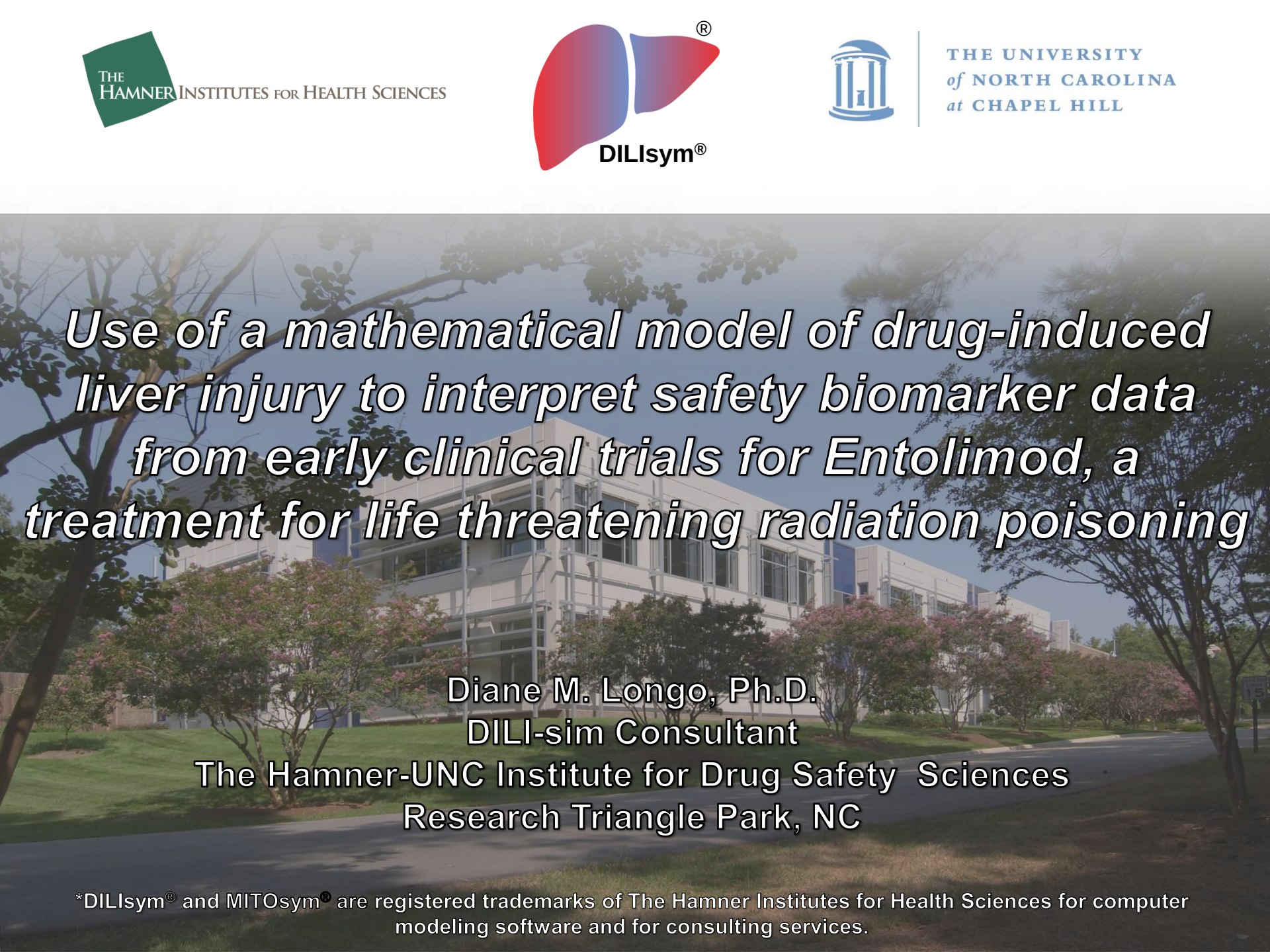


Use of a mathematical model of drug-induced liver injury to interpret safety biomarker data from early clinical trials for Entolimod, a treatment for life threatening radiation poisoning

Diane M. Longo, Ph.D.
DILI-sim Consultant
The Hamner-UNC Institute for Drug Safety Sciences
Research Triangle Park, NC

The DILI-sim Initiative Is a Partnership between the Hamner Institutes and Pharmaceutical Companies to Minimize DILI

- *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*
  - *14 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $4 million total invested in project*

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**

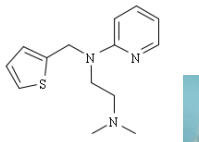
Long term goal:

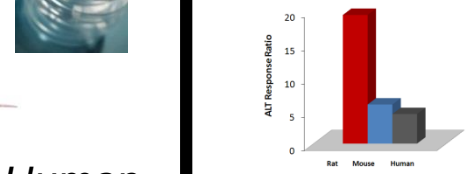
- Use DILIsym® to increase understanding of idiosyncratic DILI

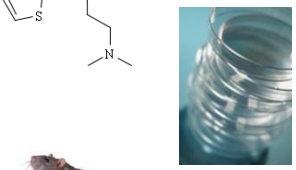
Intended application:

