SimulationsPlus

Successes and Failures of DILIsym

Scott Q Siler
(substituting for Paul Watkins)

ACS Fall 2025

Agenda

- Quantitative systems toxicology (QST) modeling of DILI
- Successes of DILIsym
- Current limitations of DILIsym and Future Directions

Every method has limitations

Understanding those limitations allows for proper interpretation of results

Two Primary Classifications of Drug Induced Liver Injury

Feature	Direct hepatotoxicity	Idiosyncratic hepatotoxicity
Dose-dependence	Dose-dependent	Usually not dose-dependent
Predictability	Predictable	Unpredictable
Incidence	High incidence	Low incidence (rare)
Latency	Short (hours to days)	Variable (days to weeks or even months)
Mechanism	Direct toxicity of the drug or its metabolites	Complex interplay of genetic, immunological, and environmental factors, often involving an immune-mediated response
Examples	Acetaminophen overdose	Amoxicillin-clavulanate, diclofenac

QST Models Predict Tox via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability

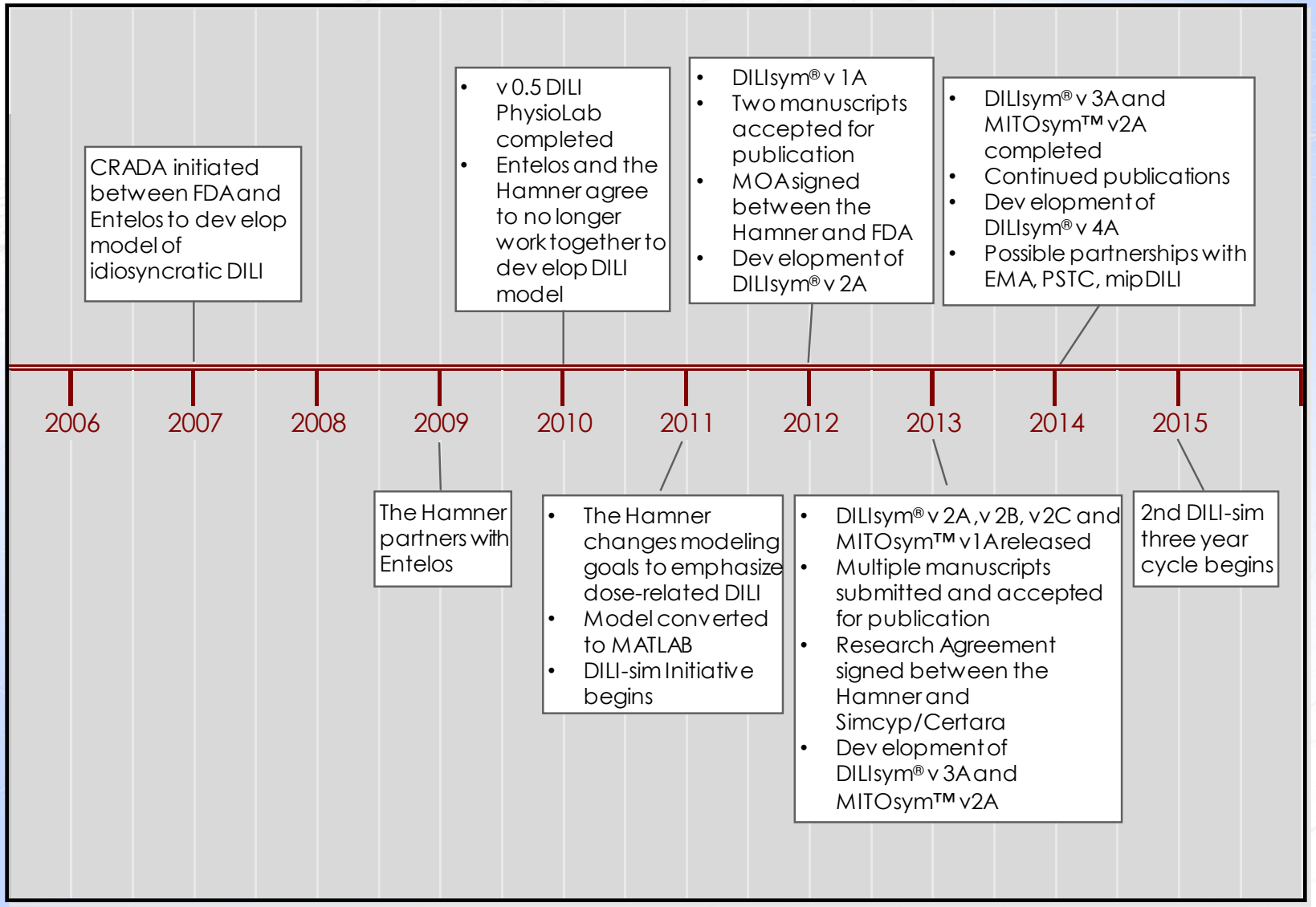
Predicted compound concentrations at site of toxicity using SLP PBPK modeling software

Toxicity mechanisms for given compounds (parent +/- metabolites) characterized by specifically-designed DILIsym input (in vitro) assays

Mechanistic representation of underlying biochemistry describing pathophysiology is foundation of QST models

