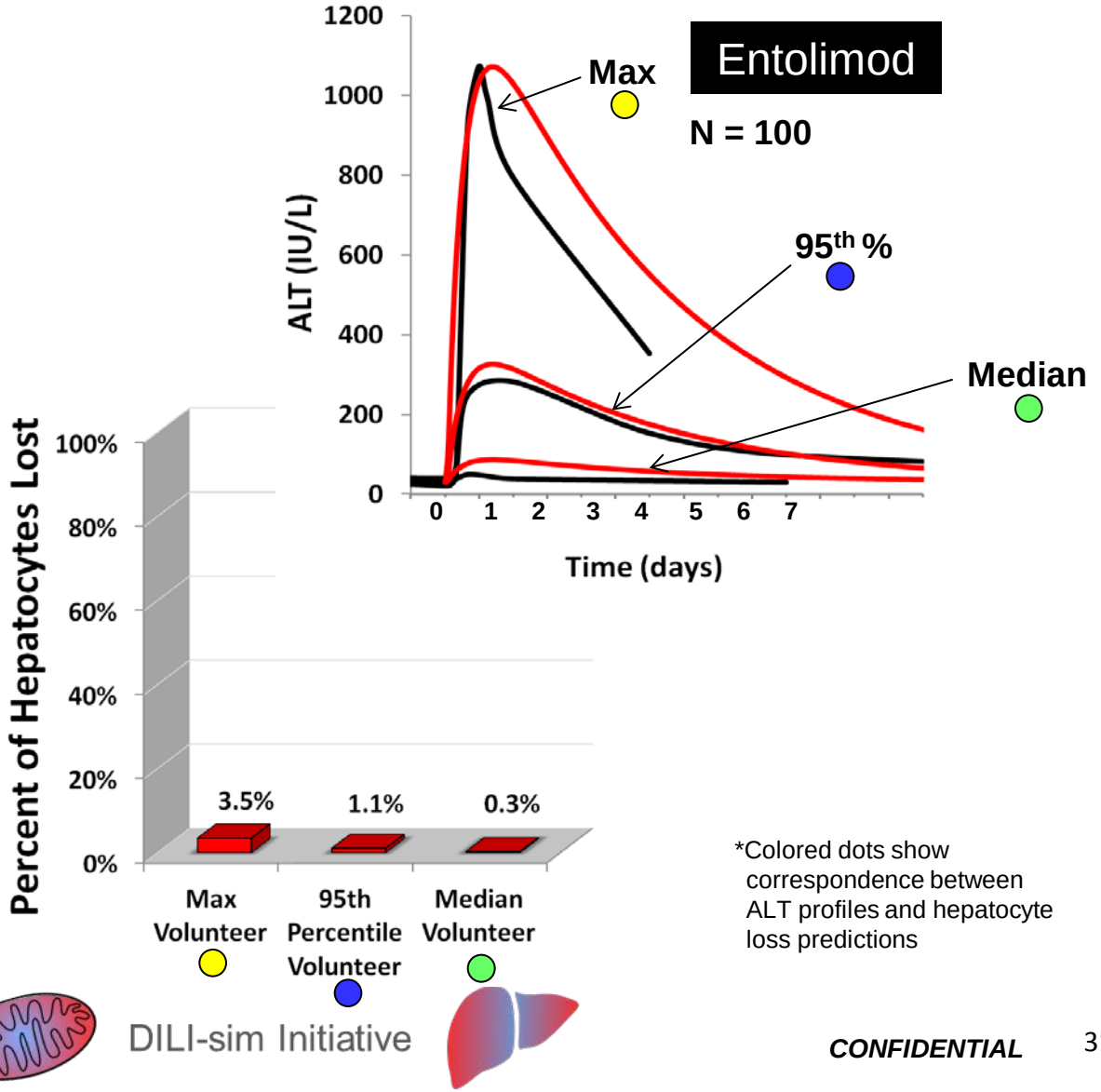
Entolimod (Cleveland BioLabs) Project Objectives

- Entolimod (single dose) reduces radiation mortality by 40%
  - Satisfies FDA's animal rule for efficacy
- Clinical Concern
  - ALT/AST elevations observed in human safety study
  - Continued development threatened
- Primary Objective
  - Use DILIsym® to infer the amount of hepatocyte necrosis necessary to achieve the ALT profiles observed after Entolimod

Howell, B. A., et al. (2014). A Mechanistic Model of Drug-Induced Liver Injury Aids the Interpretation of Elevated Liver Transaminase Levels in a Phase I Clinical Trial. CPT Pharmacometrics Syst Pharmacol 3: e98.

