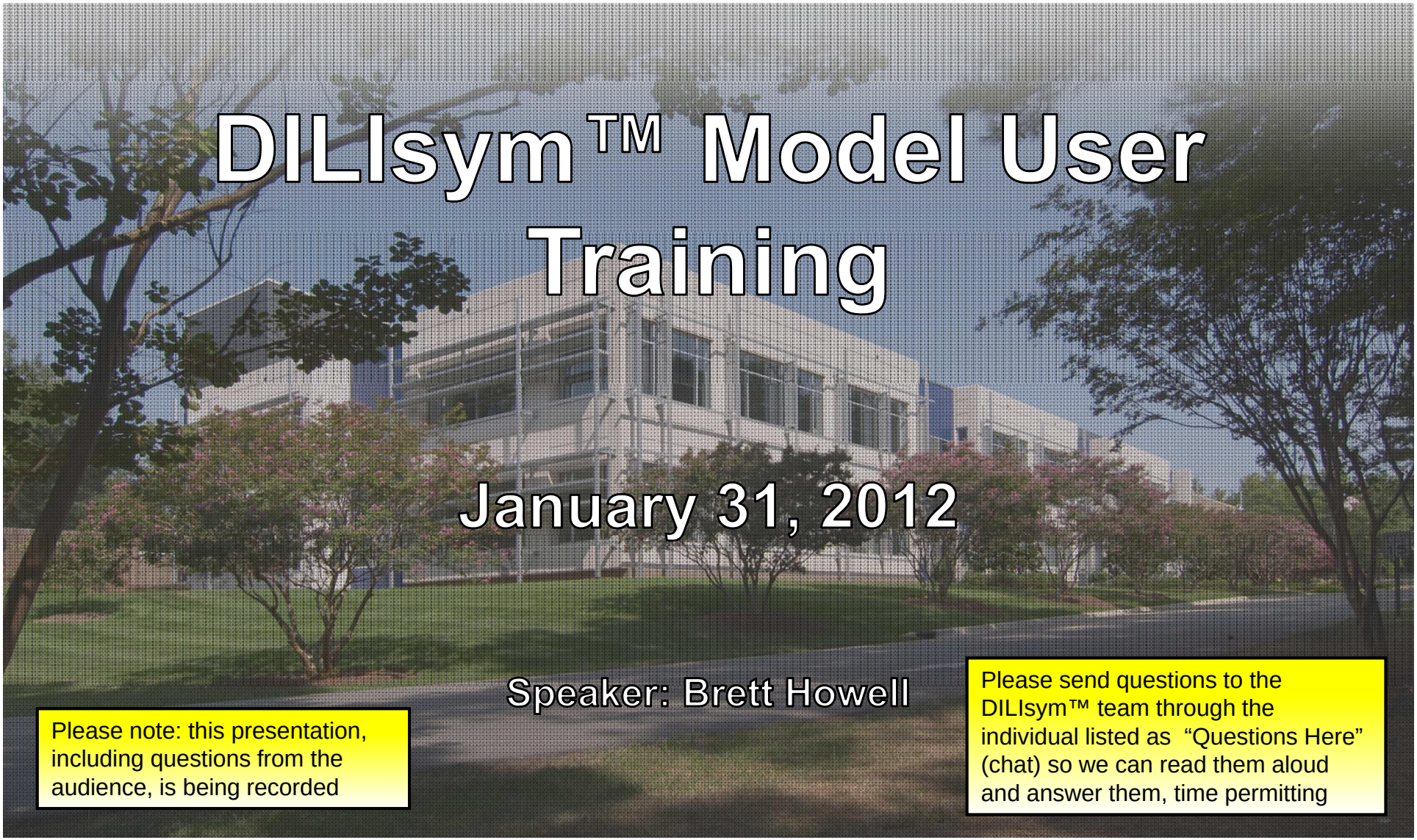


DILIsym™ Model User Training

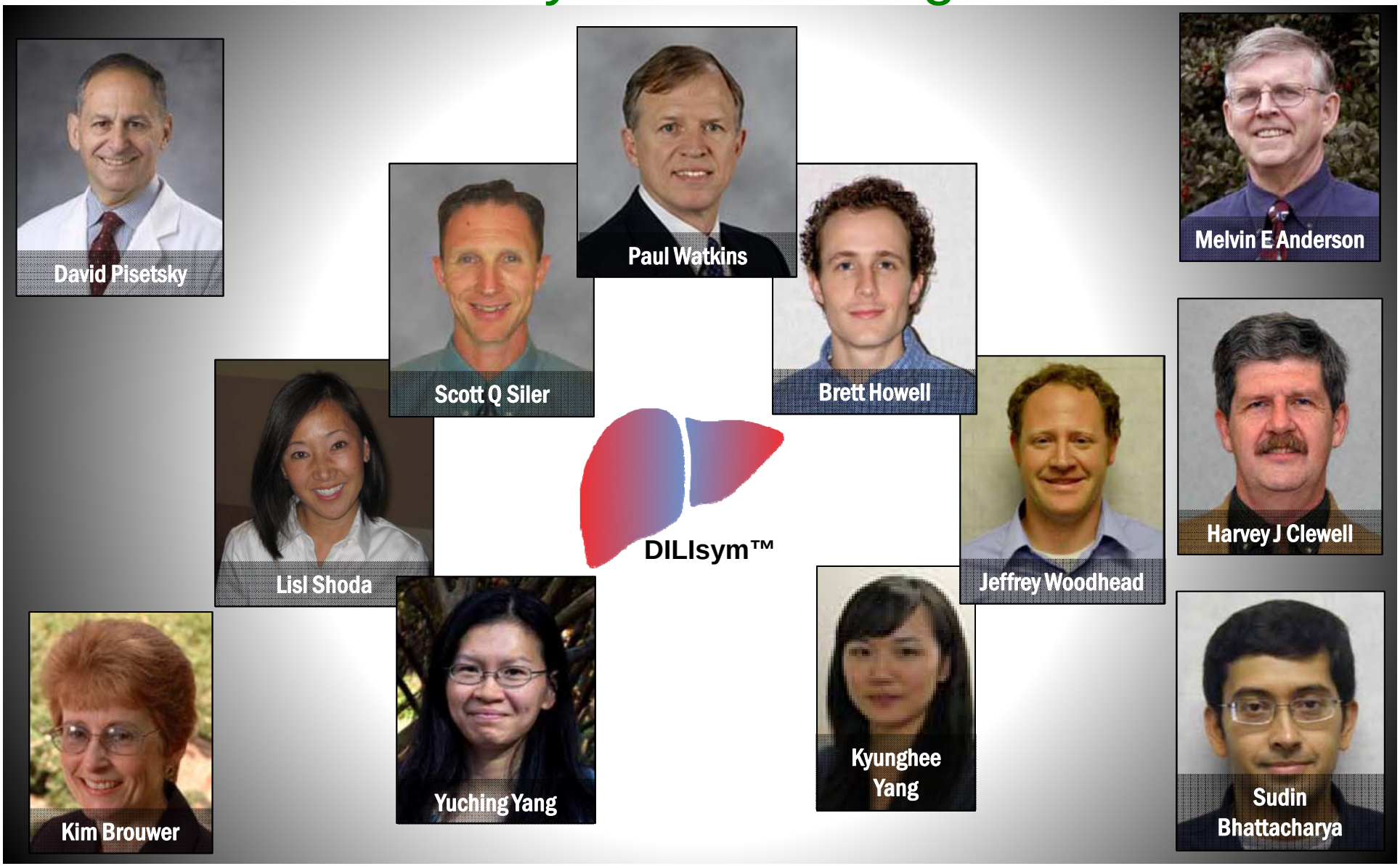
January 31, 2012

Speaker: Brett Howell

Please note: this presentation, including questions from the audience, is being recorded

Please send questions to the DILIsym™ team through the individual listed as "Questions Here" (chat) so we can read them aloud and answer them, time permitting

The DILIsym™ Modeling Team



DILIsym™ Model User Training Agenda

- DILI-sim Initiative goals overview
- DILIsym™ modeling strategy
- DILIsym™ feature highlights
- SimPops™ generation methods
- DILIsym™ model application examples
- Questions
- *Break*
- DILIsym™ documentation
- General overview of DILIsym™ and MATLAB directory structure
- Running simulations using the Graphical User Interface
- Running simulations using the MATLAB code files
- Storing and using DILIsym™ model results
- Troubleshooting
- Questions

DILI-sim Initiative Goals

Near-term

- Develop DILIsym™ model to better inform decision making concerning DILI at key points in the drug development life cycle
  - *In vitro* to *in vivo* (mouse, rat, dog) transition
  - Pre-clinical species to first-in human
- Organize DILI knowledge into a useful, dynamic format

Long-term

- Use DILIsym™ model to increase understanding of idiosyncratic DILI and how novel compounds interact with unique characteristics of individuals to evaluate hepatotoxic risk

DILIsym™ Model Context of Use

- Objective
  - Develop DILIsym™ model to aid in decision making
- Goals and Context
  - *Short term*
    - DILIsym™ provides an additional source of information for compound-progression decisions and mechanistic understanding at the in vitro and pre-clinical phases through first-in-human (FIH) studies
  - *Long term*
    - DILIsym™ may provide information on rare event frequencies and assist with clinical trial design and interpretation from the DILI perspective
- Claims
  - DILIsym™ model predictions will:
    - help balance the potential of a compound or compound class as an effective, marketable drug against DILI concerns
    - improve pre-clinical model selection and study design
    - explain species differences with respect to DILI, including which pre-clinical results are most applicable to humans
    - suggest or evaluate novel biomarkers or biomarker panels with mechanistic relevance for DILI