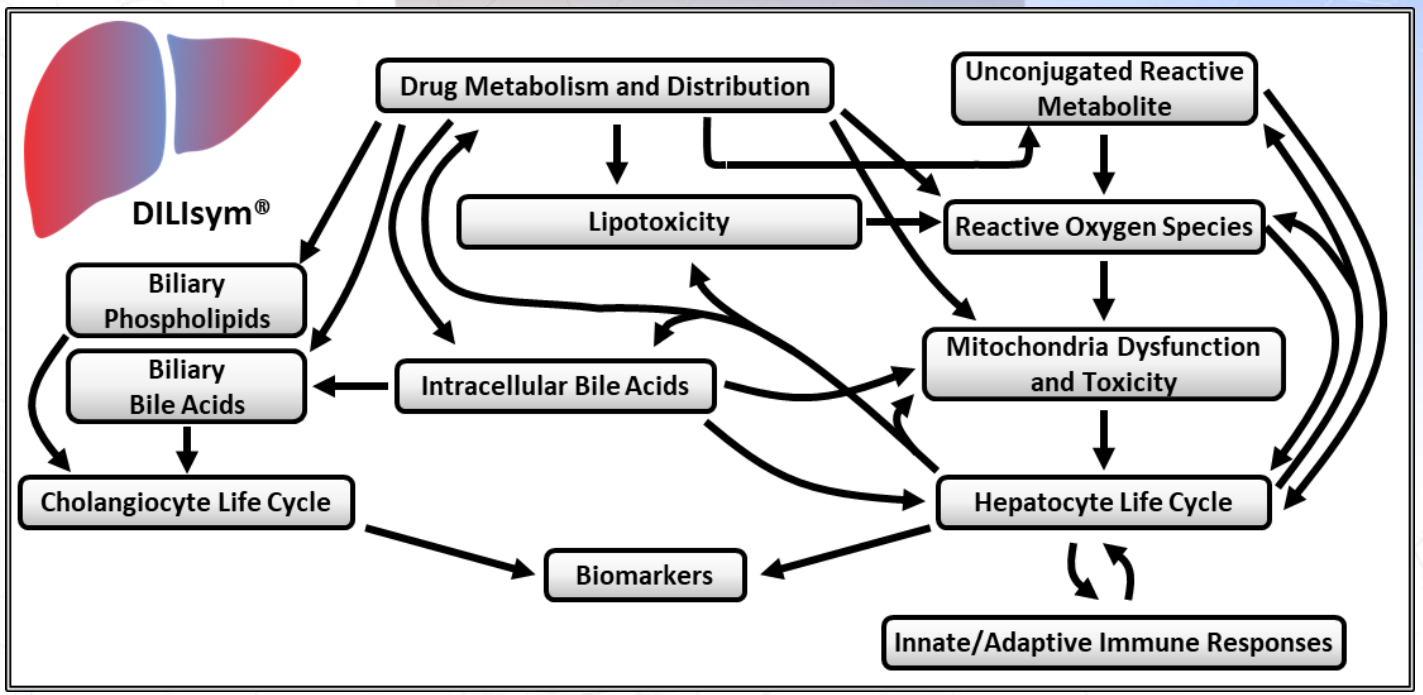
Development and use of simulated populations provides inter-patient variability

The History of DILIsym Shows the Ongoing Expansion of Capabilities…That Continues to Present Day



DILIsym Software Overview

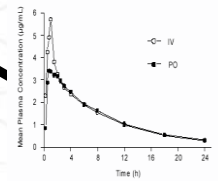
- SimPops reflecting normal liver biochemistry and multiple disease states that affect liver
  - Adults and pediatrics (normal liver)
  - Rat, mouse, dog in addition to human
- Essential cellular processes represented to multiple scales in interacting sub-models
  - Key intrinsic hepatocellular injury mechanisms
  - Cholangiocyte injury and adaptive immune response representations updated in DS11
- ~90 detailed representations of validation compounds with >80% success and **zero false positive predictions**
- Single and combination drug therapies



DILIsym Utilizes Various Data Types to Inform Decisions

Exposure (PBPK modeling)

Pharmacokinetics



Mechanisms

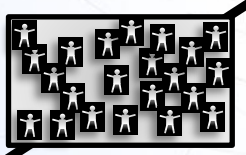
Bile Acid Transporter Inhibition

Mitochondrial Respiration

ROS Generation

Interpatient Variability

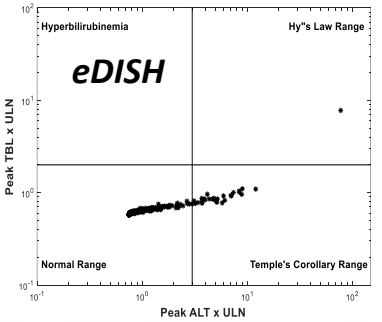
Unique Parameter Combinations

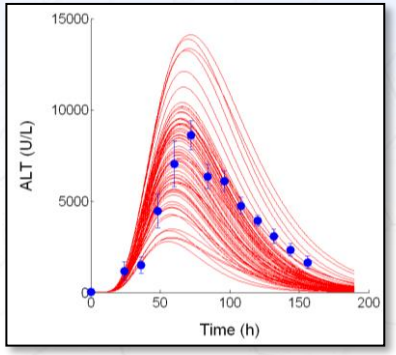


SimPops™

DILIsym®

Simulated Frequency & Severity of Liver Injury





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DILIsym on the Non-Clinical DILI Assessment List by FDA

