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In Silico and *in Vitro* Simulations to Predict Idiosyncratic DILI: What is on the Horizon?

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2019 AASLD/FDA DILI Conference

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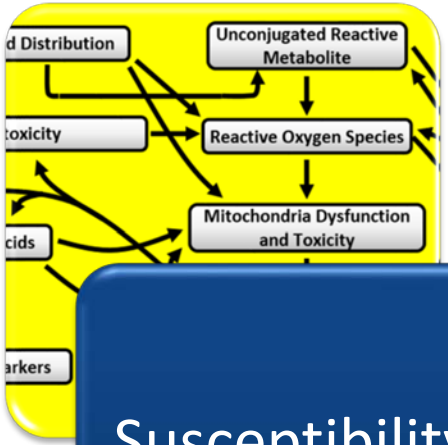
Conflict of Interest Statement

- *Brett A. Howell is an employee of DILIsym Services Inc., the producer of DILIsym software*
- *Brett A. Howell receives financial benefit from DILIsym software sales and consulting use*
- *Brett A. Howell holds Simulations Plus Inc. stock options*

Variability in Liver Response to Drugs Includes At Least Three Key Areas

Drug Exposure



Susceptibility to DILI Onset

Ability to Adapt (or Tolerate)

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

Scientific Advisory Board

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janssen
gsk GlaxoSmithKline
GILEAD
DILIsym
Mitsubishi Tanabe Pharma
Bristol-Myers Squibb
Sumitomo Dainippon Pharma
abbvie

Select Sample of Current Companies Licensing DILIsym

For a comprehensive review of progress, see *Watkins 2019: Clin Transl Sci*

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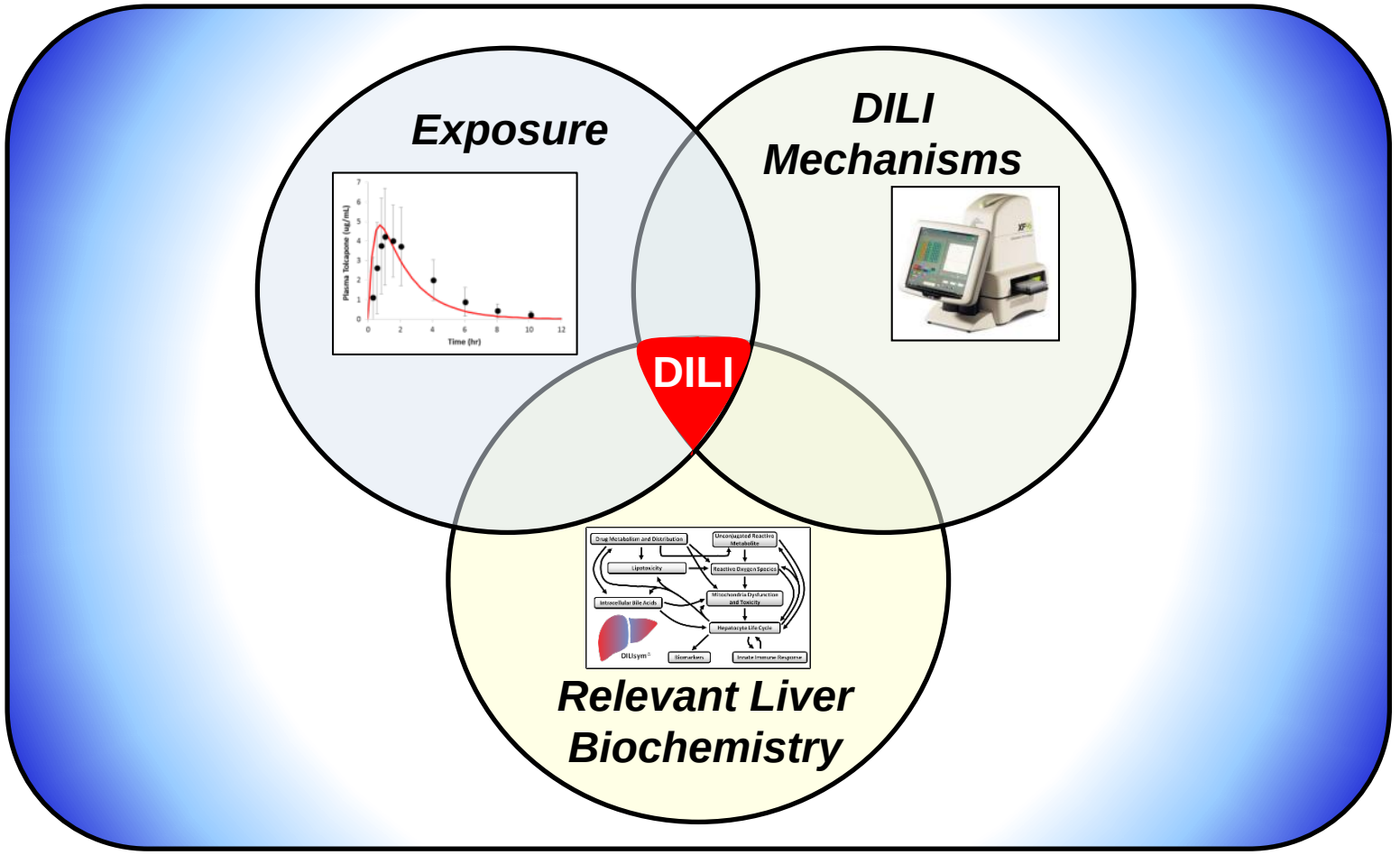
- *Overall Goals*
  - *Improve patient safety*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *19 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $9 million total invested in project*