DILIsym™ Model Will Support Decision Making at Key Points in Process

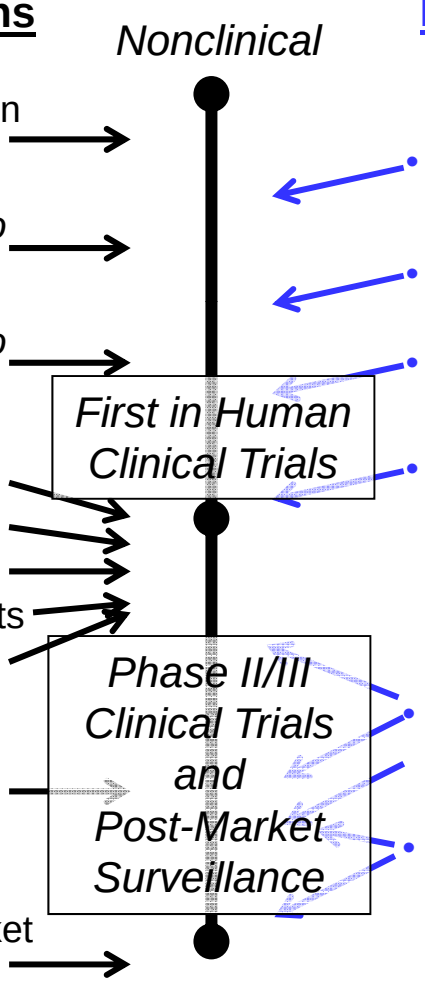
Industry hepatotoxicity decisions

- Select molecules to screen *in vitro* in microsomes and/or hepatocytes
- Select molecules to evaluate *in vivo* in rats
- Select molecules to evaluate *in vivo* in dogs

- Select molecules to use in human
- Select dose to use in human
- Select duration of trial
- Select number of patients for cohorts
- Select which biomarkers to assay

- Adjust dosing or terminate trial if clinical liver signal arises

- Add warning or withdraw from market if clinical liver signal arises



DILIsym™ model contributions

- *Simulate in vivo rat hepatotox*
- *Simulate in vivo dog hepatotox*
- *Simulate human hepatotox across wide dosing range and dosing periods*
- *Simulate biomarkers associated with hepatotox*

- *Analyze model to develop mechanistic hypotheses for observed liver signals*
- *Simulate hepatotoxicity across wide range of patient types*

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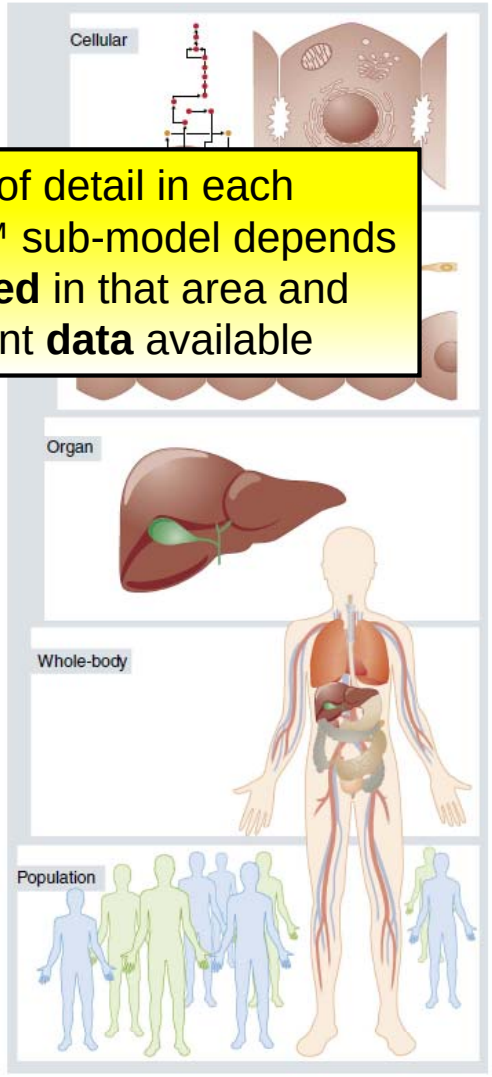
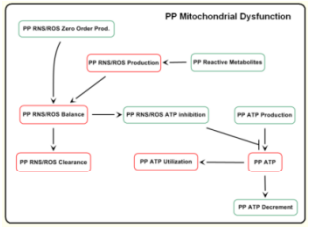
The DILIsym™ Model Is Multi-Scale

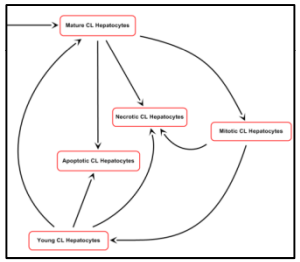
The level of detail in each DILIsym™ sub-model depends on the **need** in that area and the relevant **data** available



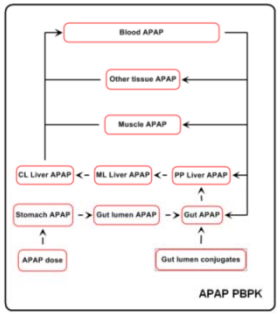
Mitochondrial dysfunction



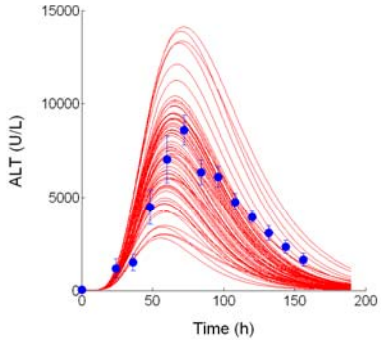
Hepatocyte life-cycle



Drug distribution & metabolism



Response variability



Kuepfer 2010, Molecular Systems Biology

DILIsym™ Model Represents Relevant Hepatic Biochemistry and Physiology

- A model of the biochemical and physiological processes involved in hepatotoxicity enables evaluation of toxicity risk of novel compounds
  - Humans, rats, mice, dogs (in progress)
- DILIsym™ model currently represents reactive metabolite-based hepatotoxicity
  - Acetaminophen literature used to determine biochemical interactions and quantitative relationships
  - Acute overdose and adaptations associated with multiple dosing
- Additional proprietary and non-proprietary compounds have been used to expand and further validate DILIsym™ model
  - Methapyrilene
  - Furosemide
  - Aflatoxin B1
  - AMAP (3'-hydroxyacetanilide)
- DILIsym™ model optimized with literature and data describing full and sub-model quantitative interactions and dynamics
  - Additional data sets used to validate full model in addition to individual sub-models

Extensive Data Comparisons Are Conducted and Included in the DILIsym™ Model

Mouse Studies

- Harrill 2009 (PK)
- Saito 2010 (GSH)
- McConnachie 2007 (GSH)
- Soga 2006 (GSH)
- James 2003 (GSH)
- Masubuchi 2009 (GSH)
- Vaquero 2007 (GSH, APAP adducts)
- Liu 1999 (GSH, RNS/ROS)
- Srinivasan 2001 (GSH)
- Muldrew 2002 (GSH, APAP adducts)
- Whitehouse 1985 (GSH)
- Michael 2001 (RNS/ROS)
- Hanawa 2008 (ATP)
- DeAngelis 2005 (regeneration rate)
- Antoine 2009 (HMGB1, K18)
- Aleksunes 2008, Fujimoto 2009, Campion 2008, Liu 2004, Liu 2006, Henderson 2007, Maddox 2010, Bourdi 2002, Dambach 2006, Srinivasan 2001, Shinohara 2010, Nakagawa 2008, James 2003, Gunawan 2006 (ALT)

Rat Studies

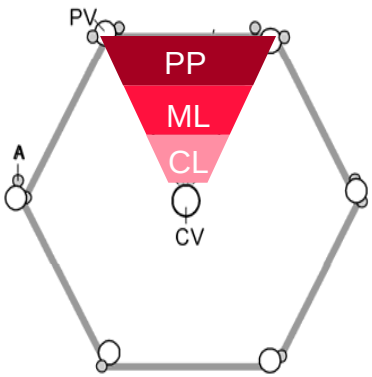
- Green 1984 (GSH)
- Galinsky 1981 dose-rsps (APAP PK, APAP sulfate PK)
- Kim 1992 dose-rsps (sulfate ratio)
- Hjelle 1984 dose-rsps (PK)
- Chen 2009 (GSH, RNS/ROS)
- Kim 2007 (GSH)
- Adams 1983 (GSH)
- Ghosh 2009 (GSH, RNS, ROS)
- Vendemiale 1996 (GSH, ATP inhibition)
- Chanda 1995 (GSH)
- Speeg 1985 (APAP adducts)
- Lauterburg 1983 (GSH)
- Zieve 1985 (regeneration)
- Hockings 2002 (regeneration)
- Kostrubsky 2003 (bile acids)
- Nirala 2008, Sawant 2004, Pooranaperundevi 2010a, b (bilirubin)
- Guerguen 2007, Chen 2009, Sugimura 1998, Wang 1999, Waters 2001, Zieve 1985, Chanda 1995 (ALT)

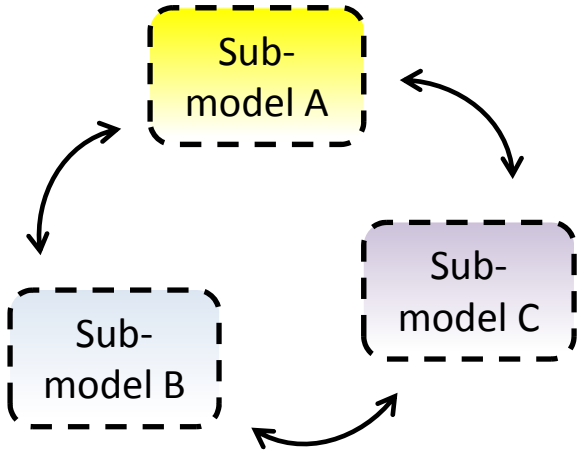
Human Studies

- Davis 1976 (bilirubin, prothrombin)
- Prescott 1980 (PK)
- Eandi 1974 (PK)
- Schiodt 2001 (ALT)
- Davidson 1976 (bilirubin)
- Singer 1995 (AST)
- Portmann 1975 (viable HC vs. bilirubin)
- Portmann 1975 (viable HC vs. prothrombin)
- Portman 1975 (regeneration time)
- Slattery 1979 (PK)
- Lauterburg 1988 (GSH)