Received: 5 November 2024 | Accepted: 6 January 2025

DOI: 10.1097/HEP.0000000000001281

REVIEW

Assessment of liver injury potential of investigational medicines in drug development

Naga Chalasani[1] | Paul H. Hayashi[2] | Debra Luffer-Atlas[3] | Arie Regev[4] | Paul B. Watkins[5]

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[2]Office of New Drugs, United States Food and Drug Administration, Silver Spring, Maryland, USA

[3]Scientific Strategy for Catalyze360, Eli Lilly and Company, Indianapolis, Indiana, USA

[4]Medical Global Patient Safety, Eli Lilly and Company, Indianapolis, Indiana, USA

[5]UNC Eshelman School of Pharmacy, Chapel Hill, North Carolina, USA

TABLE 3 Summary of nonclinical data assessed by DILI team at the U.S. Food and Drug Administration

Type	Examples of sources
In vitro	
Metabolic pathway	Dominant cytochromes, UDP
Lipophilicity	Log *P*
Reactive metabolite formation	Glutathione trapping; time-dependent CYP inhibition
Mitochondrial data	Mitochondrial injury/inhibition studies
Transporter inhibition	BSEP, MRP2, other drug transporters
In vivo (animal toxicology studies)	
Blood tests	Liver enzyme, bilirubin elevations
Liver histopathology	Inflammation, necrosis, fibrosis, zone of liver injury
Computer or modeling-based (as available and/or upon request)	
QST	DILISym
QSAR	Analysis by DARS
Rule-of-2; DILI risk score	Analysis by NCTR

Abbreviations: BSEP, bile salt export pump; CYPs, cytochrome P450 enzymes; DARS, FDA Division of Applied Regulatory Science; MRP2, multidrug resistance protein 2; NCTR, FDA National Center for Toxicological Research; QSAR, quantitative structure–activity relationship; UDP, uridine 5′-diphosphoglucuronosyltransferase.

DILIsym Performance Review – Level 1

- **Key Question: would the weight of evidence from the drug case and from the DILIsym results have led to the same overall conclusion regarding the presence or absence of a possible drug-induced liver injury liability for the compound?**

Human Simulation Scenarios		Clinical Data: DILI	Clinical Data: Clean	Sum
DILIsym Prediction	DILI Predicted	True positives **54**	False positives **0**	DILI predicted **54**
	No DILI Predicted	False negatives **17**	True negatives **24**	No DILI predicted **41**
Sum		DILI scenarios **71**	Clean scenarios **24**	Total scenarios **95**
		Sensitivity: 76%	**Specificity: 100%**	

PPV: 100%

NPV: 59%

82% (78/95) unique human simulation scenarios predicted well

**90 unique compounds*
**95 unique simulation scenarios*
**71 DILI scenarios, 24 clean scenarios*

Agenda

- Quantitative systems toxicology (QST) modeling of DILI
- Successes of DILIsym
- Current limitations of DILIsym and Future Directions

The DILI-sim Consortium Has Ensured that DILIsym QST Model Development Aligns with Industry and Regulatory Needs

Excellent Scientific Advisory Board

Current DILI-sim Members

For a comprehensive review of progress, see *Watkins 2020, Current Opinion in Toxicology (23-24:67-73)*

- *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*
  - *21 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $10 million invested in project*

DILIsym Development and Use Has Advanced the Understanding of DILI

- Primary mechanistic contributors to DILI
- Interactions between mechanisms that underpin DILI
- Contributions of adaptive responses to DILI
- Quantification of the magnitude of perturbations required to elicit DILI
- Quantification of amount of hepatocyte loss during DILI events
- Identification of DILI-susceptible patient types

Numerous DILIsym Publications Over Time

U.S. FDA Renews Annual DILIsym Software Licenses

FDA Maintains Access to Leading Liver Injury Software Program

May 06, 2020 08:30 AM Eastern Daylight Time

Comparing the Liver Safety Profiles of 4 Next-Generation CGRP Receptor Antagonists to the Hepatotoxic CGRP Inhibitor Telcagepant Using Quantitative Systems Toxicology Modeling

Jeffrey L. Woodhead,[*,1] Scott Q. Siler,[*] Brett A. Howell,[*] Paul B. Watkins [,†] and Charles Conway[‡]

First Approved Cancer Treatment for TGCT Included DILIsym Simulations in FDA Review

FDA Review Cites DILIsym Results as Part of Turalio® Submission

2020 08:30 AM Eastern Daylight Time

Application of the DILIsym® Quantitative Systems Toxicology drug-induced liver injury model to evaluate the carcinogenic hazard potential of acetaminophen

Gary Eichenbaum [a,*], Kyunghee Yang [b], Yeshitila Gebremichael [b], Brett A. Howell [b], F. Jay Murray [c], David Jacobson-Kram [d], Hartmut Jaeschke [e], Edwin Kuffner [a], Cathy K. Gelotte, John C.K. Lai [f], Daniele Wikoff [g], Evren Atillasoy [f]

Assessing Liver Effects of Cannabidiol and Valproate Alone and in Combination Using Quantitative Systems Toxicology

Vinal V. Lakhani[1], Grant Generaux[1], Brett A. Howell[1], Diane M. Longo[1] and Paul B. Watkins[2,3,*]

Pharm Res (2019) 36: 48
https://doi.org/10.1007/s11095-019-2582-y

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RESEARCH PAPER

Analyzing the Mechanisms Behind Macrolide Antibiotic-Induced Liver Injury Using Quantitative Systems Toxicology Modeling