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



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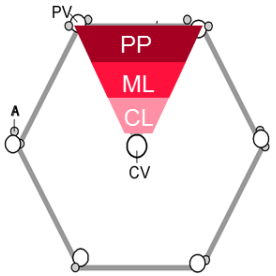
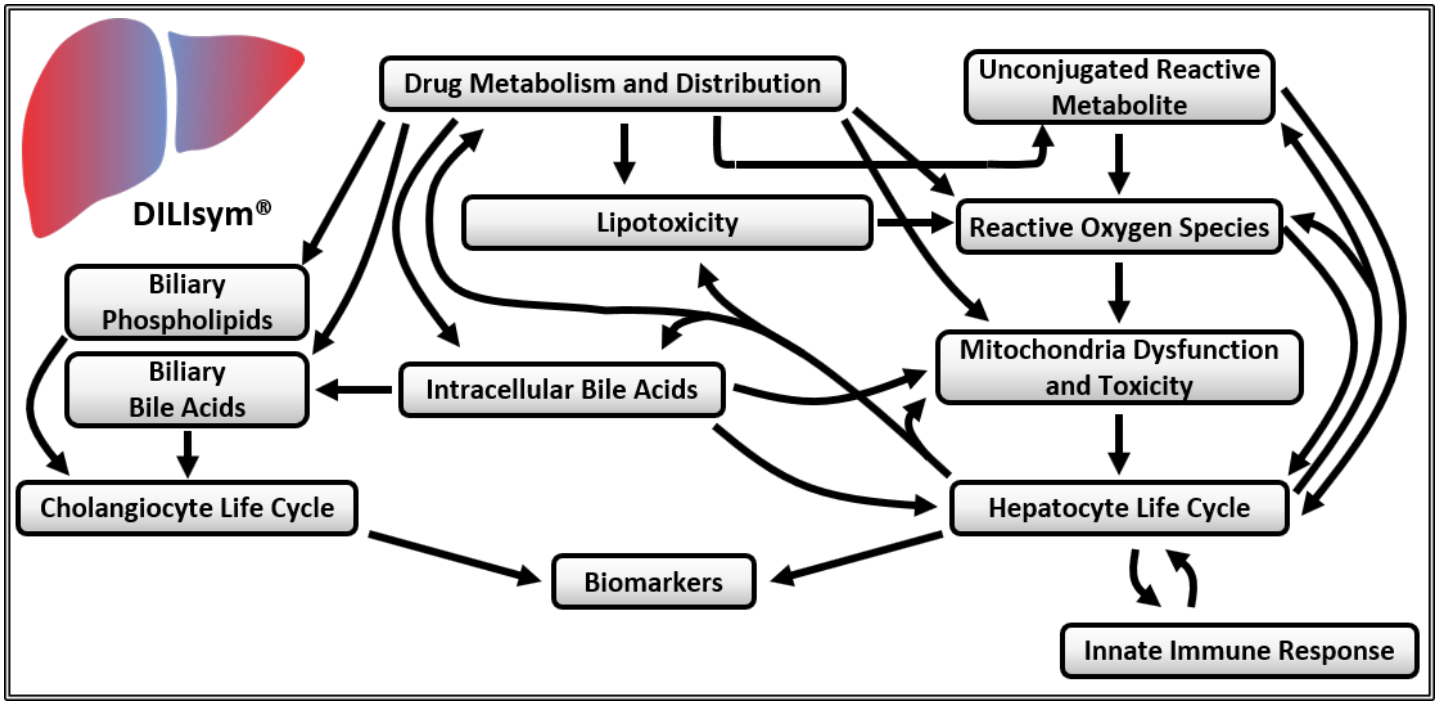
DILIsym Tool Overview

- **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**
- **Over 60 detailed representations of optimization or validation compounds with 80% success**
- **Single and combination drug therapies**





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DILIsym Utilizes Various Data Types to Inform Decisions

Exposure Data

PBPK Modeling
- Compound Properties
- Tissue penetration studies
- Pharmacokinetic data
- *in vitro* data

Modeling & Simulation

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
- Mitochondrial toxicity
- Bile acid / phospholipid transporter inhibition
- Bilirubin transport/metabolism

Clinical Data

- Dosing Protocols, fasting/fed state, meal times
- Anthropometric data
- Pharmacokinetic data