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

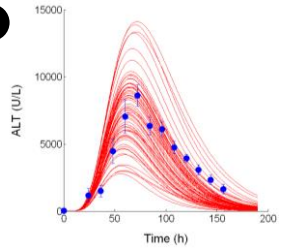
Preclinical

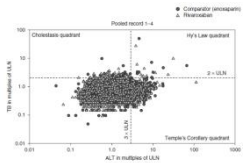


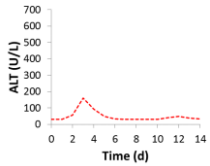




First in Human Clinical Trials

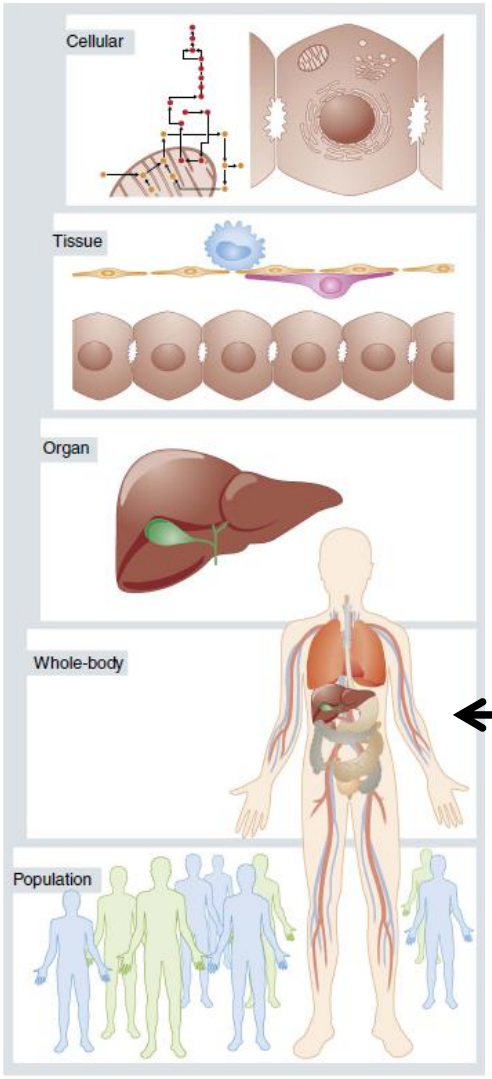




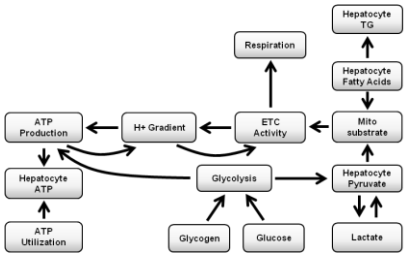


Phase II/III Clinical Trials and Post-Market Surveillance

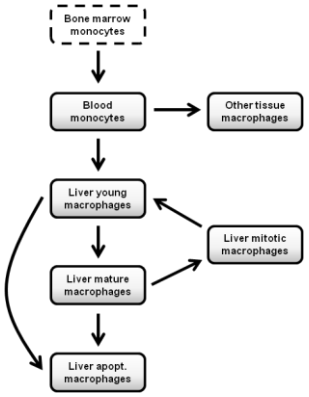
DILIsym®: 'Middle Out' and Multi-Scale



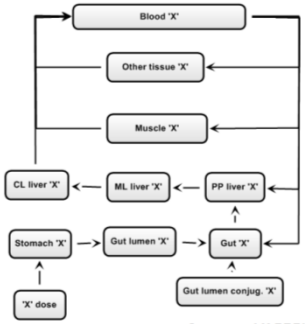
Mitochondrial dysfunction



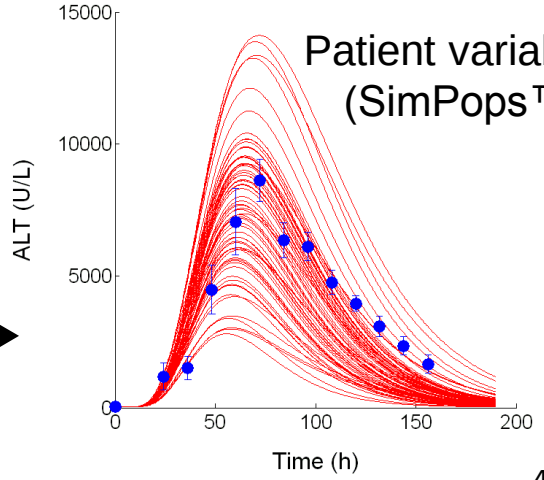
Cellular life-cycle



Drug distribution & metabolism



Compound X PBPK

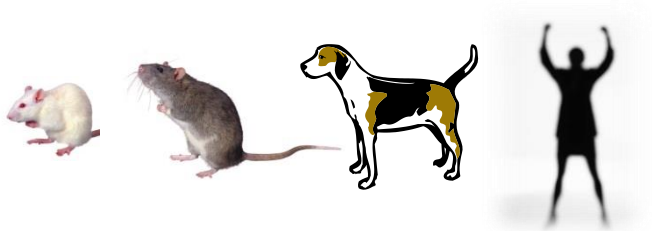


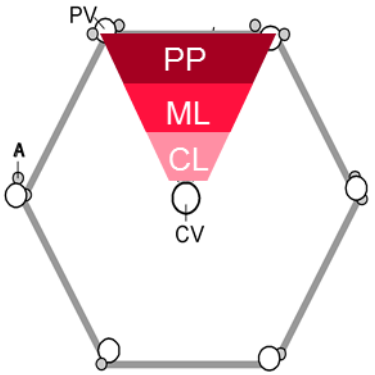
Patient variability (SimPops™)

Kuepfer 2010, Molecular Systems Biology

DILIsym® Overview

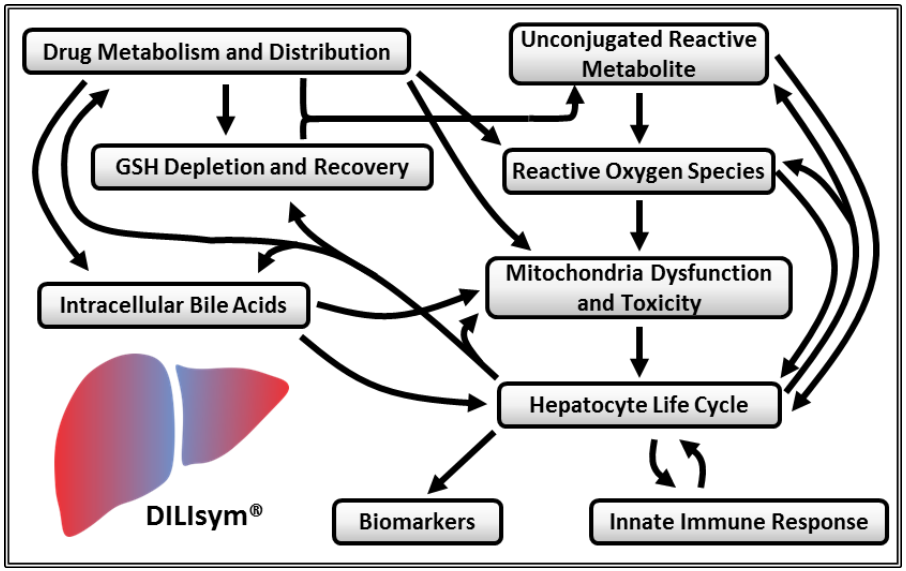
- **Multiple species: human, rat, mouse, and dog**
  - Population variability