Quantitative Systems Toxicology Modeling Predicts that Reduc… Biliary Efflux Contributes to Tolvaptan Hepatotoxicity

James J. Beaudoin, William J. Brock, Paul B. Watkins, Kim L. R. Brouwer

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

DILIsym: Quantitative systems toxicology impacting drug development

Paul B. Watkins

Quantitative Systems Toxicology Identifies Independent Mechanisms for Hepatotoxicity and Bilirubin Elevations Due to AKR1C3 Inhibitor BAY1128688

Mechanistic Investigations Support Liver Safety of Ubrogepant

Brenda Smith,[*] Josh Rowe,[*,1] Paul B. Watkins,[†] Messoud Ashina,[‡] Jeffrey L. Woodhead,[§] Frank D. Sistare,[¶] and Peter J. Goadsby[‖]

Prediction of the liver safety profile of a first-in-class myeloperoxidase inhibitor using quantitative systems toxicology modeling

Jeffrey L. Woodhead[a], Yeshi Gebremichael[b], Joyce Macwan[a], Irfan A. Qureshi[c], Richard Bertz[c], Victoria Wirtz[c] and Brett A. Howell[a]

Assessment of the Mechanism for Remdesivir-Associated Clinical ALT Elevations Using DILIsym Quantitative Systems Toxicology Modeling

Kyunghee Yang[1], Brett A Howell[1], Joy Y. Feng[2], Darius Babusis[2], Tomas Cihlar[2], Scott Q Siler[1]

Introduction | Parameterization of Clinical PK Data | Parameterization of *in vitro* Toxicity Data | Methods | Simulation Results | Conclusions | Acknowledgements

Quantitative systems toxicology (QST) reproduces species differences in PF-04895162 liver safety due to combined mitochondrial and bile acid toxicity

Grant Generaux[1] | Vinal V. Lakhani[1] | Yuching Yang[1] | Sashi Nadanaciva[2] | Luping Qiu[3] | Keith Riccardi[4] | Li Di[4] | Brett A. Howell[1] | Scott Q. Siler[1] | Paul B. Watkins[5,6] | Hugh A. Barton[7] | Michael D. Aleo[3] | Lisl K. M. Shoda[1]

Modeling and Simulation of Acetaminophen Pharmacokinetics and Hepatic Biomarkers After Overdoses of Extended-Release and Immediate-Release Formulations in Healthy Adults Using the Quantitative Systems Toxicology Software Platform DILIsym

Quantitative Systems Toxicology Modeling Informed Safe Dose Selection of Emvododstat in Acute Myeloid Leukemia Patients

Kyunghee Yang[1,*], Ronald Kong[2], Robert Spiegel[2], John D. Baird[2], Kylie O'Keefe[2], Brett A. Howell[1] and Paul B. Watkins[3]

Comparison of the Hepatotoxic Potential of Two Treatments for Autosomal-Dominant Polycystic Kidney Dis… Using Quantitative Systems Toxicology Modeling

J. L. Woodhead[1] • L. Pellegrini[2] • L. K. M. Shoda[1] • B. A. Howell[1]

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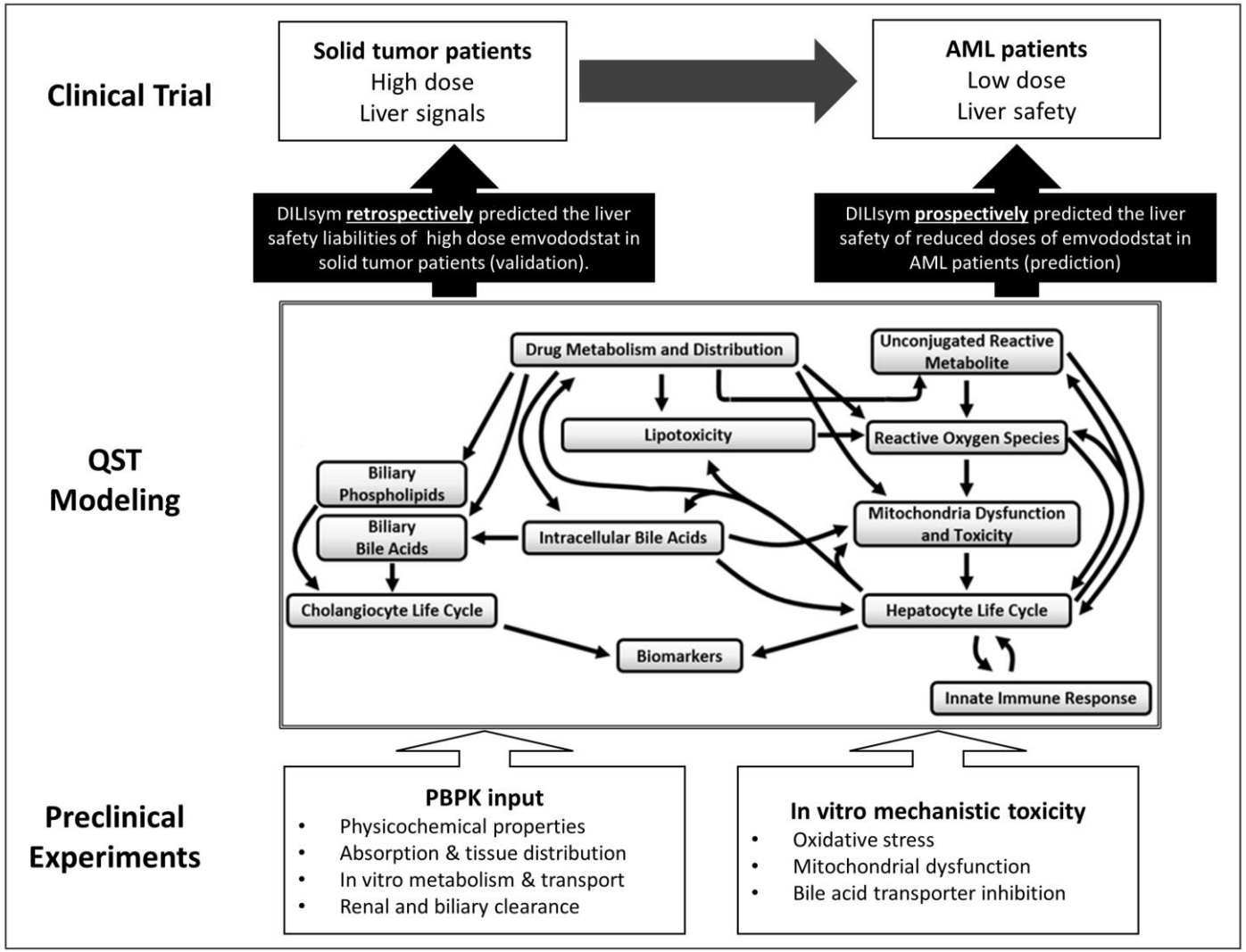
QST Modeling Informed Safe Dose Selection of Emvodostat in Acute Myeloid Leukemia (AML) Patients