Baseline Human Simulations Indicate Minimal Hepatocyte Loss with Entolimod

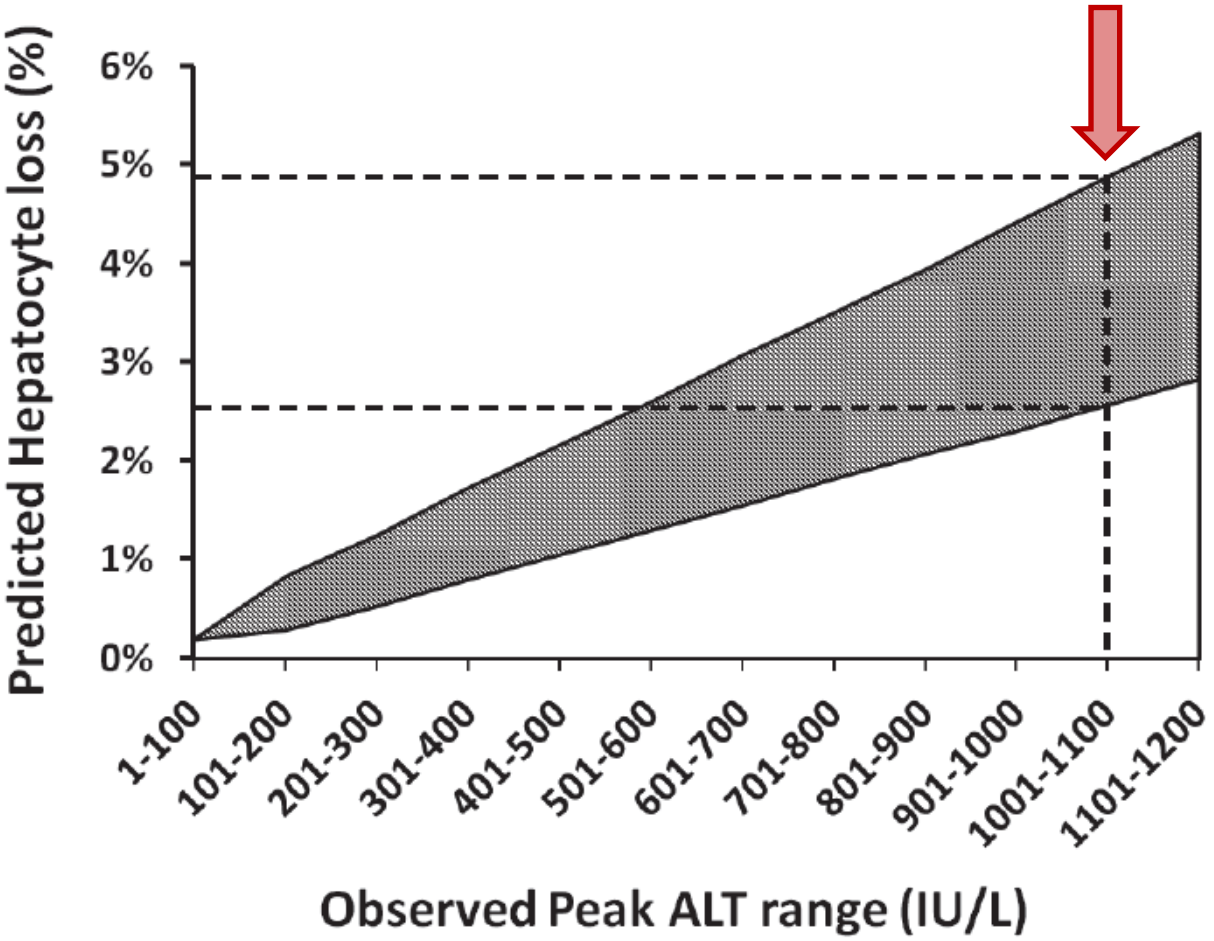
- ALT clinical data
  - Mostly minor elevations
  - Few higher elevations
- Focused on max, 95th percentile, and median ALT levels
- Simulations agree with ALT clinical data by design
- Minimal hepatocyte inferred from ALT profiles



*Colored dots show correspondence between ALT profiles and hepatocyte loss predictions

Minimal Range of Hepatocyte Loss Predicted for Entolimod Using Population Sample

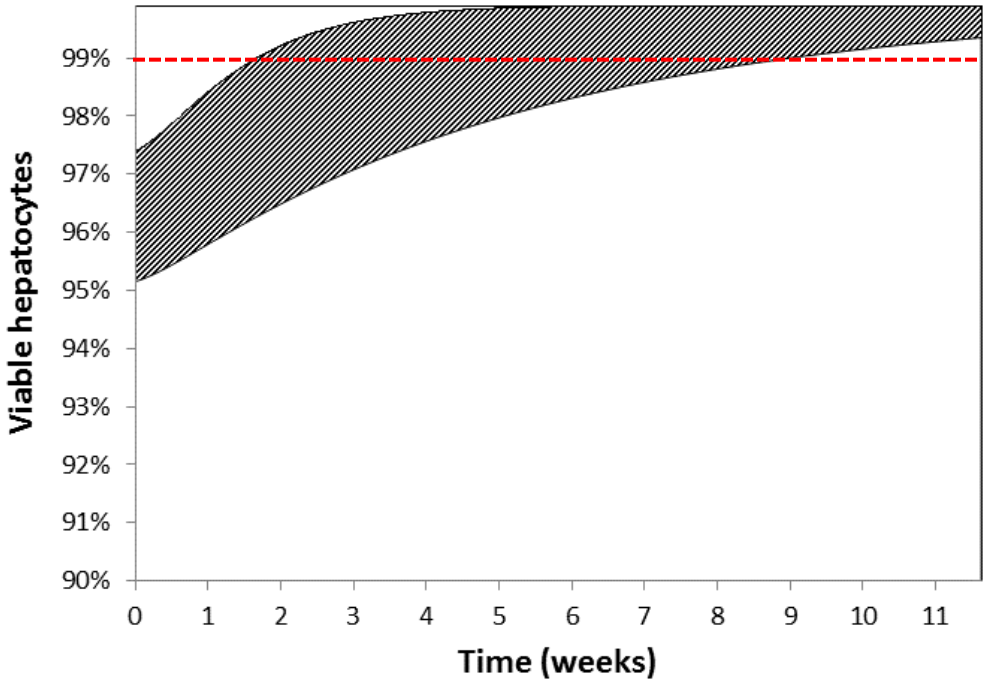
- Various levels of necrosis simulated for population sample
- Max observed ALT (1001-1100 U/L) corresponds with 2.6-4.6% predicted hepatocyte loss*
- DILIsym® simulation results were submitted to the FDA in support of the safety of Entolimod