- **The three primary acinar zones of liver represented**

- **Essential processes represented to multiple scales in interacting sub-models**



Biomarkers of Hepatocellular Death Are Outputs of DILIsym®

- Biomarkers are outputs of model
  - Used for validation of DILIsym® model
  - Used for comparison with clinical and preclinical data
- More biomarkers being added as data are becoming available
  - SDH, miR-122 latest examples
- 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 (direct/conjugated)[1,5]	Function/Cholestasis
Bilirubin (total)[1,2,5]	Function/Cholestasis
Aspartate aminotransferase (AST)[1,2,3,4,5]	Necrosis
Prothrombin time [1,2]	Function
HMGB1[1,10]	Necrosis
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

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

DILI Dose Response Estimation

Clinical biomarker analysis

Predicting variability in response

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

Clin Pharmacol Ther 96(5):589-98. 2014

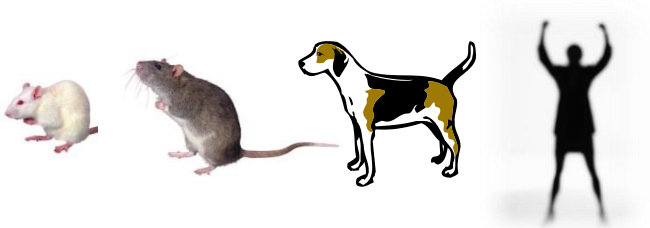
Entolimod (Cleveland BioLabs) Project Objectives

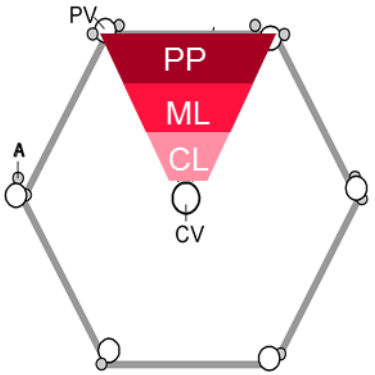
- Entolimod (single dose) reduces radiation mortality by 40%
  - Satisfies FDA's animal rule for efficacy
- Clinical Concern
  - ALT/AST elevations observed in human safety study
  - Continued development threatened
- Primary Objective
  - Use DILIsym® to infer the amount of hepatocyte necrosis necessary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

DILIsym® Overview

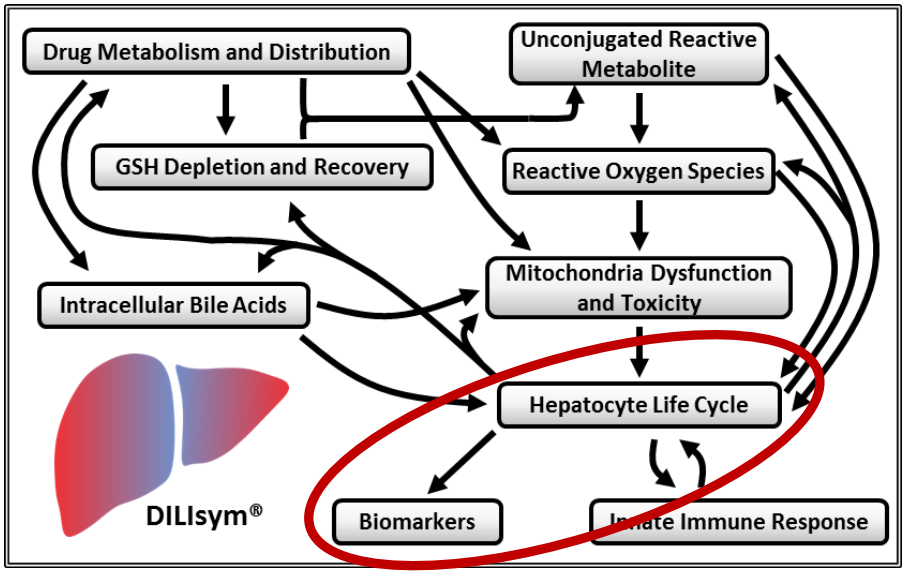
- **Multiple species: human, rat, mouse, and dog**
  - Population variability





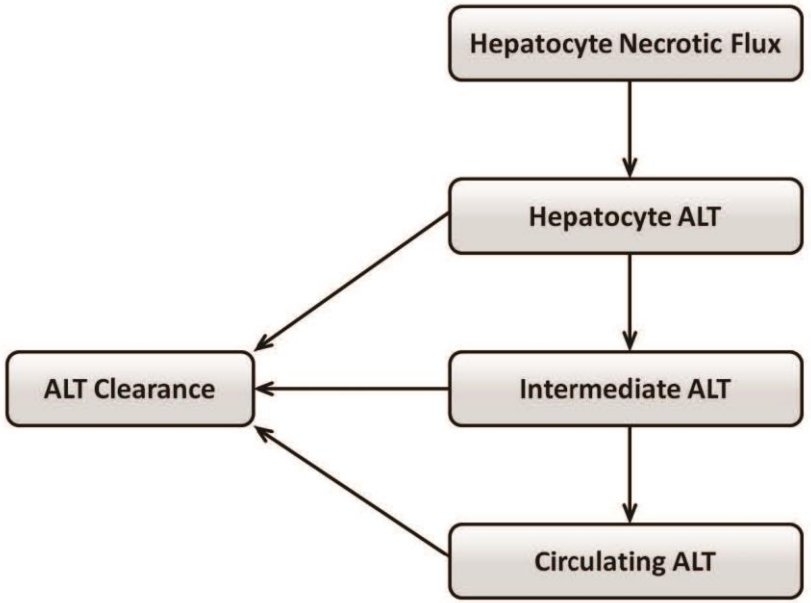
- **The three primary acinar zones of liver represented**