Case 2

- Clinical investigation of emvododstat for the treatment of solid tumors was halted after two patients experienced drug-induced liver failure
- Preclinical investigations supported that emvododstat at lower doses might be effective in treating AML patients
- **Retrospective** DILIsym simulations adequately predicted the liver safety liabilities of emvododstat in solid tumor trials and **prospective** simulations predicted the liver safety of reduced doses in an AML clinical trial
- Liver safety was confirmed in a subsequent clinical trial



Yang et al., CPT (2023)
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Potential Reasons for DILIsym False Negative Predictions

- Incomplete input datasets (including PBPK modeling)
- Lack of toxicity signals in in vitro assays
- Untracked but potentially toxic metabolite(s)
- Potential alternate mechanisms
- Population-specific susceptibility
- Some cases may fit in more than one category

Potential Reasons for DILIsym False Negative Predictions: Incomplete Input Datasets

- Prescribed DILIsym input data includes:
  - Preclinical/clinical PK data to develop and validate PBPK models
  - In vitro assays to determine drug effects on key intrinsic toxicity pathways: oxidative stress, mitochondrial dysfunction, bile acid transporter inhibition
- Not all compounds had prescribed input datasets
  - Compound L hepatic exposure was inferred using a liver partition coefficient (PBPK model was not developed)
  - Standard in vitro toxicity assays were not performed for crizotinib
- Collection of complete standard input datasets recommended to increase predictivity

Drug	Exposure	Mito	BA	ROS/RNS	RM
Compound A (DILI)					
Compound P (DILI)					
Telithromycin (DILI)					
Azithromycin (DILI)					
MK-0536 (DILI)					
Riluzole (DILI)					
Compound L (DILI)					
Compound U (DILI)					
Compound V (DILI)					
Crizotinib (DILI)					
Ketoconazole (DILI)					
Compound KK (DILI)					
Compound MM (DILI)					
Compound QQ (DILI)					
Compound SS (DILI)					
Compound A4 (DILI)					

Color Key – Data Quality	
Excellent	
Good	
Fair	
Unavailable	

Potential Reasons for DILIsym False Negative Predictions: Lack of Toxicity Signals In in vitro Assays

- DILIsym leverages in vitro mechanistic toxicity signals to predict clinical hepatotoxicity
- Some DILI compounds showed no in vitro mechanistic toxicity signals, leading to false negative DILI predictions
- Lack of in vitro signals is likely to be due to:
  - Potential contribution of metabolite(s) as assays were performed in metabolically incompetent cells or vesicles (by design)
  - Potential alternate mechanisms

Mechanism	Assay	System
Oxidative stress	High content imaging to evaluate drug effects on oxidative stress using probes such as dihydroethidium (DHE)	HepG2 and HepaRG spheroids
Mitochondrial dysfunction	Seahorse XF analyzer assays to evaluate drug effects on mitochondrial respiration	HepG2
Bile acid transporter inhibition	Transporter inhibition assays to determine IC_{50} of the compound interest for inhibition of BSEP, MRP3, MRP4, and NTCP	Membrane vesicles or transfected cell lines

Potential Reasons for DILIsym False Negative Predictions: Untracked but Potentially Toxic Metabolite(s)