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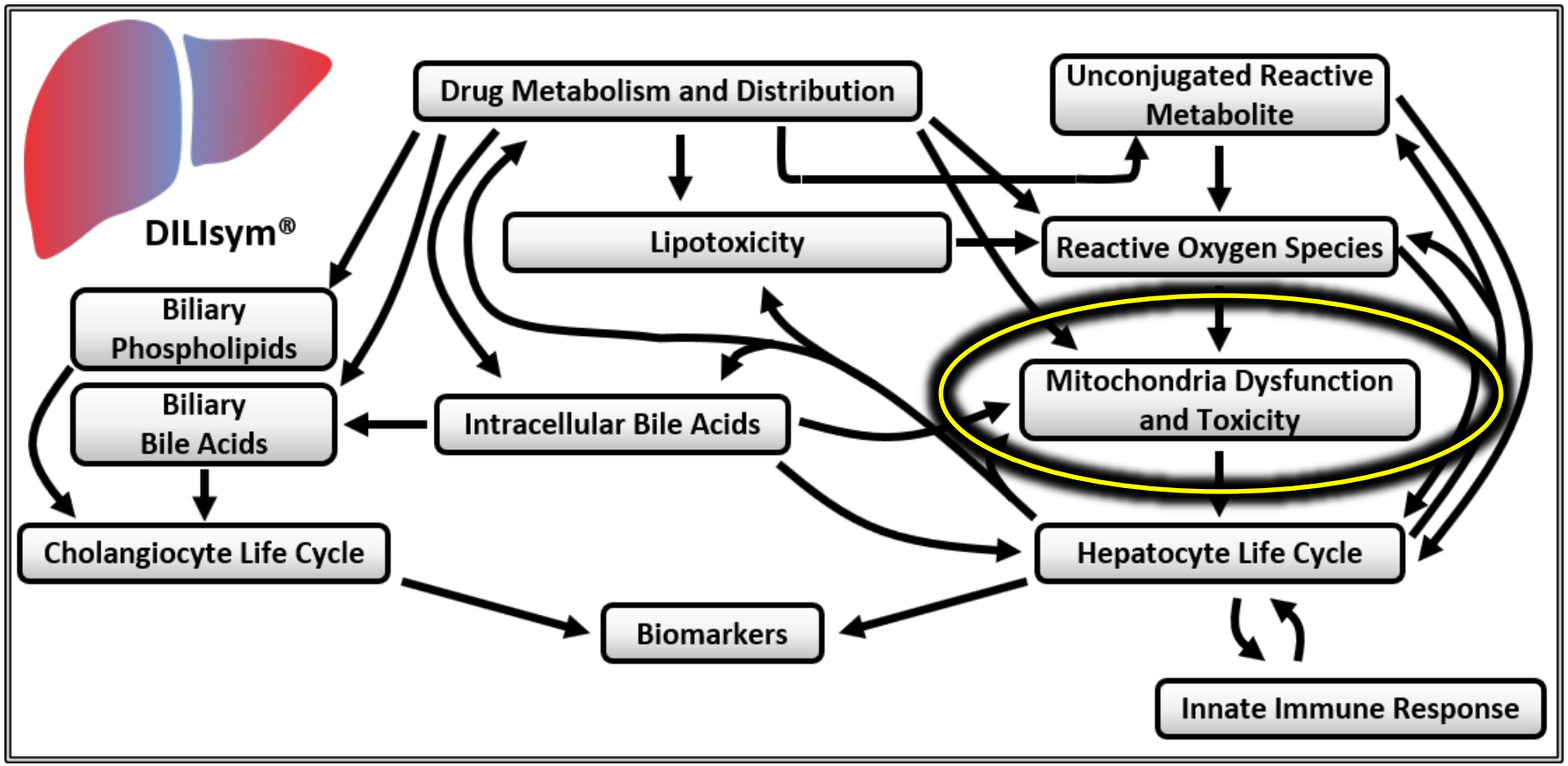
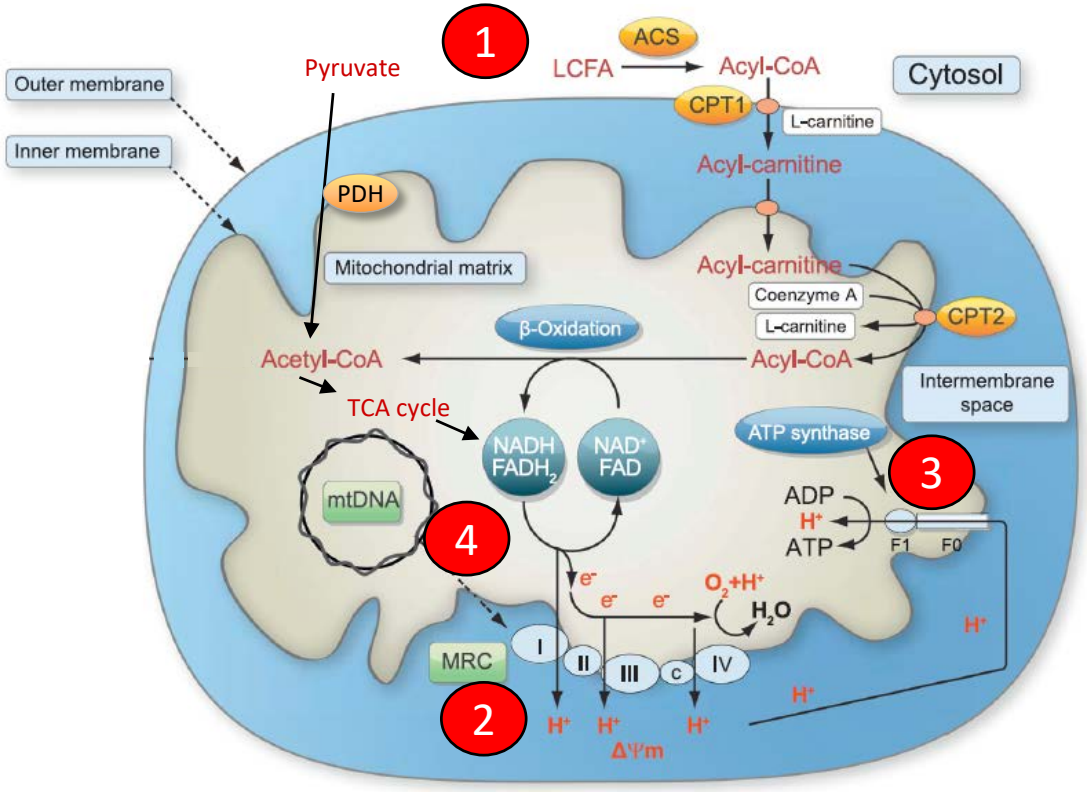
Drug Effects on Hepatocyte Mitochondria is An Area of DILI Adaptation Investigation



Overview of Mitochondria Bioenergetics Biochemistry

1. Metabolic substrate
2. Electron Transport Chain (ETC)
3. ATP synthesis
4. Mitochondrial DNA encodes multiple mitochondria proteins



Adapted from Begriche 2011

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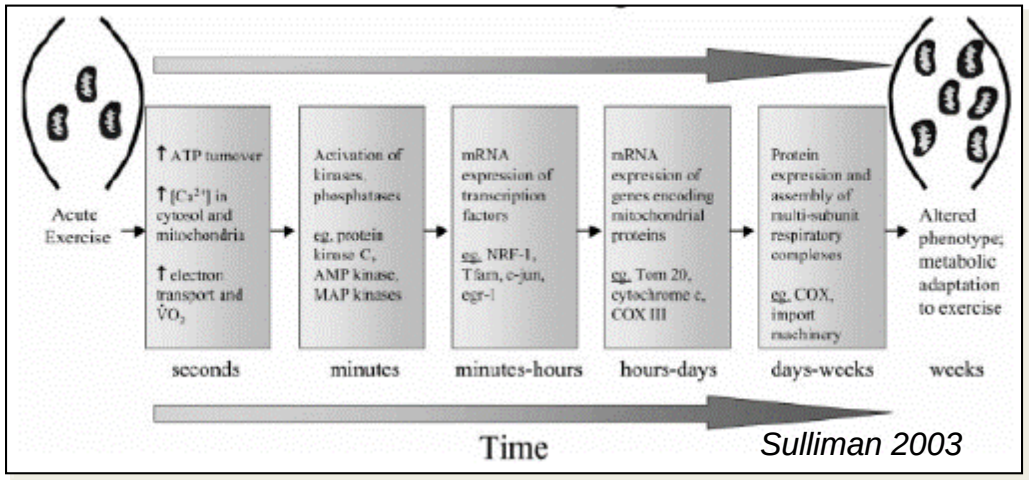
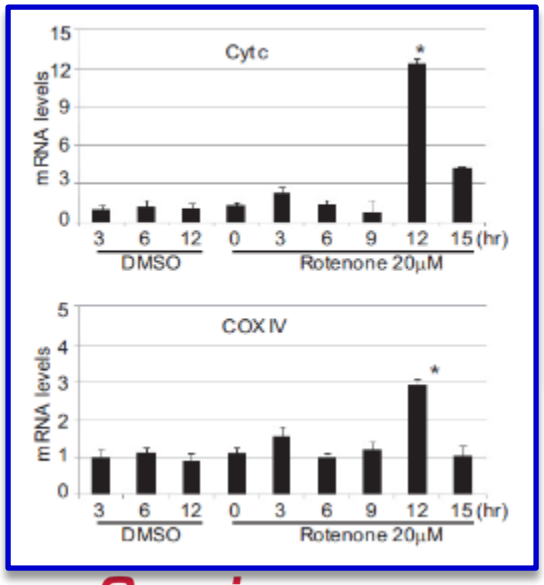
Mitochondrial Biogenesis Can Help Overcome Bioenergetic Duress