- **Essential processes represented to multiple scales in interacting sub-models**
  - **Hepatocyte life cycle**
  - **Biomarkers**



Approach for Using Simulations to Analyze Entolimod Clinical Data

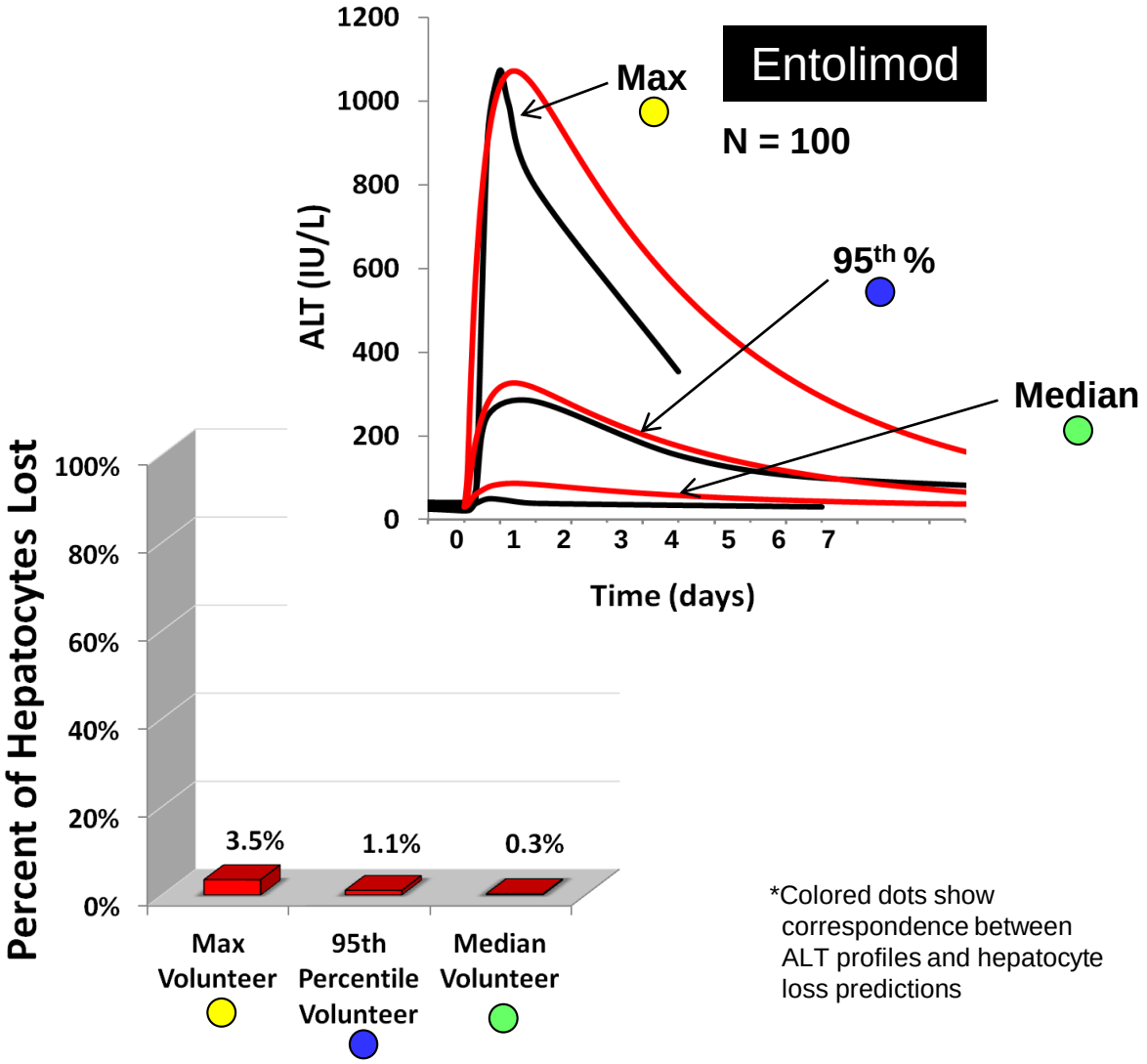
- Approach: use ALT dynamics to infer hepatocyte loss
  - ALT content per cell based on cellular measurements
    - *Boyd 1983, Remien 2012, Lindblom 2007*
  - ALT release occurs upon hepatocyte necrosis
  - ALT elimination half-life based on clinical data
    - *Nicoll 1997*



- Initial simulations in DILIsym® baseline normal healthy volunteer (NHV)

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



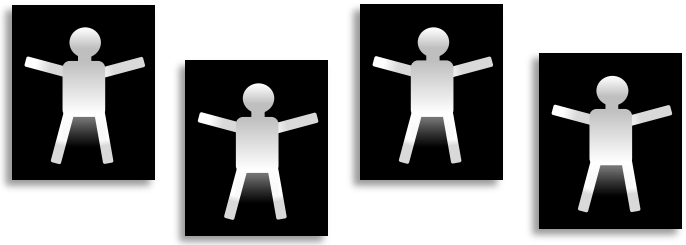
*Colored dots show correspondence between ALT profiles and hepatocyte loss predictions