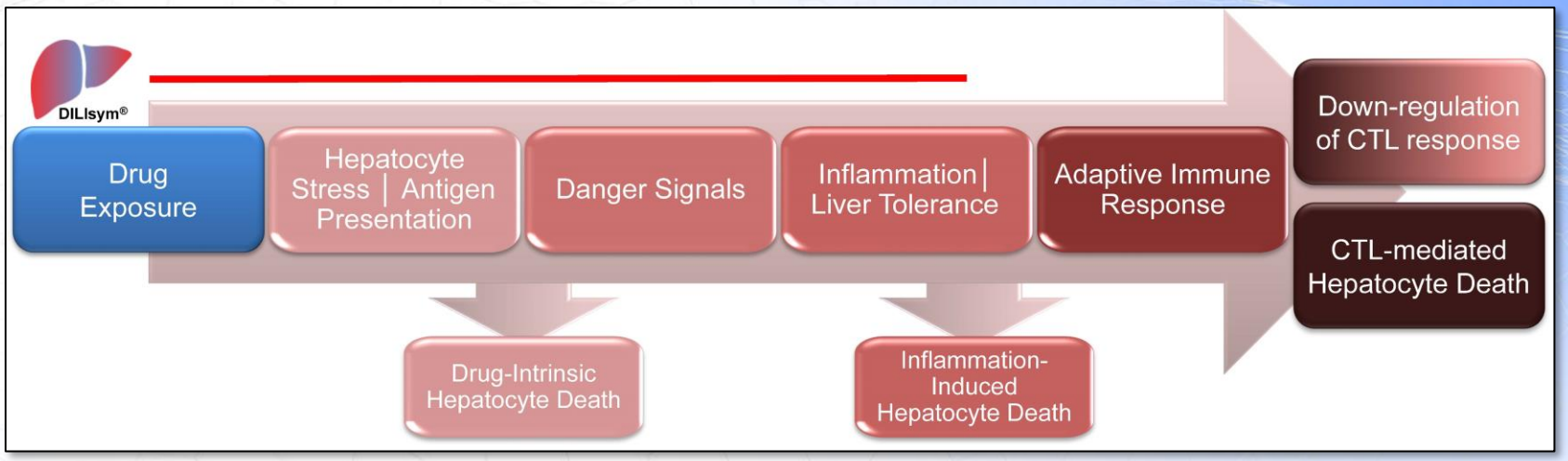
- DILIsym tracks the exposure of each chemical entity (e.g., parent and metabolites) and links these exposures to toxicity parameters specific to each entity
- DILIsym in vitro mechanistic toxicity assays are performed in cells and vesicles with minimal metabolic capacity
  - To evaluate the specific effects of each chemical entity (i.e., parent and metabolites)
  - If available, metabolites are tested separately
- Some false negative compounds have known major metabolites which were not represented in DILIsym due to limited datasets
  - Compound A, azithromycin, telithromycin, ketoconazole, compound QQ
- In recent years, HepaRG spheroids have been added to oxidative stress to evaluate effects of potentially unidentified metabolites
  - Compound QQ showed no toxicity signals in HepG2 but showed ROS signal in HepaRG spheroids
- Future directions
  - In vitro testing in metabolically competent systems (requires quantification of parent and metabolites to tease out respective effects)
  - ML/AI to identify mechanistic signals of potential metabolites (e.g., Liver Safety Plus)

Potential Reasons for DILIsym False Negative Predictions: Potential Alternate Mechanisms

- DILIsym represents three intrinsic mechanisms of hepatocellular injury
  - oxidative stress, mitochondrial dysfunction, bile acid transporter inhibition
- No DILI prediction indicates there is no “direct” effects on hepatocytes causing injury through three investigated mechanisms at simulated doses
  - It does not rule out the potential hepatocellular/cholestatic injury mediated by mechanisms not yet included in DILIsym (e.g., adaptive immune reaction, cholestatic injury, ER stress)
  - Compound L showed cholestatic liver signals
  - Compound SS and compound A4 had target-mediated immune mechanisms

Idiosyncratic DILI Frequently Includes the Adaptive Immune System

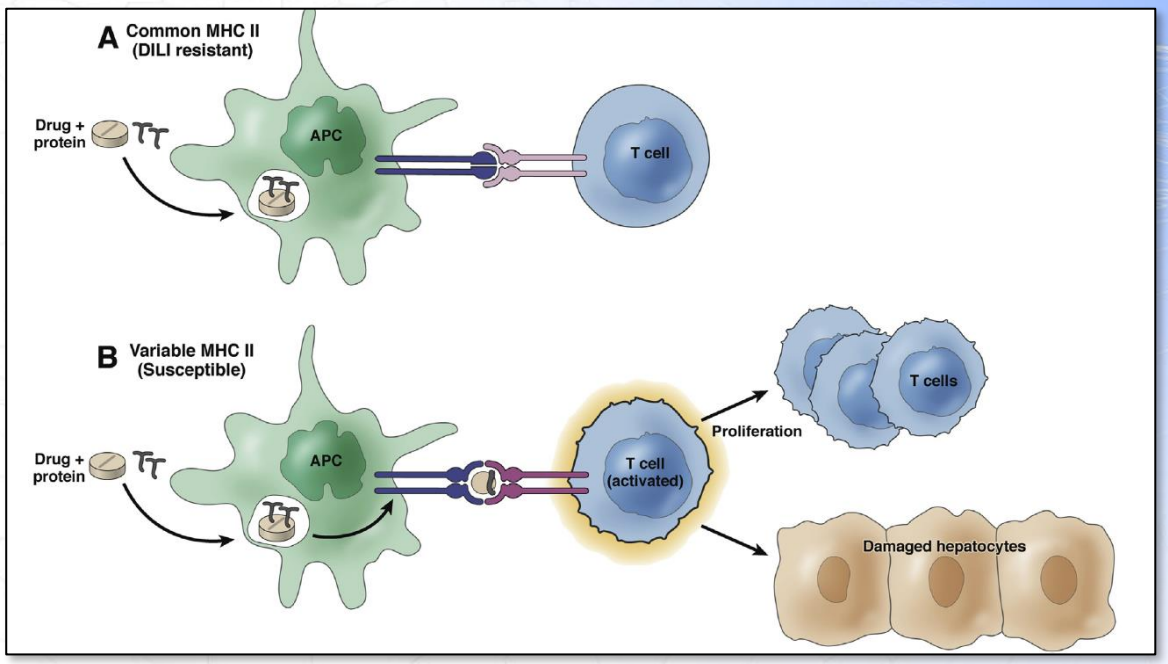
- Low frequency, delayed DILI is often associated with an adaptive immune response
- Initial step includes release of antigen from hepatocyte into circulation
- Subsequent presentation of same antigen at a later time can engage the cytotoxic T cells leading to hepatocyte death
- <u>Challenge for predicting potential for a compound to elicit idiosyncratic DILI:</u> No assays can be used to identify potential antigens of compounds at the present time