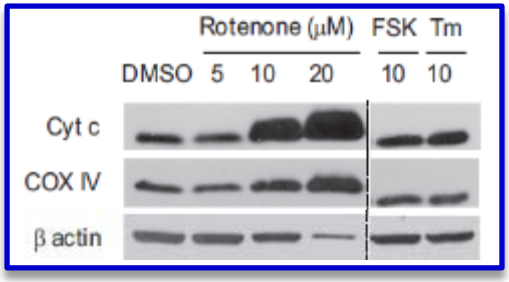
- Well documented that adaptive mitochondrial biogenesis helps compensate for bioenergetic stress in muscle
  - ***A primary initiating signal is ATP loss***
- Some evidence that similar adaptations occur in liver



Sulliman 2003



MOUSE HCs



Than 2011

Preclinical Data

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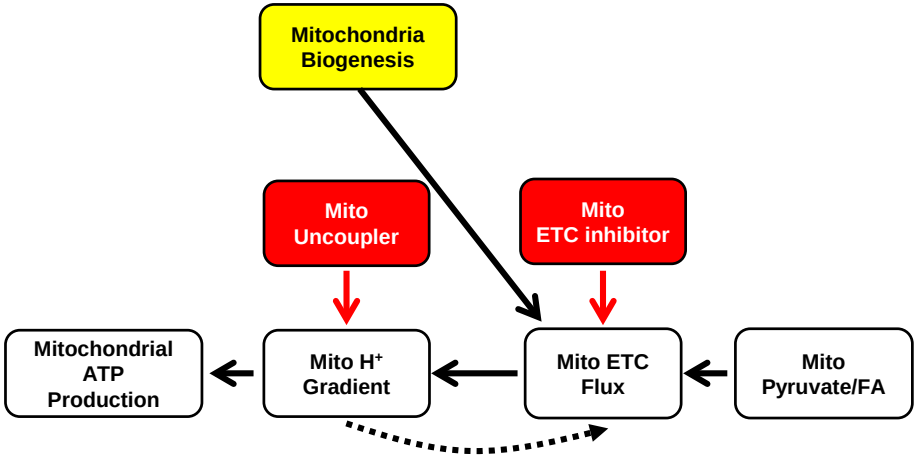
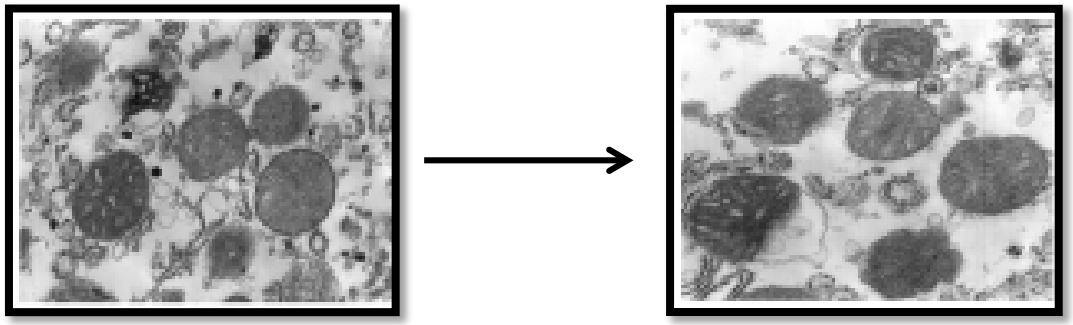
Mitochondrial Biogenesis Can Reduce Sensitivity to DILI

- Increased number and size of mitochondria can partially offset mitochondrial dysfunction