Clinical Data and Simulation Results

Approach for Introducing Population Variability into Simulations

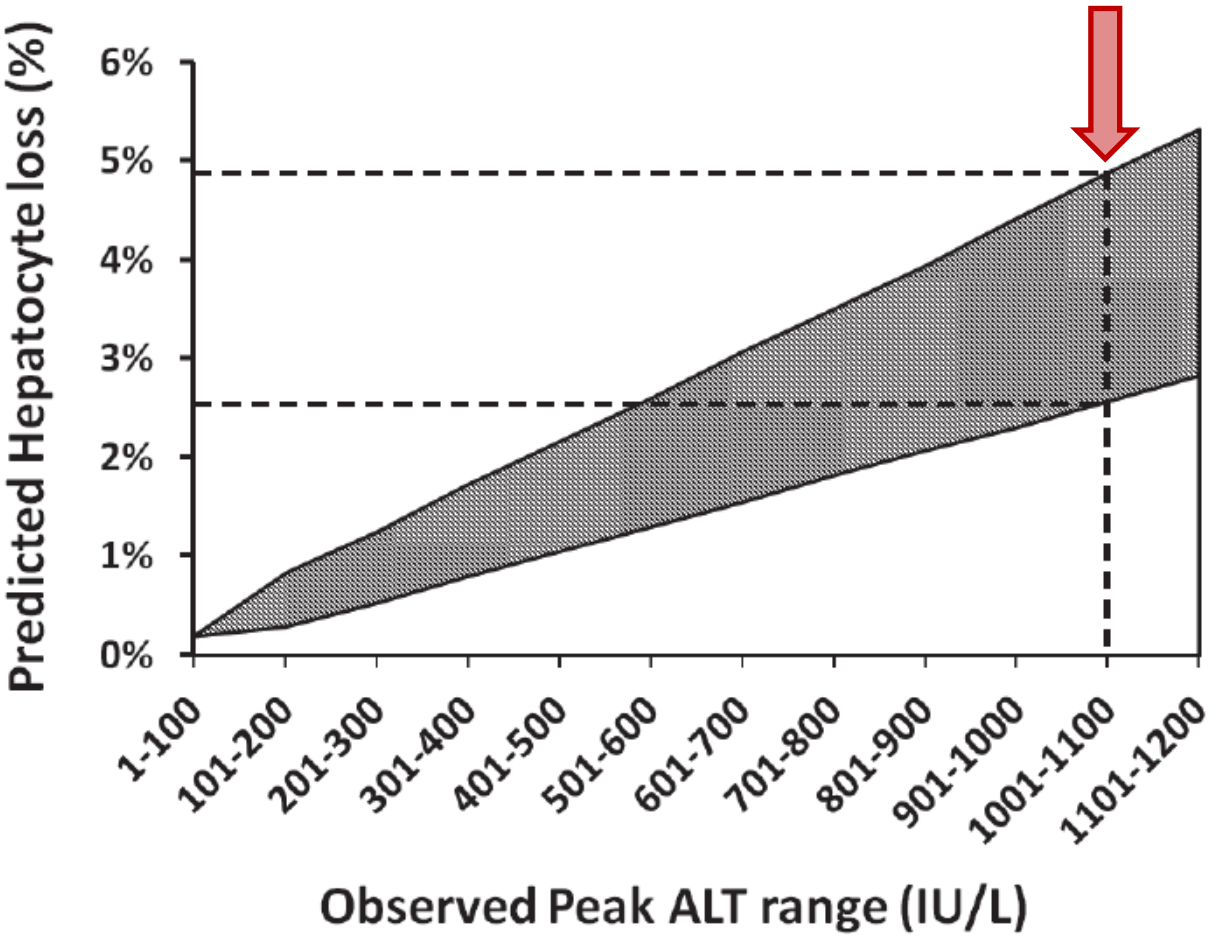
- Varying parameters associated with ALT dynamics in accordance with variance described in literature
  - *Remien 2012, Nicoll 1997, Portmann 1975, Prescott 1979*
- Compared simulated humans (N ≈ 300) with clinical data from Prescott 1979 and Portmann 1975
  - Indirect link between ALT and necrosis
- Simulated humans used to simulate Entolimod trial protocol

Variables Used to Construct Population Sample for Entolimod Application
Hepatocellular ALT content
ALT $t_{1/2}$
ALT transport rate
Hepatocyte proliferation rate



Minimal Range of Hepatocyte Loss Predicted for Entolimod Using Population Sample

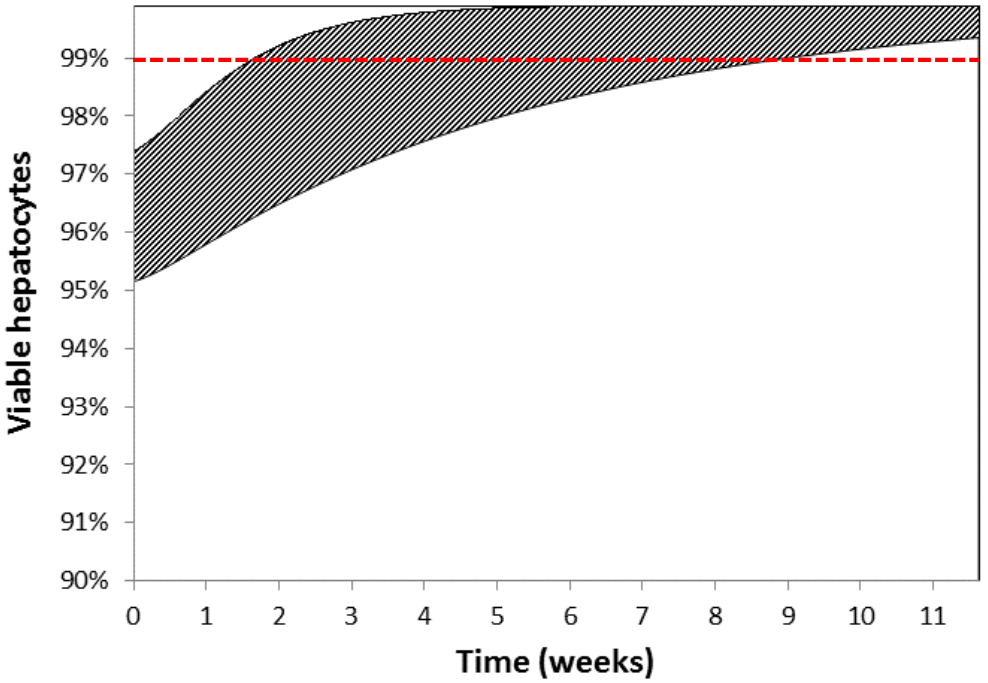
- Various levels of necrosis simulated for population sample
- Max observed ALT (1001-1100 U/L) corresponds with 2.6-4.6% predicted hepatocyte loss