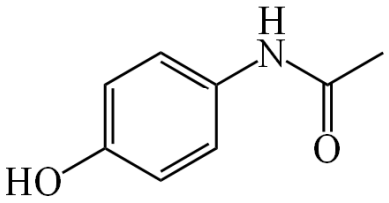
DILIsym™ Model v1A Overview

- Multiple species: human, rat, mouse, and dog
  - Population variability
- The three primary acinar zones of liver represented
- Essential cellular processes represented in interacting sub-models
  - Hepatocyte life-cycle
  - GSH depletion
  - Mitochondrial dysfunction
  - Cellular energy balance
  - Immune mediators
  - Dosing (IP, IV, Oral)
  - Drug metabolism
  - Pharmacokinetics
  - Transporter Inhibition
  - Biomarkers (ALT, AST, INR, bilirubin)





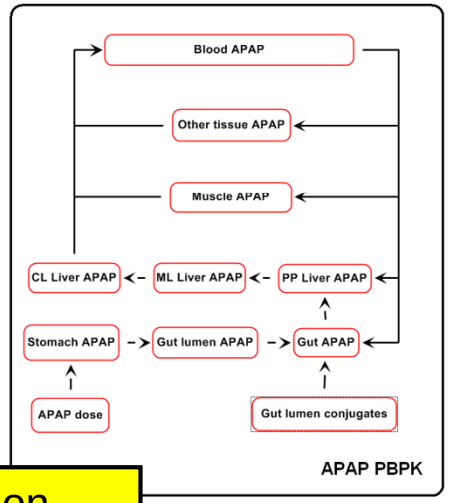
- Exemplar compounds
  - Acetaminophen



  - Methapyrilene
  - Furosemide
  - Other compounds to be added
- Compartment-based modeling
  - >280 state variables
  - 'Form to function' connection
  - Ordinary differential equations
  - May expand to include alternative mathematical approaches

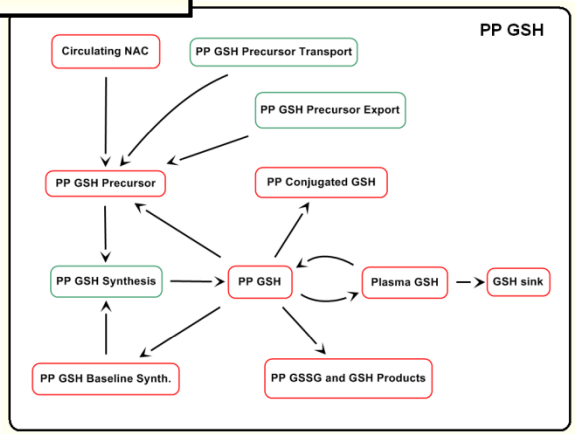
DILIsym™ Model v1A Sub-model Interactions: Drug Metabolism, GSH, and Mito. Dysfunction

Drug distribution & metabolism



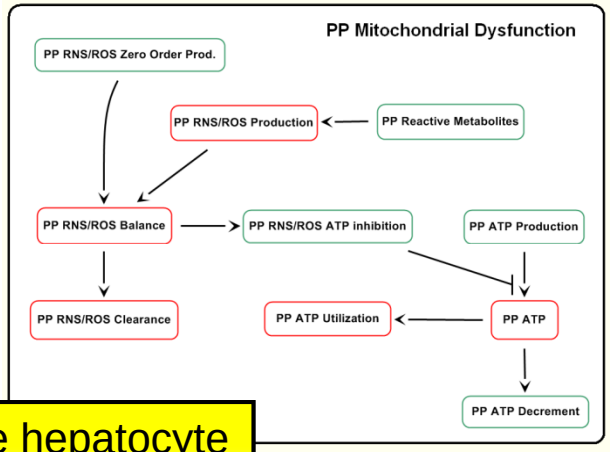
Conjugation occurs until the antioxidant is depleted

GSH depletion & recovery



Reactive metabolite

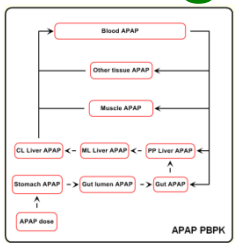
Mitochondrial dysfunction



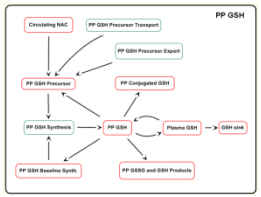
The hepatocyte energy balance is disrupted