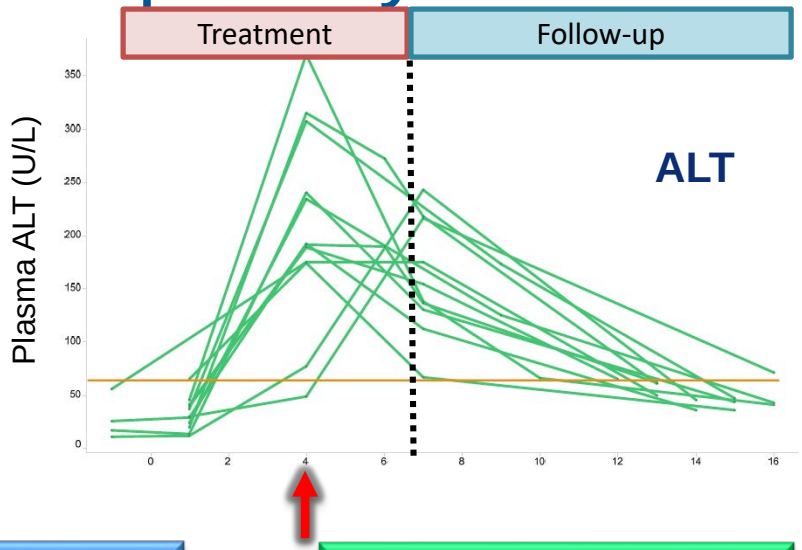


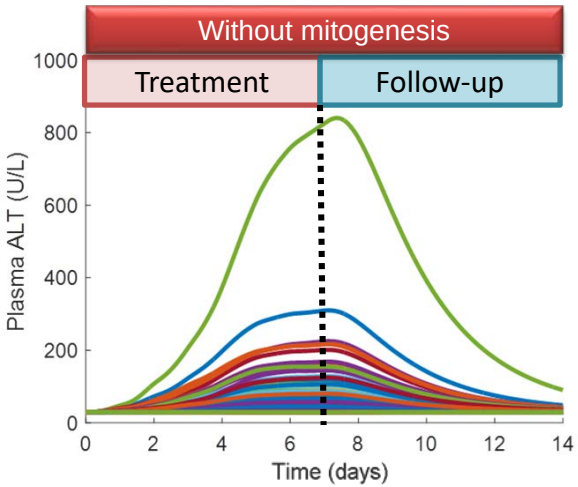


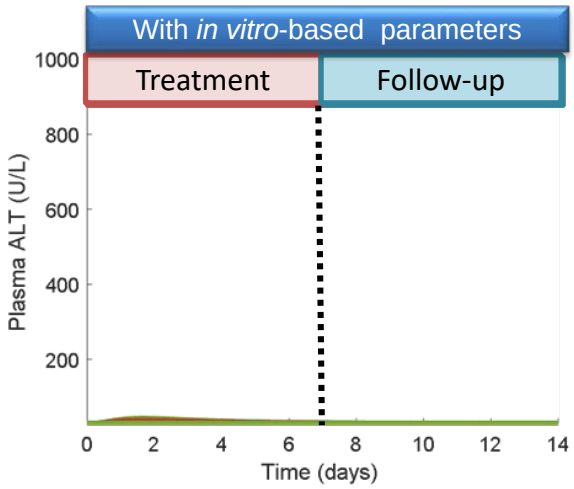
Justo 2005

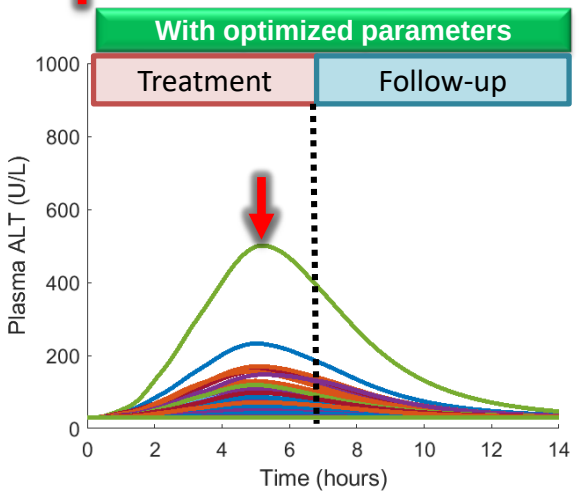
DILIsym Mitogenesis Parameters Optimized to Recapitulate Solithromycin Data With Biogenesis Effects on Respiratory Reserve

- Patients treated with ***solithromycin*** for 7 days
- ALT and AST elevations were asymptomatic
- Solithromycin initially simulated in SimPops with parameters based on in vitro data
- Optimization of parameters to clinical data yields similar behavior to clinic
  - ***Can we validate the parameter values independently?***









Simulation Results

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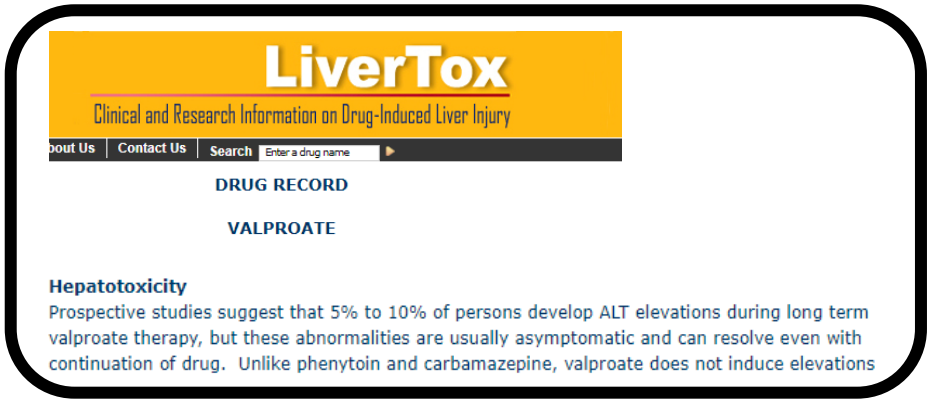
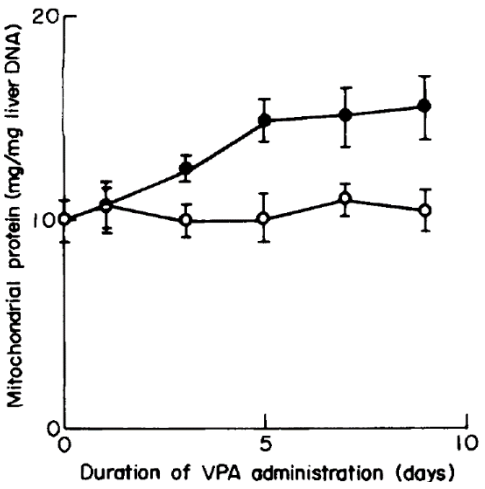
HUMAN

Valproate Data Can be Used to Validate Mitochondrial Biogenesis Parameters



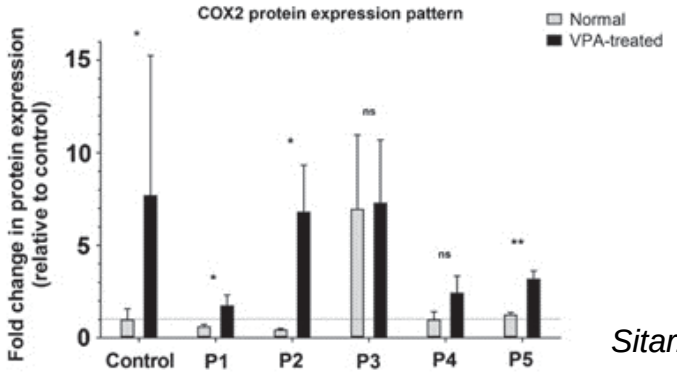
- Valproate causes ALT elevations in 5-10% of patients during long-term therapy
  - *In vitro* data indicates that valproate elicits mild mitochondrial ETC inhibition (Komulainen 2015 and internal data)
- *In vivo* and *in vitro* data indicate valproate causes mitochondrial biogenesis