*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)

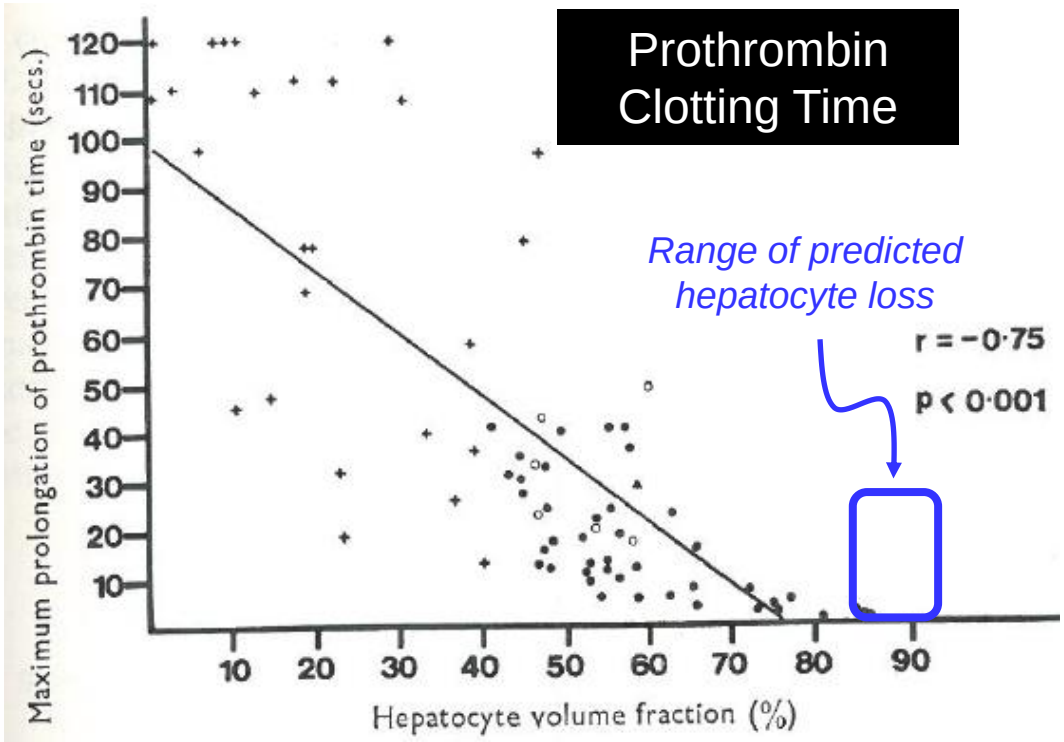


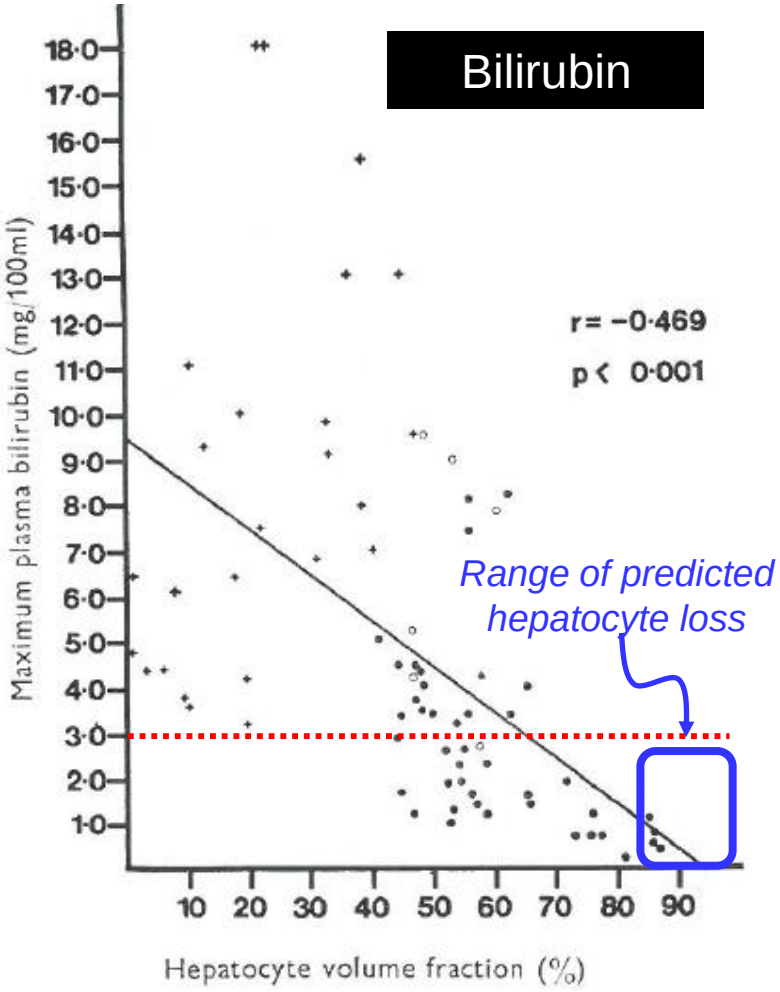
Evidence from Literature to Support Safety of Minimal Hepatocyte Loss with Entolimod

- Excision of up to 60% of liver volume in living human donors commonly performed for autologous transplantation
  - (*Florman 2006, Lee 2010*)

- Clinical data from literature indicate that approximately <25% loss of hepatocytes has no detectable effect on liver function

Minimal Symptom Presentation Likely with ≤25% Hepatocyte Loss: Portmann 1975 Clinical Data