Form to Function Approach Links Dynamic Changes in Hepatocytes to Liver Function

Drug distribution & metabolism

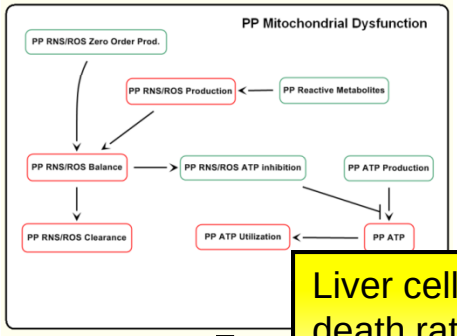


GSH depletion & recovery



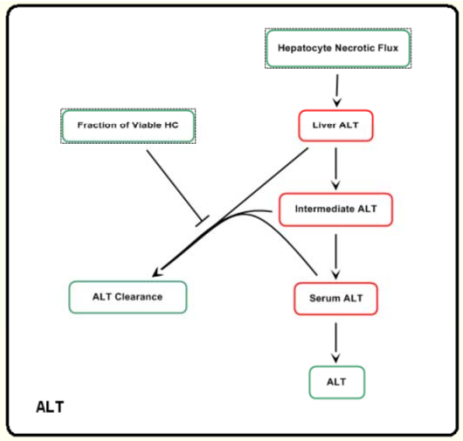
RM

Mitochondrial dysfunction

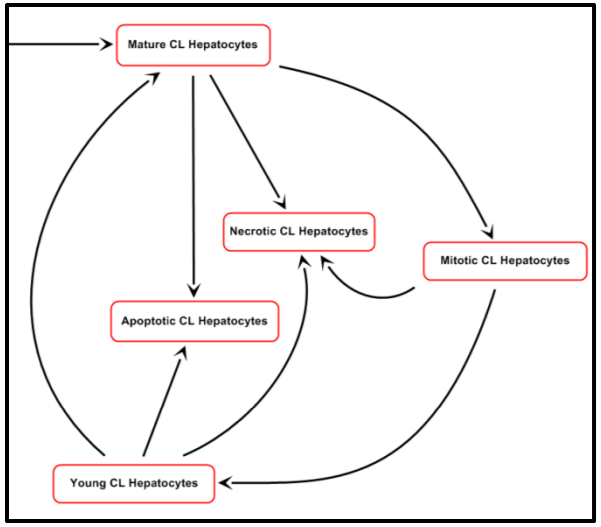


Liver cell death rates increase

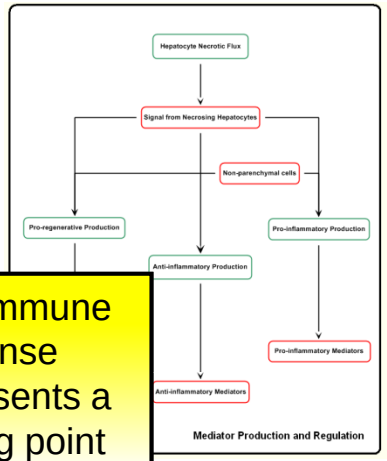
Biomarkers



Hepatocyte life-cycle



Immune mediators

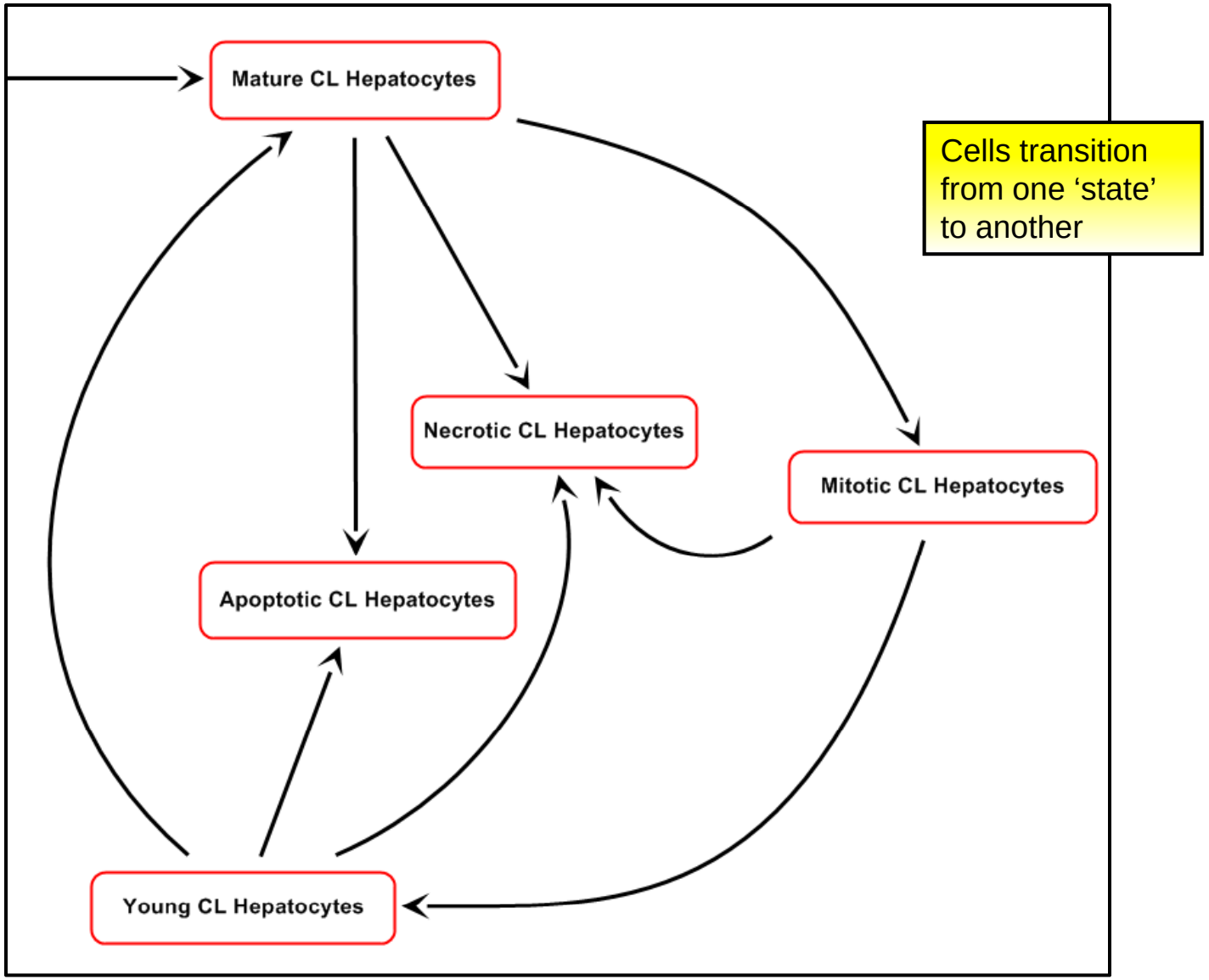


The immune response represents a tipping point

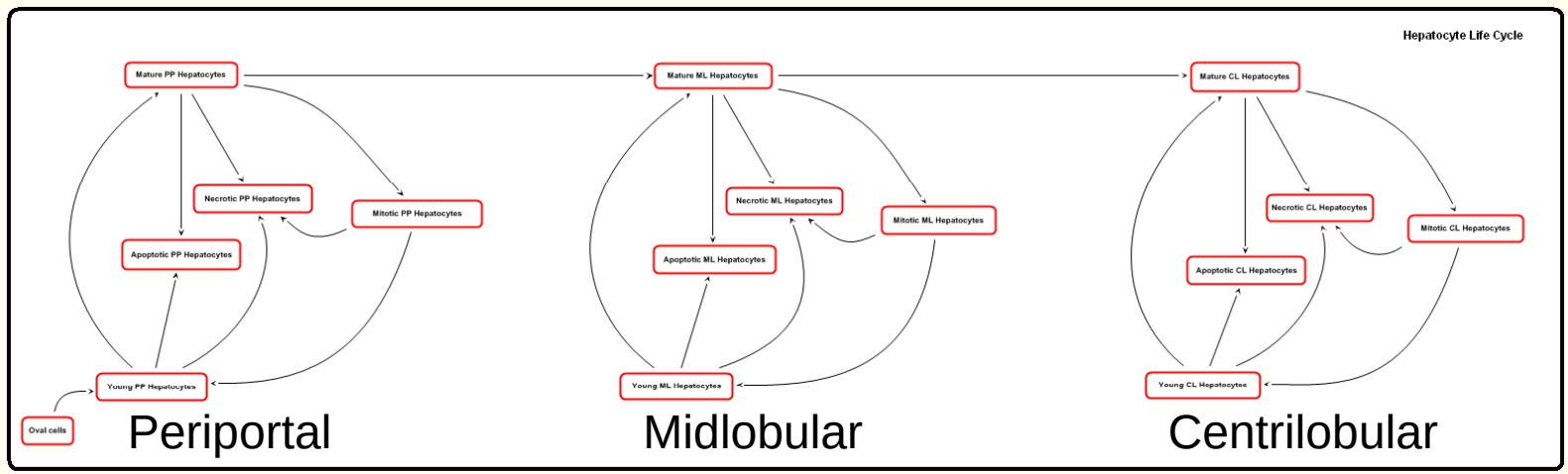
'Form to function'

Liver health affects liver function dynamically

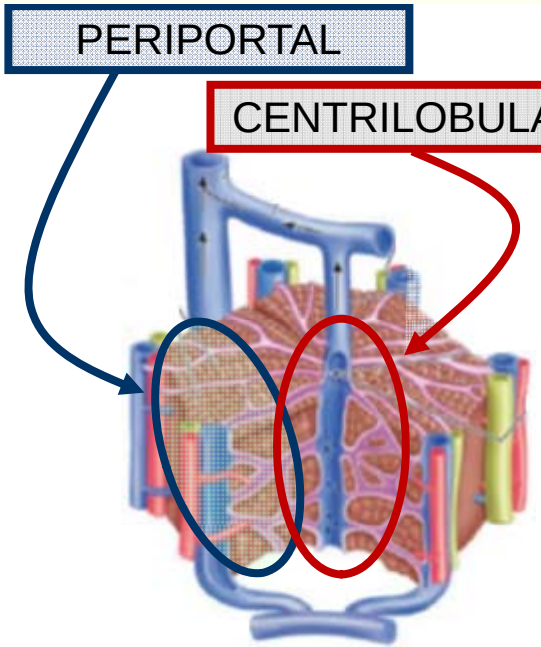
Representation of Hepatocyte Life Cycle

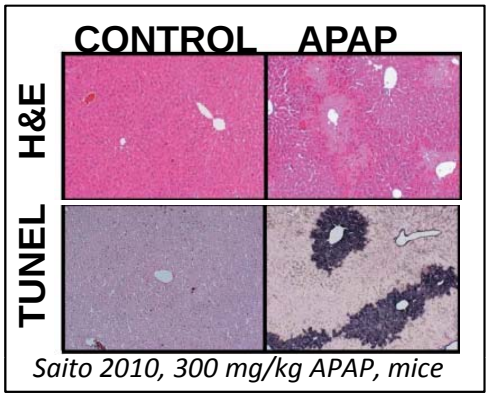


Design Considerations Include Acinar Zonal Liver Toxicity Observed in APAP-DILI



- DILIsym™ model has been designed to include centrilobular, mid-lobular, and periportal regions
- O_2 tension across hepatic acinus provides zonation of enzymes and cellular function
- Cytochrome P450 enzymes tend to be more heavily expressed in centrilobular region
- Many drugs cause zone-specific injury patterns (i.e., centrilobular APAP DILI)





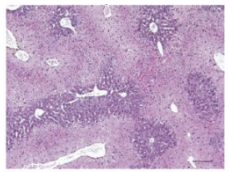
Saito 2010, 300 mg/kg APAP, mice

Preclinical data

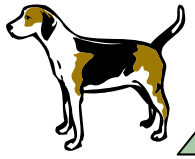
Complementary Partnership between Simulations and Laboratory Increases Efficiency

Use DILIsym™ model to make predictions from minimal initial data inputs

Laboratory Experiments and Data



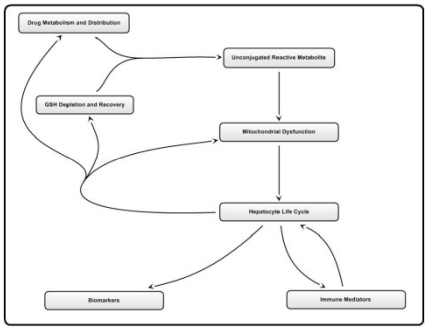
Vaquero 2007

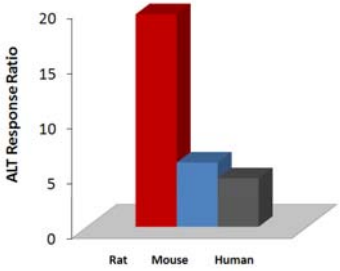


Multiple approaches to manage limitations in available data:

- *Infer from existing data*
- *Perform simulations with alternative hypotheses*
- *Collect additional data*

Modeling & Simulation





Use DILIsym™ model predictions to guide experimental design and data collection

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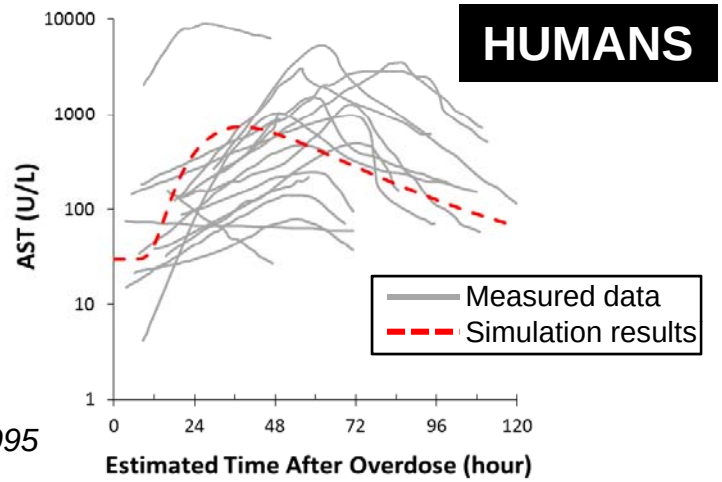
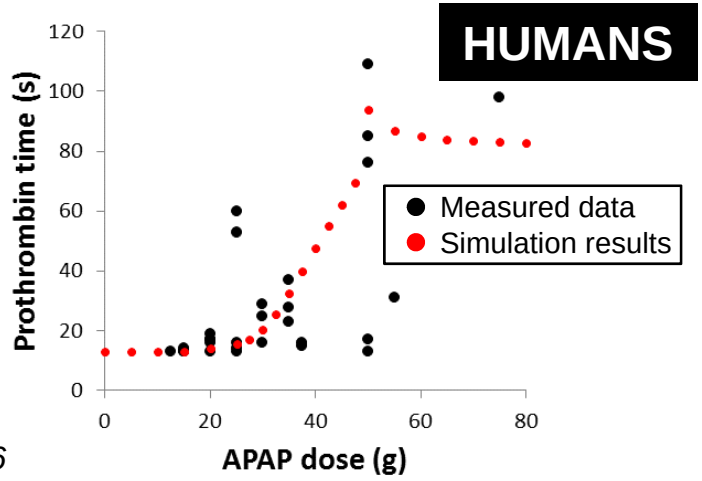
DILIsym™ Model Includes DILI Biomarkers AST and Prothrombin