RAT



Hayasaka 1986

Human Fibroblasts



Sitarz 2013

Preclinical Data

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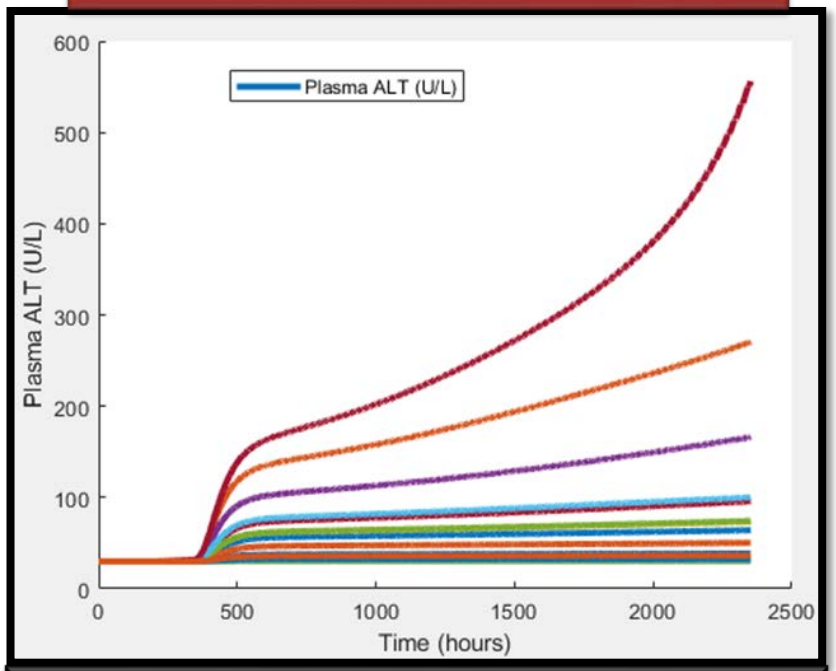
DILIsym Valproate Simulations With Mitogenesis Shows Adaptation Similar to Clinic

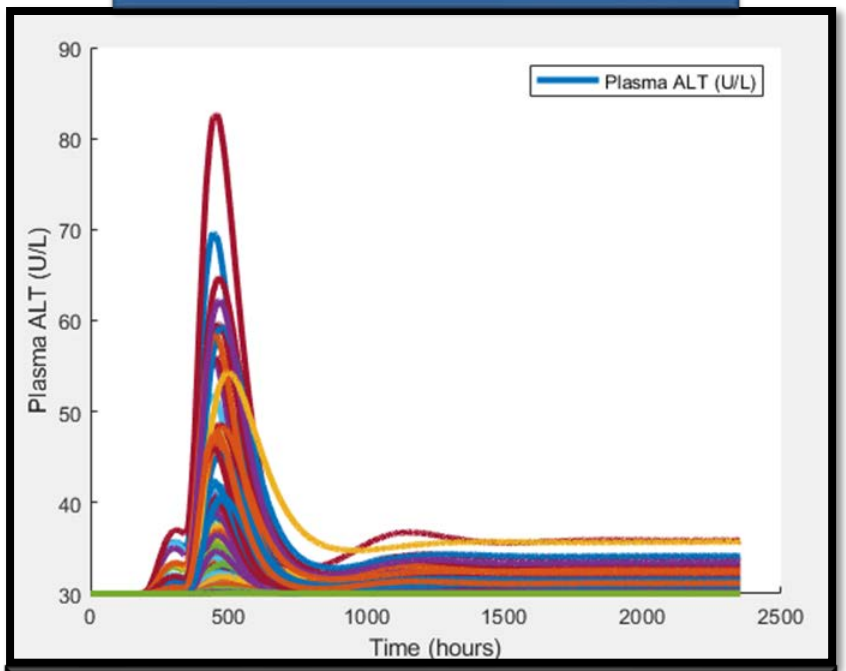
- Valproate titrated from 5 mg/kg BID to 15 mg/kg BID over 3 weeks
- Without biogenesis, ALT does not look like clinical presentation
- With biogenesis, ALT resolves, looks similar to clinical presentation

WITHOUT MITOGENESIS



VPA Titrated from 5 mg/kg to 15 mg/kg BID

WITH MITOGENESIS



VPA Titrated from 5 mg/kg to 15 mg/kg BID

Simulation Results

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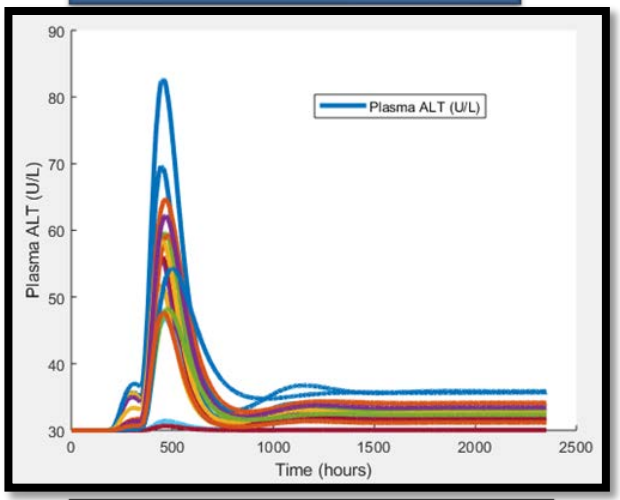
Plasma ALT from Compound X + Valproate Simulations Show Synergy and Adaptation

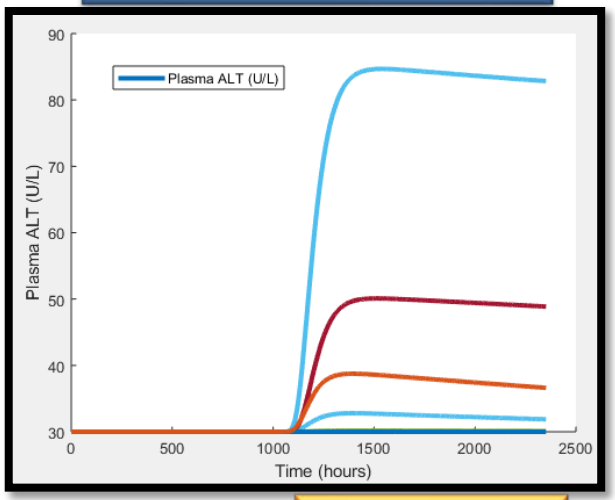
- Compound X in isolation causes minimal, mild ALT elevations
- Compound X + valproate leads to DILI DDI, with more bumps in ALT
- Resolution occurs
- One confidential case of two compounds leading to this response has already been noted – both compounds cause mild ETC inhibition