Clemens 2025

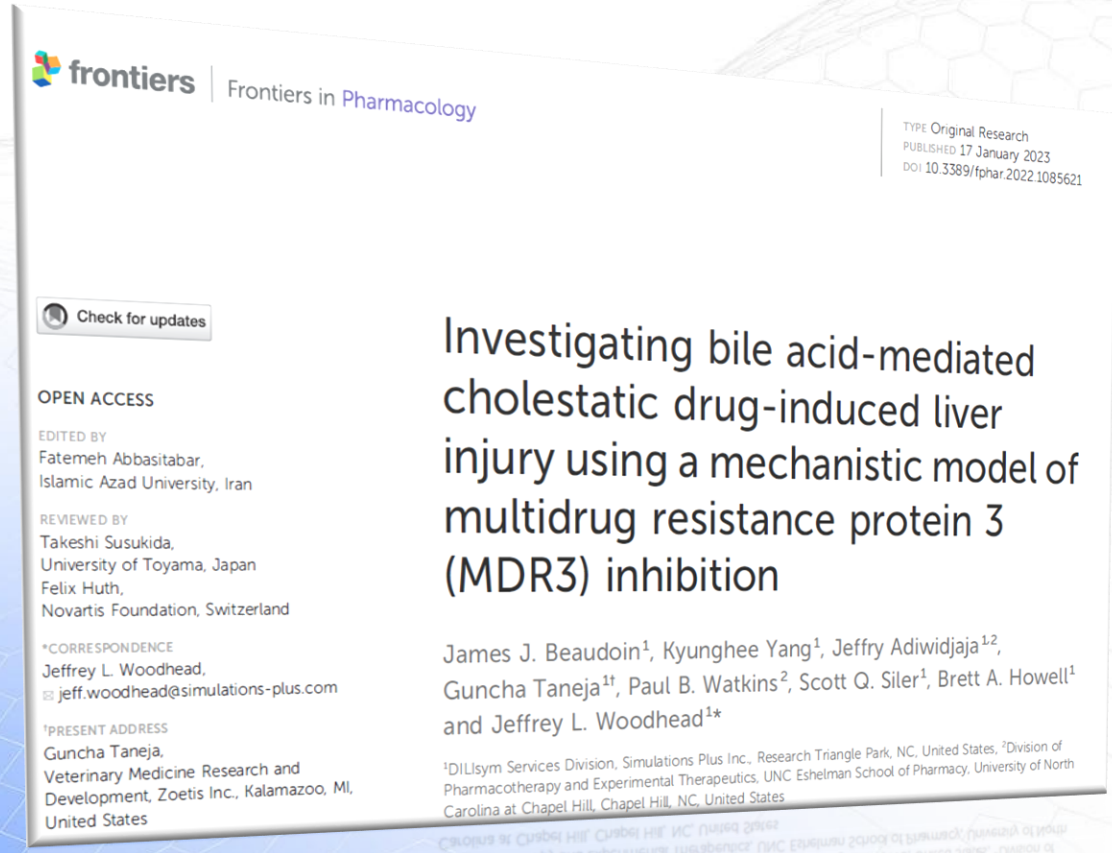
Susceptibility to Idiosyncratic DILI Frequently May Have Roots in Genetic Variability within the Adaptive Immune System

- Not all antigen presenting cells (APC) interact with antigens released from hepatocytes
- Genotypic variability in the major histocompatibility complex (MHC) II plays a role
- Challenge for predicting potential for a compound to elicit idiosyncratic DILI: The relationship between hepatocyte antigens and varied MHC II has not been established yet



Fontana 2014

Representations of Bile Acid and Phospholipid Disposition and Cholestatic DILI Were Updated for DILIsym 11



frontiers | Frontiers in Pharmacology

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Investigating bile acid-mediated cholestatic drug-induced liver injury using a mechanistic model of multidrug resistance protein 3 (MDR3) inhibition