- Entolimod safety study confirms lack of significant bilirubin elevations
- Risk of jaundice and bruising/bleeding likely to be low in range of predicted hepatocyte loss (*Portmann 1975*)

Evidence from Literature to Support Safety of Minimal Hepatocyte Loss with Entolimod

- Excision of up to 60% of liver volume in living human donors commonly performed for autologous transplantation
  - (*Florman 2006, Lee 2010*)

- Clinical data from literature indicate that approximately <25% loss of hepatocytes has no detectable effect on liver function

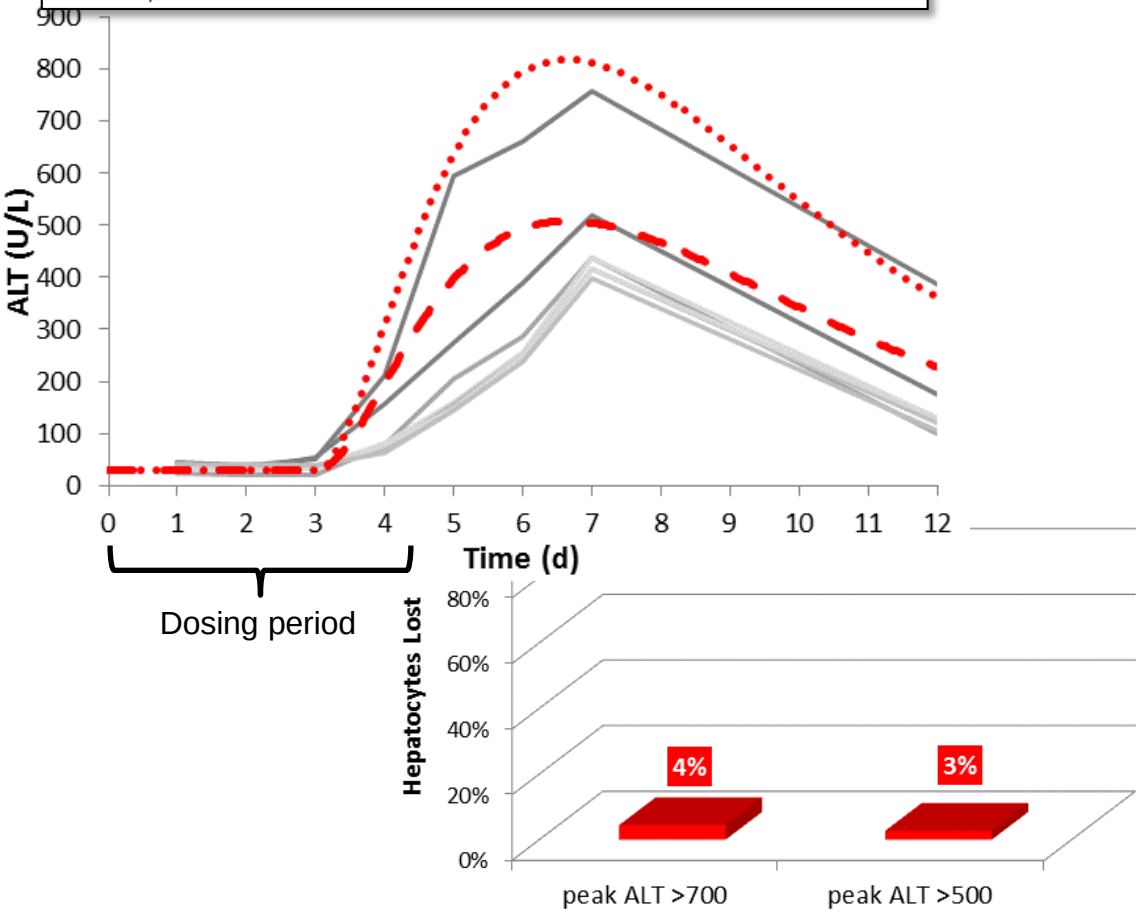
- Heparins are safe despite causing hepatocyte necrosis

Simulations Predict Minimal Hepatocyte Loss with Heparin Treatment

- Transient, benign ALT increases observed with heparin treatment in NHV
  - Additional biomarkers indicated necrosis
- Simulations performed with DILIsym® NHV
  - Focused on highest responders
- Similar hepatocyte loss predicted from observed ALT profiles as Entolimod

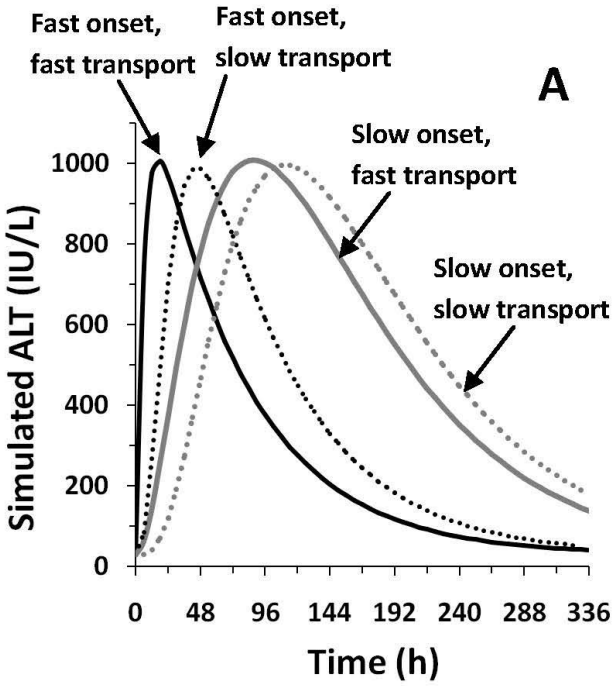
The Effects of Heparins on the Liver: Application of Mechanistic Serum Biomarkers in a Randomized Study in Healthy Volunteers