WITH MITOGENESIS



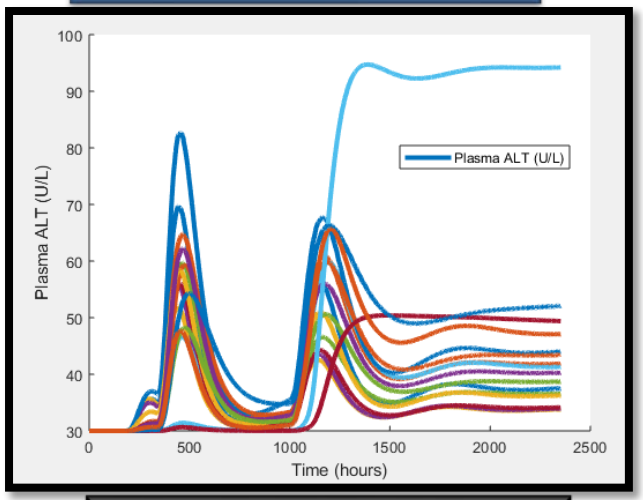
VPA Titrated to 15 mg/kg BID

WITH MITOGENESIS



Compound X

WITH MITOGENESIS



VPA Titrated to 15 mg/kg BID

Compound X

Simulation Results

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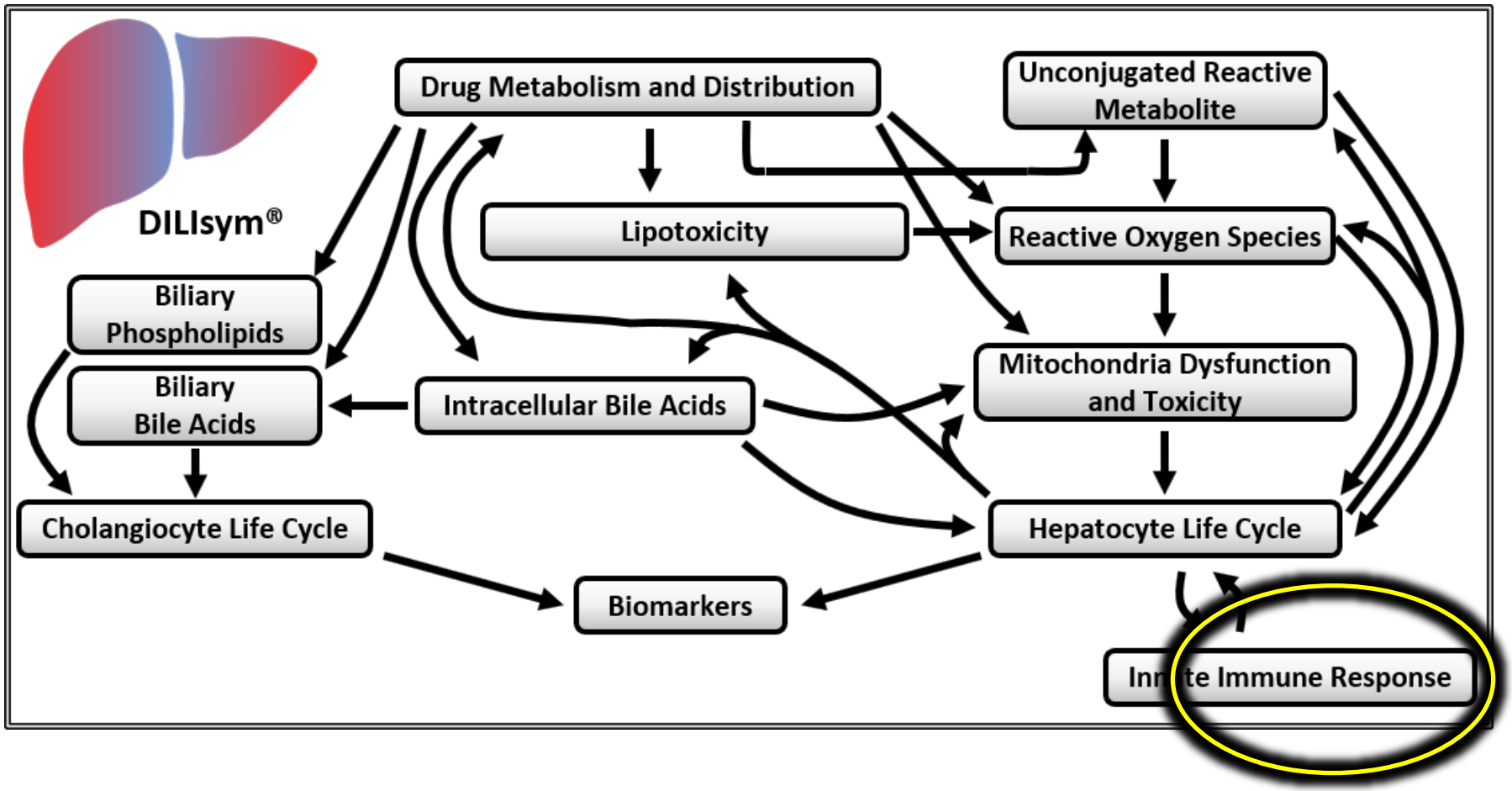
Drug Effects on Hepatocyte Mitochondria is An Area of DILI Adaptation Investigation