- DILIsym™ model includes ALT, AST, bilirubin, and prothrombin time as biomarkers of liver injury
  - ALT and bilirubin included in previous DILIsym™ model versions
- AST release reflects rate of hepatocyte necrosis in DILIsym™ model
  - Simulated AST consistent with Singer 1995 individual patient data
- Prothrombin clotting time reflects amount of viable hepatocytes
  - Simulated prothrombin clotting time consistent with Davis 1976 individual patient data
- Additional emerging biomarkers being incorporated into model as well
  - HMGB1 (necrosis)
  - K18 fragments (apoptosis)



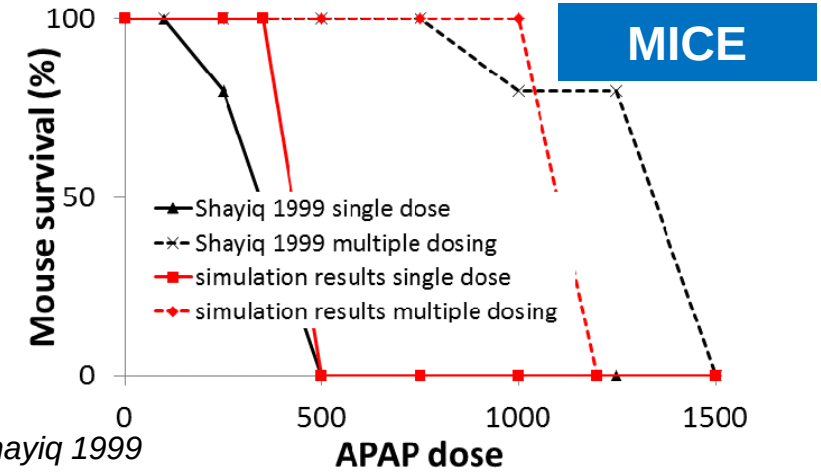
Singer 1995



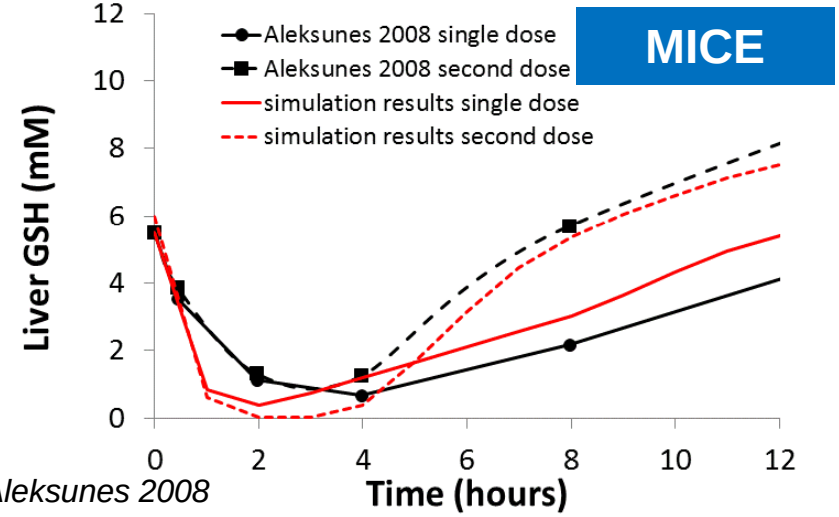
Davis 1976

Hepatic Autoprotection Components Enable Multiple Dosing Predictions

- Some of the key elements of hepatic autoprotection have been incorporated into the DILIsym™ model
  - Adaptations with multiple dosing decrease hepatotoxicity, lethality
- Simulated nrf-2 mediated increases in glutathione synthesis with repeat exposure are consistent with reported changes
  - Multiple studies in different species
- Post-injury hepatocyte proliferation offsets drug-induced hepatotoxicity with multiple dosing
  - Simulated proliferation indexes consistent with measured data in multiple species (*not shown*)
- Other mechanistic hypotheses being explored as well
  - Resistant young hepatocytes, mitochondrial proliferation, increased phase II enzyme synthesis



Shayiq 1999

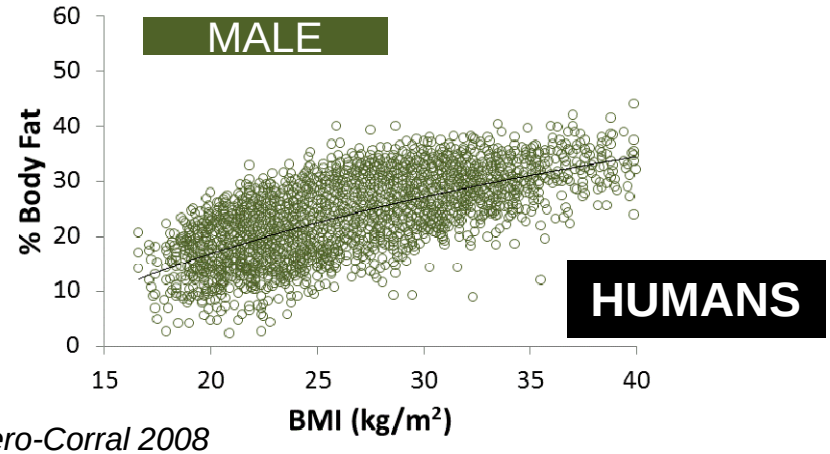


Aleksunes 2008

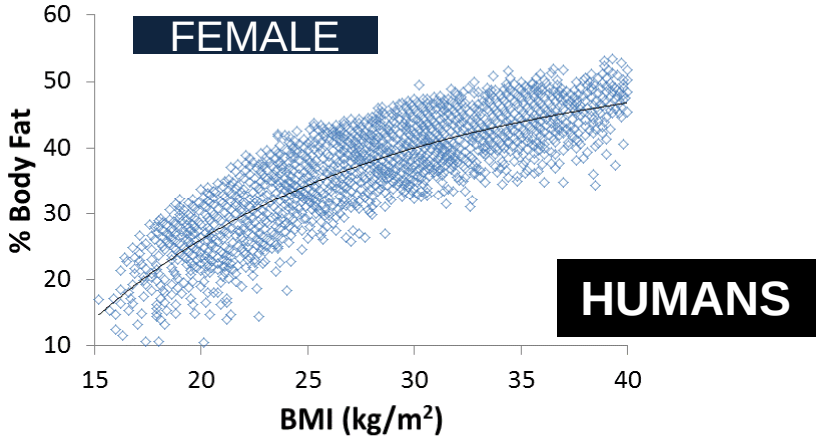
Preclinical Data and Simulation Results

Inclusion of Patient Body Composition Facilitates Predictions of Variability in Compound Exposure

- DILIsym™ model includes body composition impact on drug distribution via user inputs
  - Body weight
  - Gender
- Enables simulated patients and SimPops™ to reflect observed patient characteristics
  - Increases in patient body fat levels over the last several decades
  - Drug tissue distribution
- Inputs are based on publicly-available population data relationships
  - Body weight-BMI
  - BMI-lean body mass



Romero-Corral 2008



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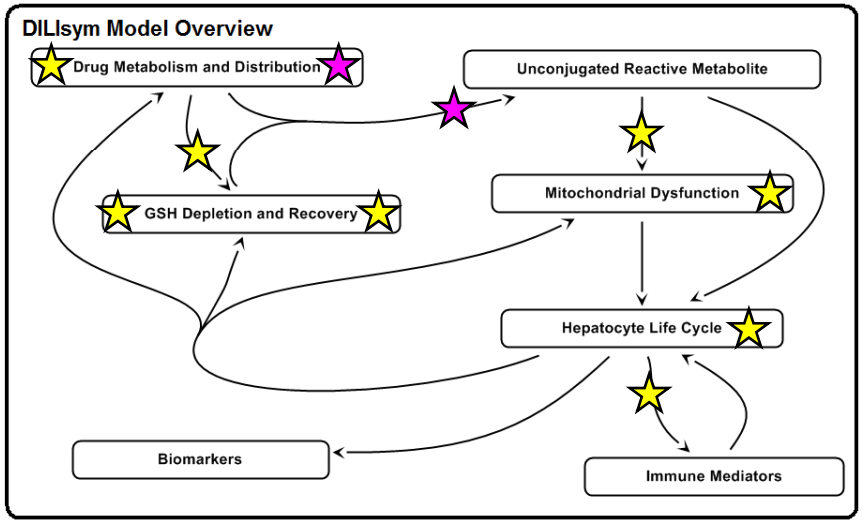
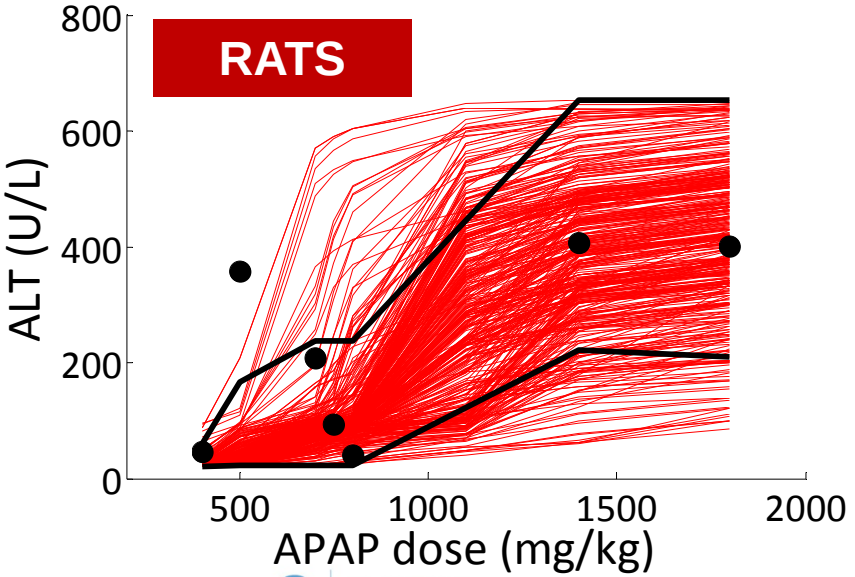
Range of Hepatotoxic Responses in SimPops™ Due to Variability in Underlying Biochemistry