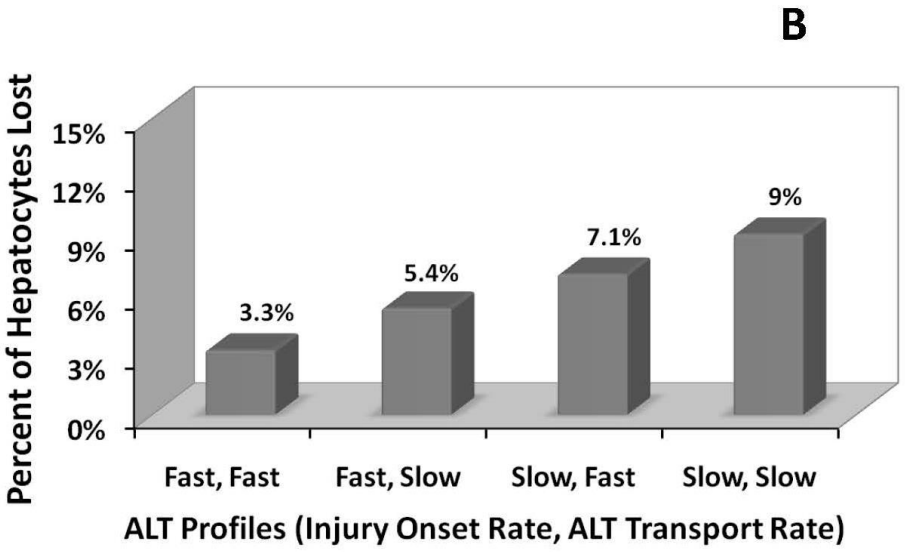
AH Harrill[1,2], J Roach[3], I Fier[3], JS Eaddy[1], CL Kurtz[1], DJ Antoine[4], DM Spencer[5,6], TK Kishimoto[3], DS Pisetsky[5,6], BK Park[4] and PB Watkins[1,2,7]



Clinical Data and Simulation Results

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





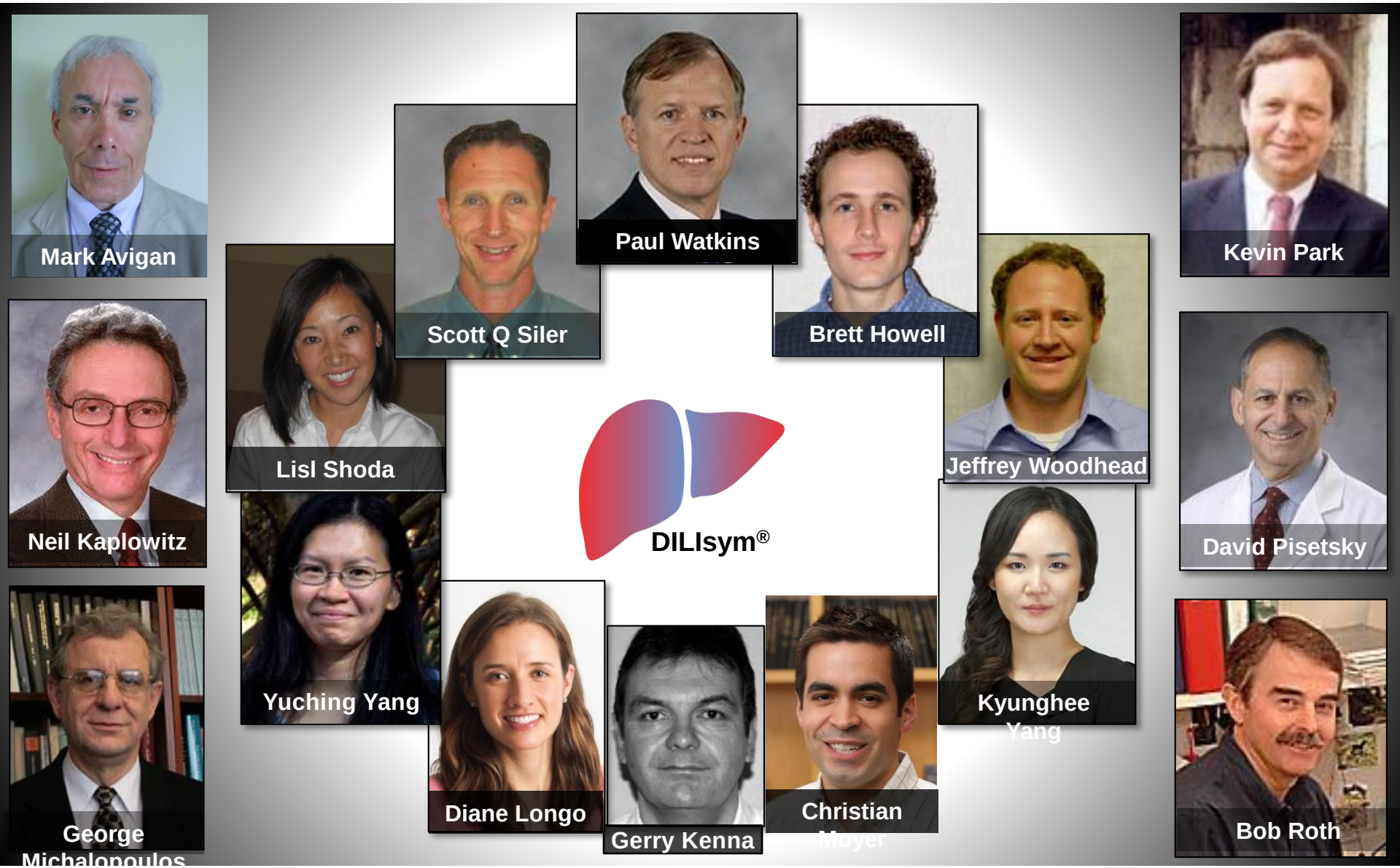
- Various ALT profiles shown with different kinetics, same peak
- Rapid rises and early peaks in ALT lead to less predicted hepatocyte loss
- The detailed time course of liver enzymes is important

Howell *et al.* 2014 - *CPTPSP*

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

The DILI-sim Team and the SAB



Acknowledgements

- Cleveland BioLabs
  - Sponsored work on Entolimod
  - Allowed the presentation of the materials

- DILI-sim members