*Predictions only valid for time courses similar to those observed with Entolimod

Regenerative Hepatocyte Proliferation Predicted to be Complete 2-9 Weeks after Entolimod Dosing

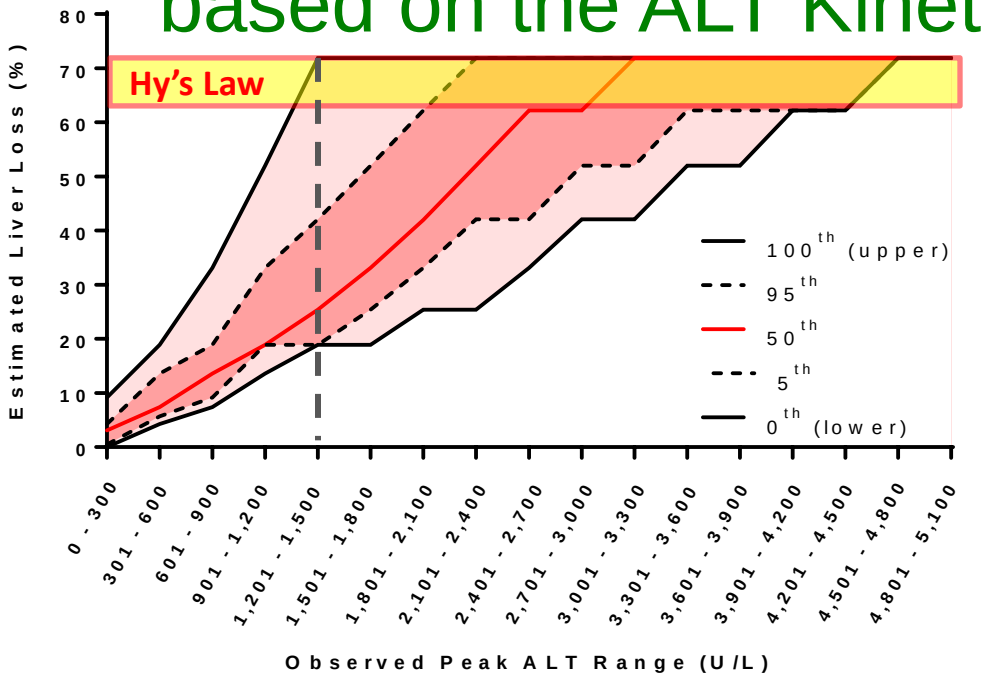
- Population sample included variability in hepatocyte proliferation
- Hepatocyte restoration complete within ~2-9 weeks after onset of injury (median human prediction - 3 weeks)



Project Summary

- Analyses indicate that volunteers with ALT elevations following Entolimod administration likely incurred hepatocyte losses of ≤5%
- The liver should have completely recovered in 2-9 weeks
- Literature review and modeling heparin-induced ALT profiles support the conclusion that the potential hepatocyte loss occurring in the Entolimod clinical trial did not represent a serious health threat
- DILIsym® simulation results were submitted to the FDA in support of the safety of Entolimod

Current DILIsym® Projects are Focused on Quantifying the Probability of Serious Liver Injury based on the ALT Kinetics and Peak Values



Peak ALT Range (U/L)	Likelihood of Hy's Law (%)
1 - 300	0
301 - 600	0
601 - 900	0
901 – 1,200	0
1,201 – 1,500	2.0
1,501 – 1,800	3.3
1,801 – 2,100	11
2,101 – 2,400	31
2,401 – 2,700	57
2,701 – 3,000	81
3,001 – 5,100	> 90

- ALT time course kinetics specific to a given compound and clinical study are analyzed
- Information regarding the extent of hepatocyte loss required for seriously compromised liver function already incorporated into DILIsym® is used to estimate the probability of serious liver injury having occurred at each ALT level for a given kinetic profile
- This information helps decision makers understand how close or far their cases may have been to serious liver injury, and risk/benefit can be assessed