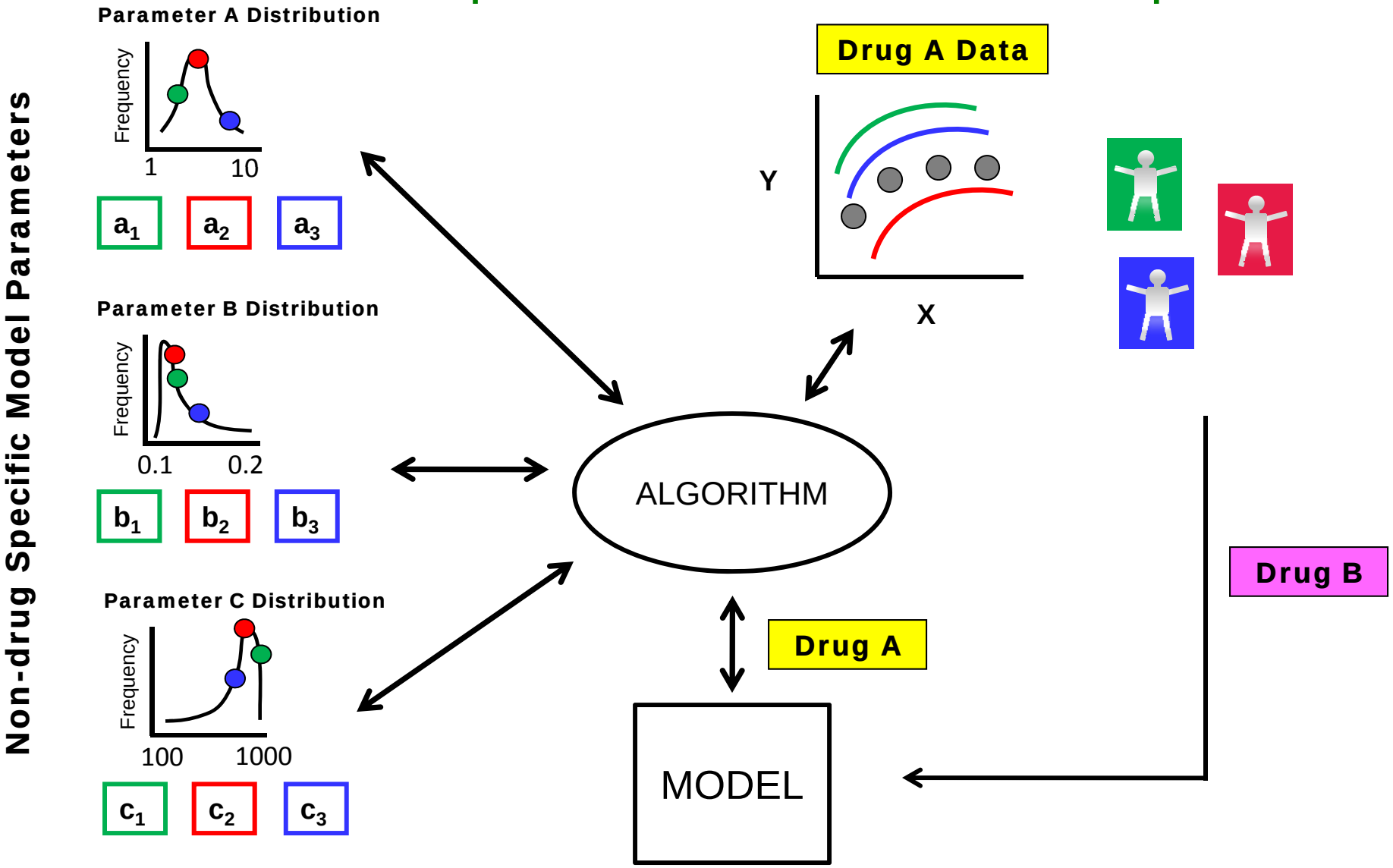
- SimPops™ are population samples with variability in hepatotoxic drug responses
- Eleven general and APAP-specific SimPops™ are included in DILIsym™ model v1A
  - Humans, rats, mice
- All SimPops™ include variability in key cellular biochemical pathways (yellow stars ★)
  - Quantitative pathway ranges determined from literature, documentation included
  - Can be applied to other drugs as well
  - Drug (APAP) specific SimPops also include variability in conjugation and Cyp pathways (pink stars ★)
- Simulated individual animals capture dose-response range (and other responses) indicated by measured data
  - Similar for patients
  - Generate simulated animals (and patients) with responses on outer ranges to provide information about possible extremes within experimental cohorts



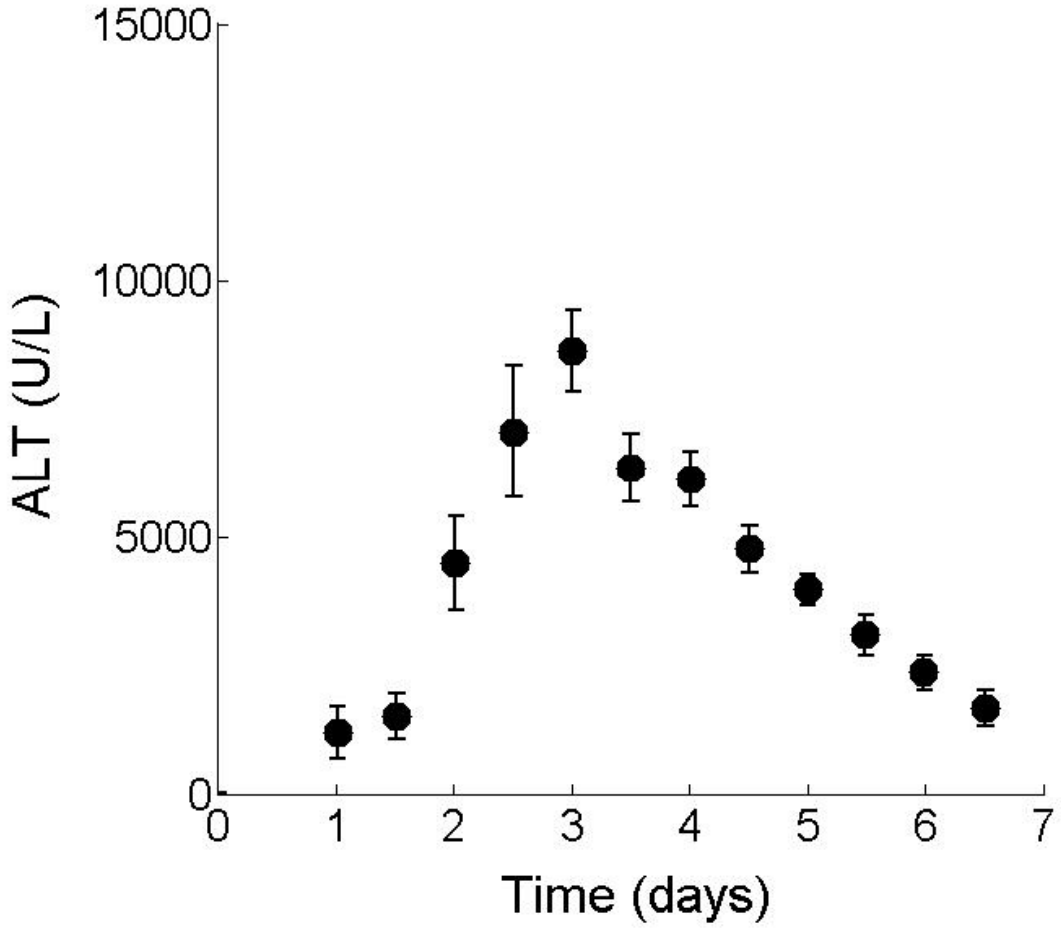


Simulation Results and Preclinical Data

Genetic Algorithm Includes Parameter Ranges and Measured Responses to Generate SimPops™