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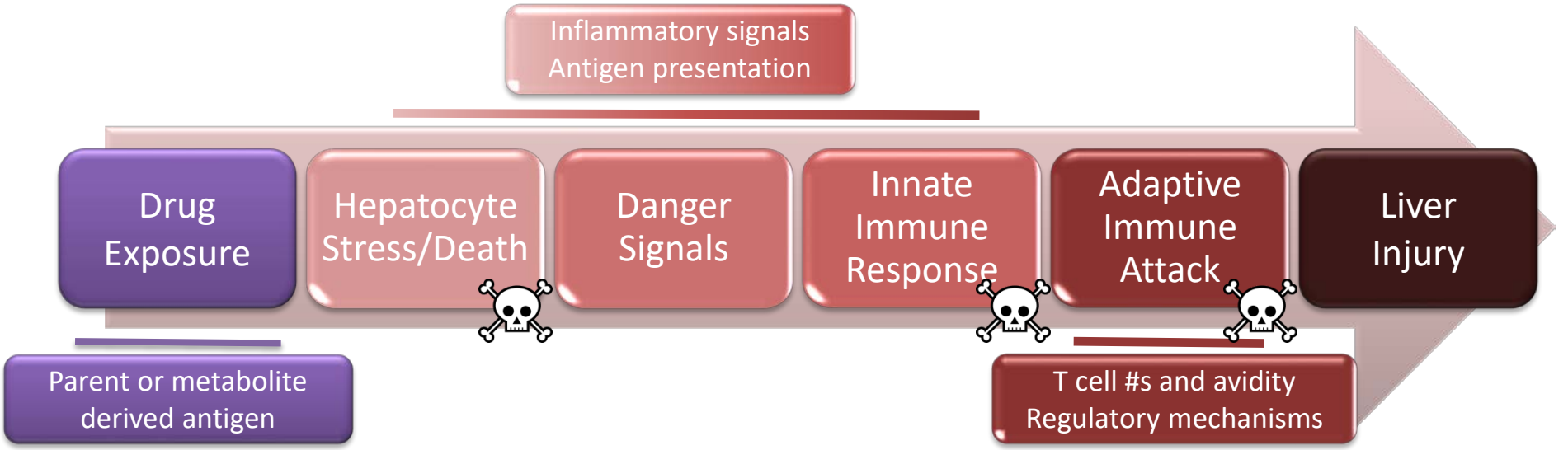
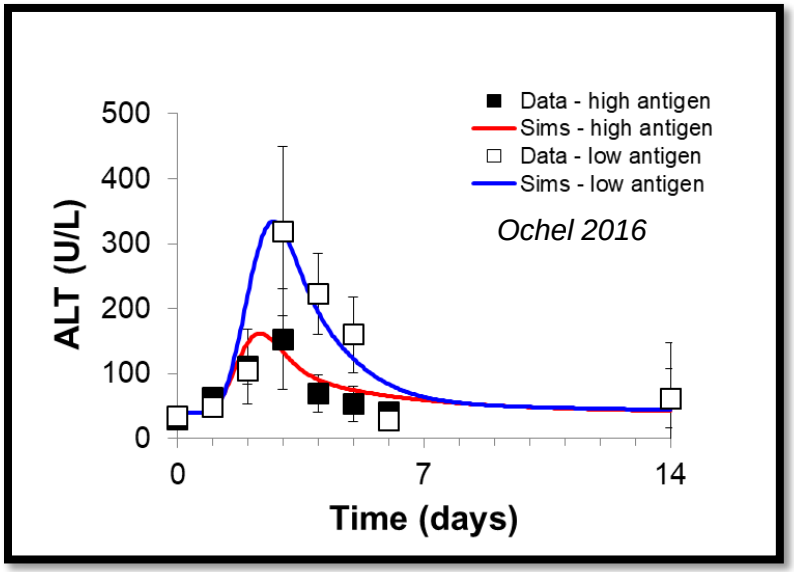
T cell Mediated Hepatotoxicity Plausibly Occurs Within Permissive Liver Environment

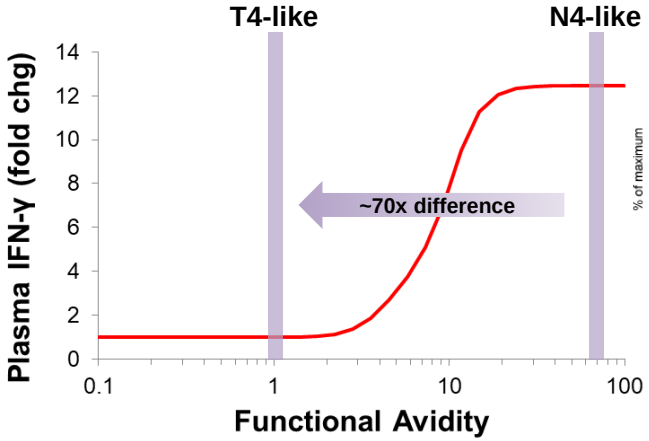


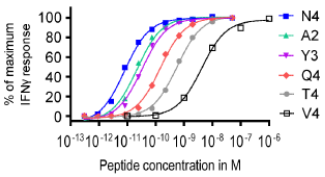
- Liver generally a tolerogenic environment (likely related to gut antigen exposure)
- Intrinsic drug toxicity and local inflammation can drive hepatocyte injury
- T cell mediated cytotoxicity postulated to depend on breaking tolerance, including
  - Generation of drug-dependent antigen
  - Inflammatory conditions (altering normally tolerogenic environment)
  - T cell availability and avidity
  - Overcoming other regulatory mechanisms (*e.g.,* inhibitory receptors, regulatory T cells)

Simulations Reproduce Differential T cell Response to Persistent Ag or Variable Avidity

- Simulations reproduce milder injury in the presence of persistent Ag
  - Differential ALT response despite similar T cell expansion
  - Reproduces evidence for exhaustion, i.e., differentiation to “exhausted” T cell populations
  - Reproduces T cell dysfunction, i.e., diminished cytokine production, cytotoxicity
- Simulations reproduce loss of functional T cell response with lower T cell avidity, consistent with data
  - Also reproduces less injury with lesser T cell avidity, consistent with data
- Current efforts focused on incorporation of AQ and reproducing delayed AQ-specific CD8+ T cell mediated ALT elevations







Zehn 2009

MICE

Preclinical Data and Simulation Results

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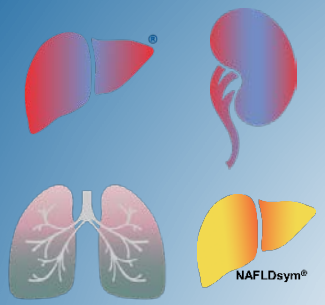
Acknowledgments – DILIsym Team and Partners



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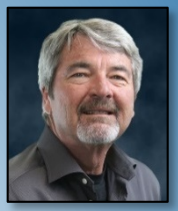

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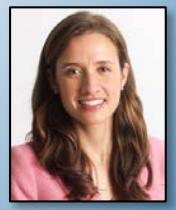

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Thank You - Questions?

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