SimPops™ Variability in Data Considered in Population Sample Generation - Humans



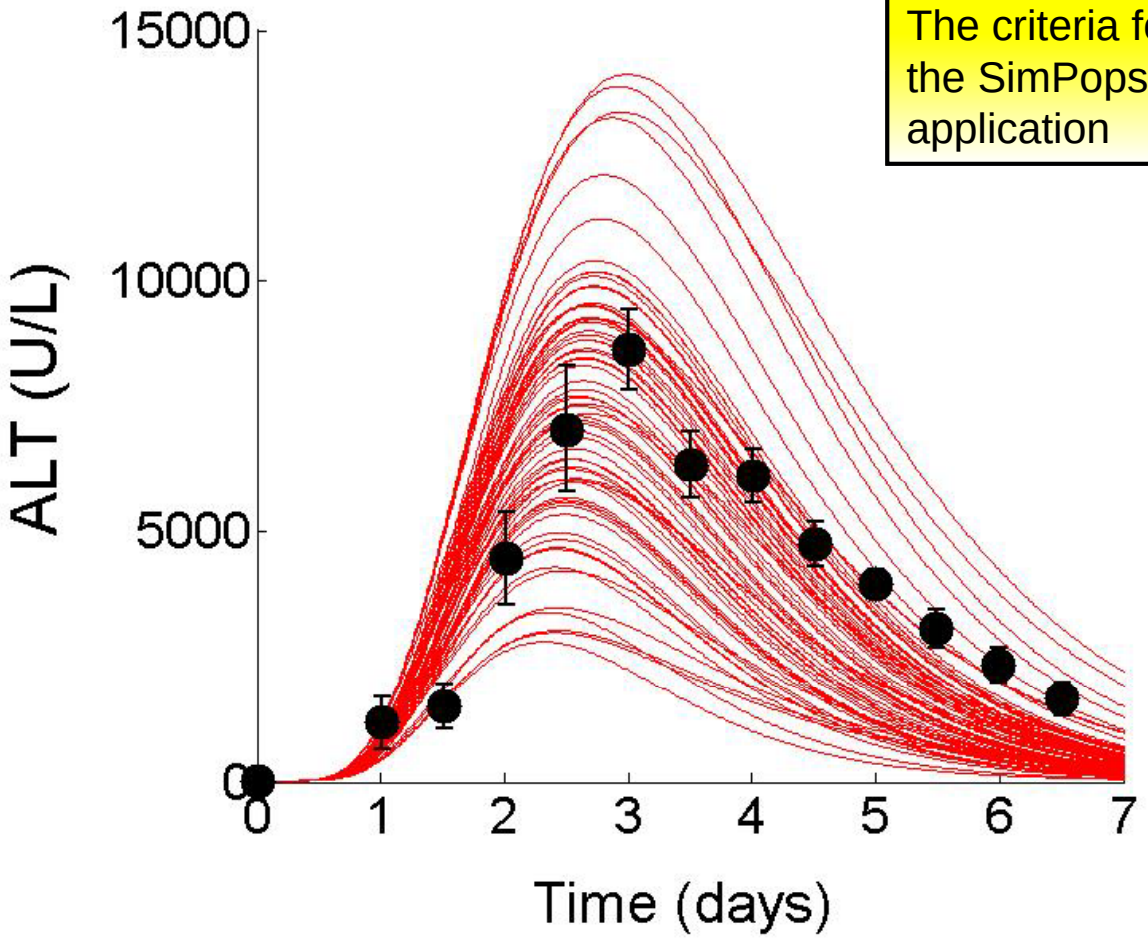
HUMANS

Schiodt 2001

39g mean APAP dose
34 hr mean NAC delay

n = 37

Range of Variability in Measured Data Captured by SimPops™



The criteria for acceptance into the SimPops™ depends on the application

HUMANS

Schiodt 2001

39g mean APAP dose
34 hr mean NAC delay

n = 37

** Red lines indicate simulated individual patients*

DILIsym™ Model User Training Agenda

- DILI-sim Initiative goals overview
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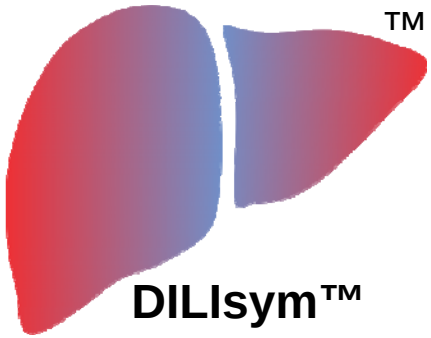
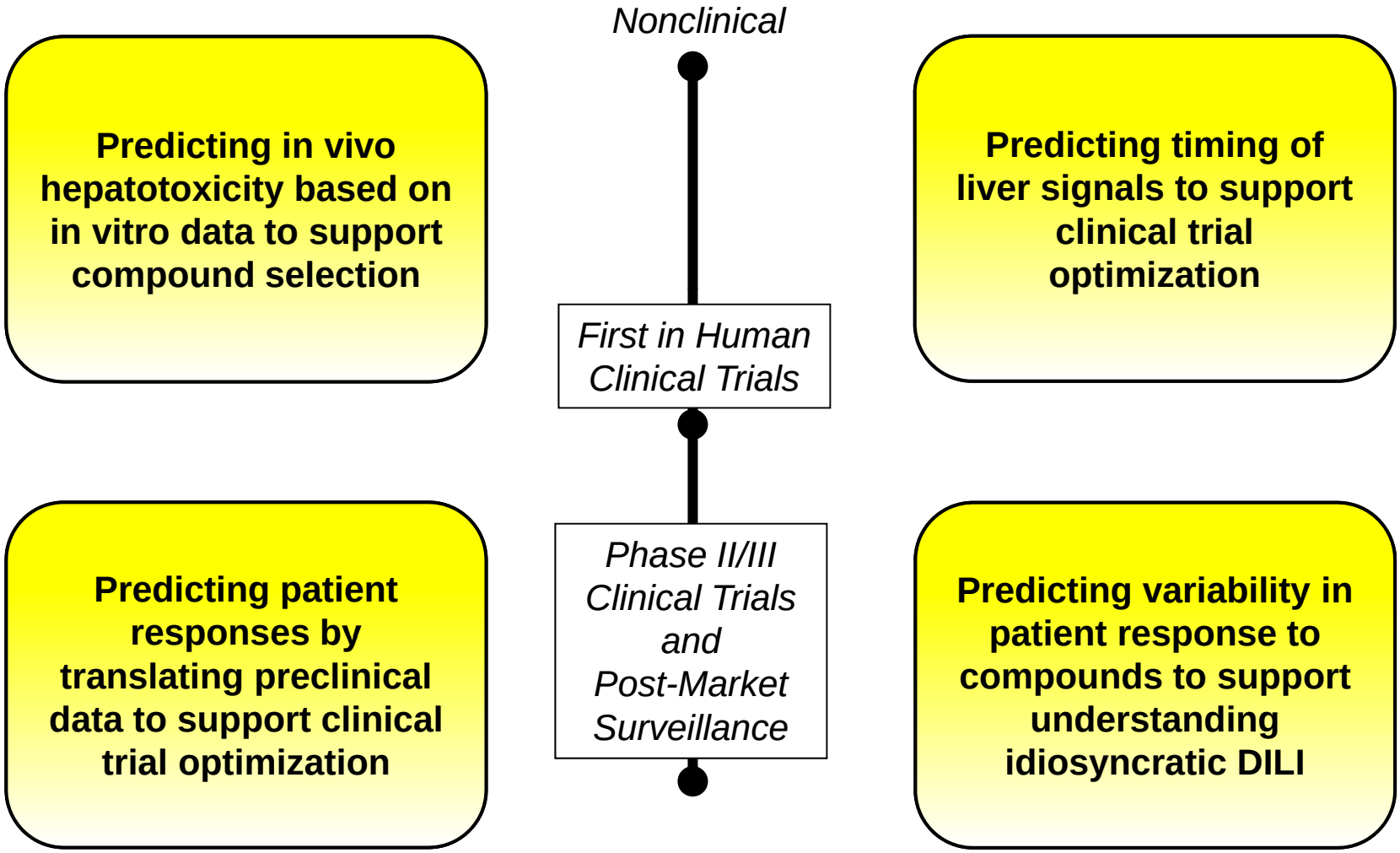


Examples of How DILIsym™ Model Can Support Decision Making



Highlights of 2012 DILIsym™ Modeling Plans

- DILIsym™ modeling team has developed plans for Q1/Q2
  - Emphasis on mechanisms suggested in Sept 2011 kickoff meeting
  - Exemplar compounds from literature
- Q3/Q4 plans in progress
  - Based on direct input from DILI-sim Initiative members
- Mitochondrial toxicity
  - Disturbances in mitochondrial ATP production
  - Buprenorphine and panadiplon are exemplar compounds
  - Additional proprietary compounds may also be added
- BSEP inhibition
  - Interaction with bile acid dynamics and effects on hepatocellular life cycle
  - Bosentan is exemplar compound
  - Additional proprietary compounds may also be added
- Innate immunity
  - KC, LSEC life cycle, mediator production and effects
  - Build upon 2011 scoping efforts
- Model release tasks
  - SimPops™ development
  - v2A release
- Manuscripts and presentations
  - Target of 2-4 manuscripts, presentations in 2012

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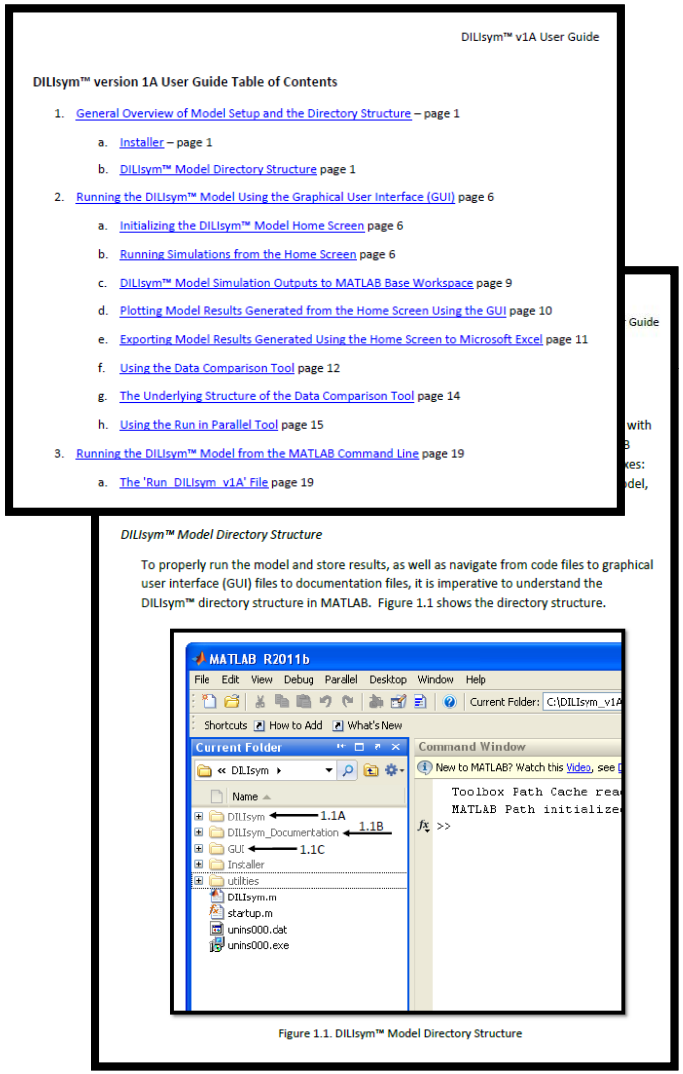
DILIsym™ version 1A User Training

- DILIsym™ version 1A WebEx Training Session
  - January 31st, 2012, 9AM EST – 12 PM EST
  - Training session is being recorded
  - Member companies may attend
  - Future members may review the WebEx
  - Contact DILIsym™ Modeling team for WebEx information
- DILIsym™ version 1A documentation
  - User manual
  - Complete, importable reference library
  - Qualitative diagram and corresponding notes
  - SimPops™ information
    - General overview of data used
    - Parameter distributions



DILIsym™ v1A User Guide

DILIsym™ version 1A User Guide Table of Contents

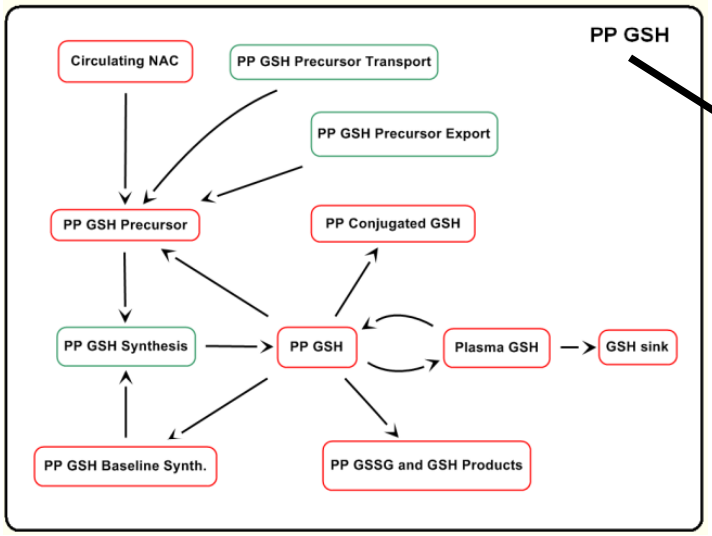
DILIsym™ Model Directory Structure

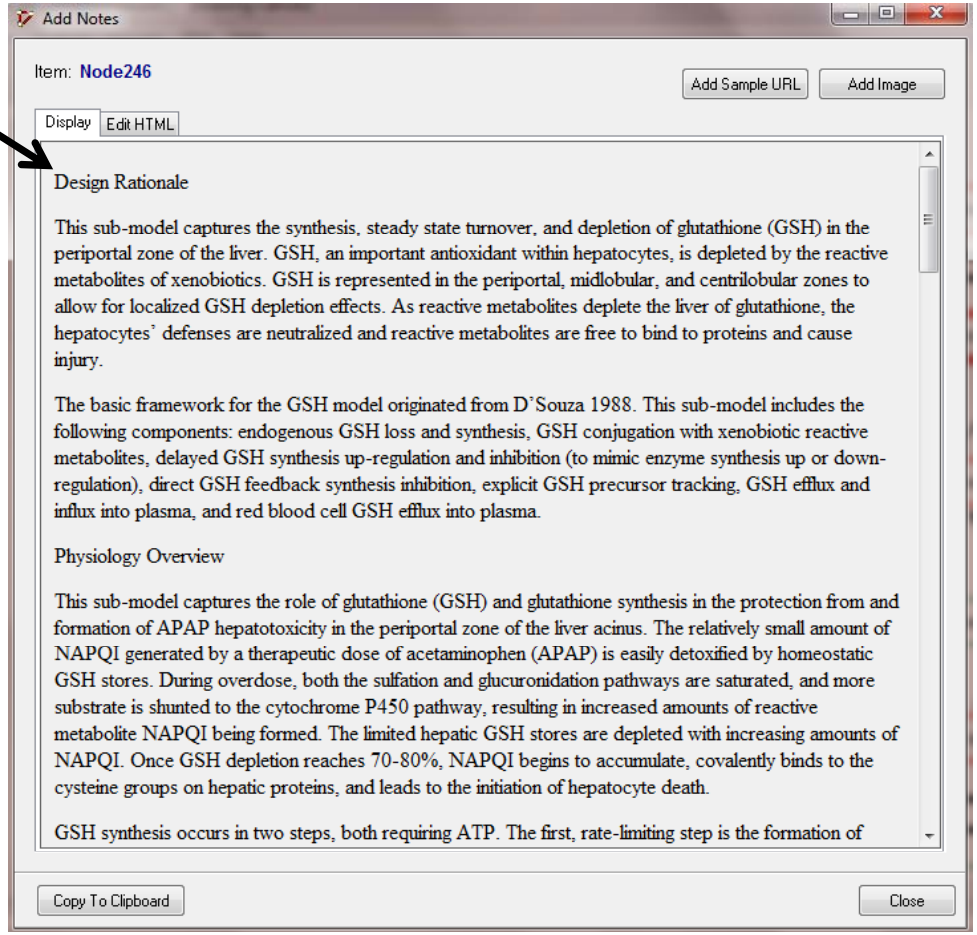
To properly run the model and store results, as well as navigate from code files to graphical user interface (GUI) files to documentation files, it is imperative to understand the DILIsym™ directory structure in MATLAB. Figure 1.1 shows the directory structure.

Figure 1.1. DILIsym™ Model Directory Structure

DILIsym™ v1A Qualitative Diagram

- J-Designer license not included, currently available at no cost
- Illustrates mechanisms with corresponding documentation





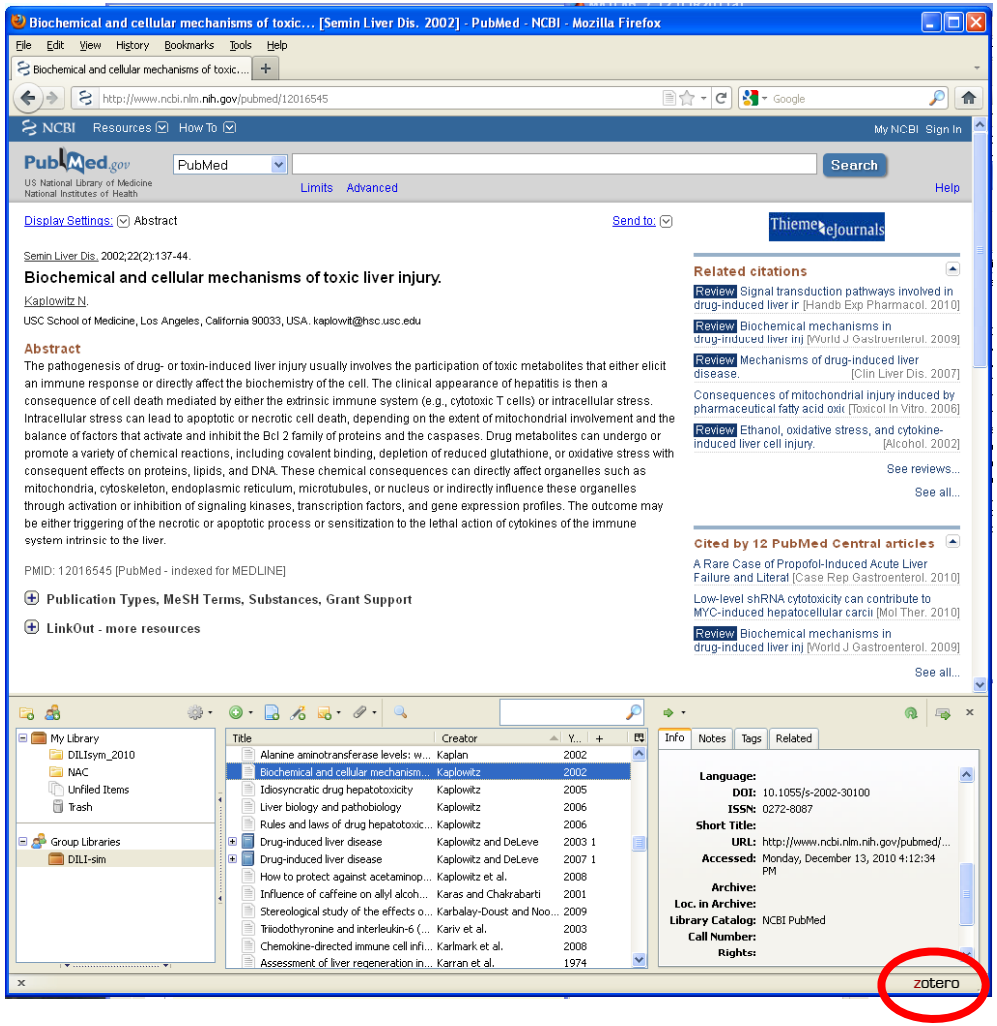
J-Designer
Systems Biology Workbench

http://sys-bio.org/

Sauro Lab, University of Washington

DILIsym™ v1A Model Reference Database

- Zotero license not included, currently available at no cost
- Zotero runs inside of Mozilla Firefox
- Over 2500 references currently in DILIsym™ model database
- Complete reference library is also available in other reference programs via export from Zotero



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