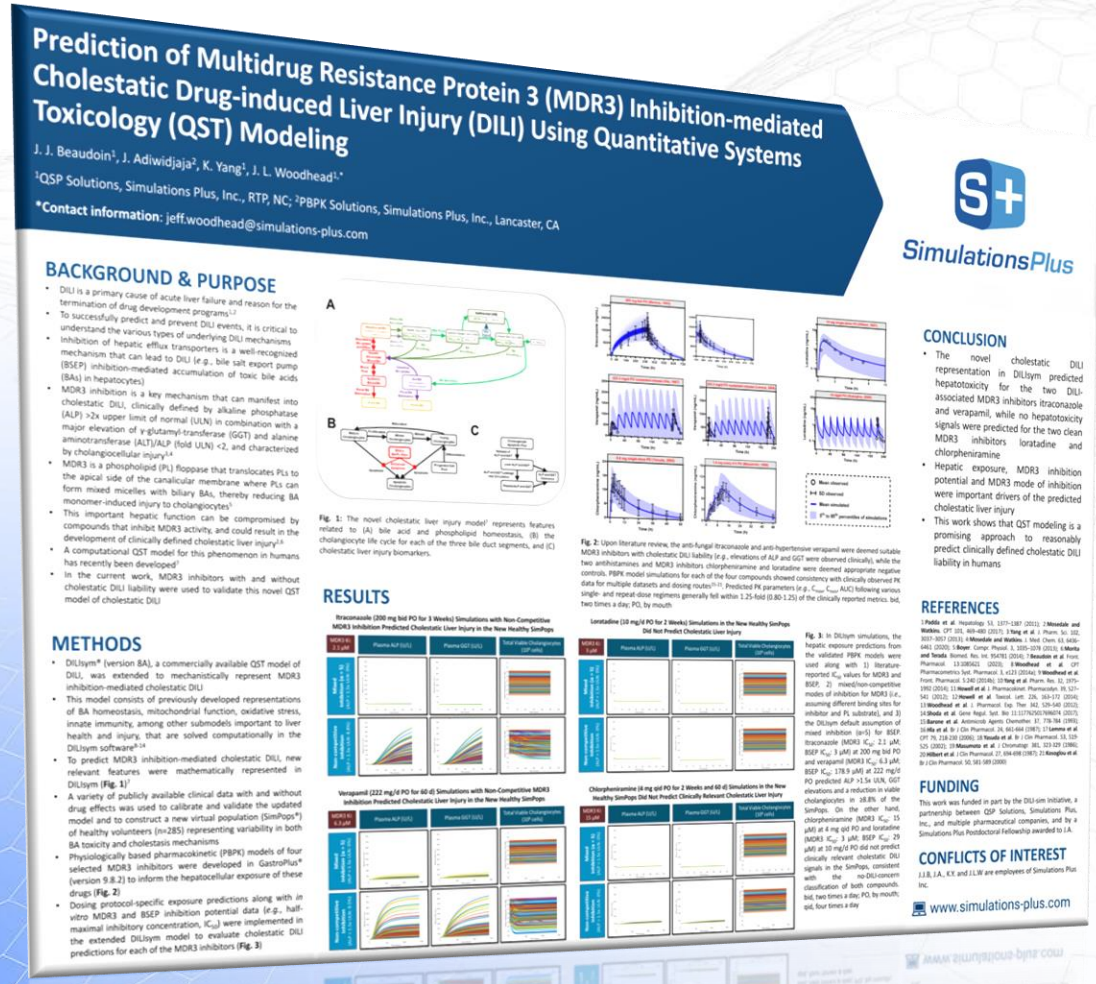
James J. Beaudoin[1], Kyunghee Yang[1], Jeffry Adiwidjaja[1,2], Guncha Taneja[1†], Paul B. Watkins[2], Scott Q. Siler[1], Brett A. Howell[1] and Jeffrey L. Woodhead[1*]

[1]DILIsym Services Division, Simulations Plus Inc., Research Triangle Park, NC, United States, [2]Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

- The human bile acid (BA) and phospholipid (PL) submodels within DILIsym have been updated with new features relevant to cholestatic liver injury
  - Cholehepatic shunting of BAs
  - Biliary HCO3- secretion
  - Different modes of MDR3 inhibition
  - Non-MDR3-mediated PL efflux
  - Cholangiocyte regeneration
- New SimPops with variability in both BA toxicity and cholestasis mechanisms was developed and validated
  - 30+ clean/DILI-associated exemplar compounds have been tested
  - Five previously developed human SimPops (e.g., NHV, NAFLD, T2D) and one rat SimPops were updated and validated
  - Previously developed sensitive SimCohorts based on these SimPops have been updated accordingly
  - In addition: a post-menopausal women (PMW) SimPops has been developed and validated

**Please see our manuscript for more information*

Simulations of MDR3 Inhibition Illustrate the Predictive Capabilities of the Cholestatic Liver Injury Updates for DILIsym 11



- Using the updated BA and PL sub-models and SimPops in DILIsym:
  - Simulations predicted absence of clinically relevant cholestatic liver injury for the two clean MDR3 inhibitors loratadine and chlorpheniramine
  - Simulations predicted occurrence of clinically relevant cholestatic liver injury for the two DILI-associated MDR3 inhibitors itraconazole and verapamil
- Hepatic exposure, MDR3 inhibition potential and MDR3 mode of inhibition are important drivers of the predicted cholestatic liver injury
- Mixed inhibition ($\alpha = 30$) of MDR3 may be the recommended mode of inhibition to reasonably predict cholestatic DILI liability based on additional simulations
  - Likely compound-specific; potential MDR3 mode of inhibition studies necessary

**Please see our poster presentation for more information*

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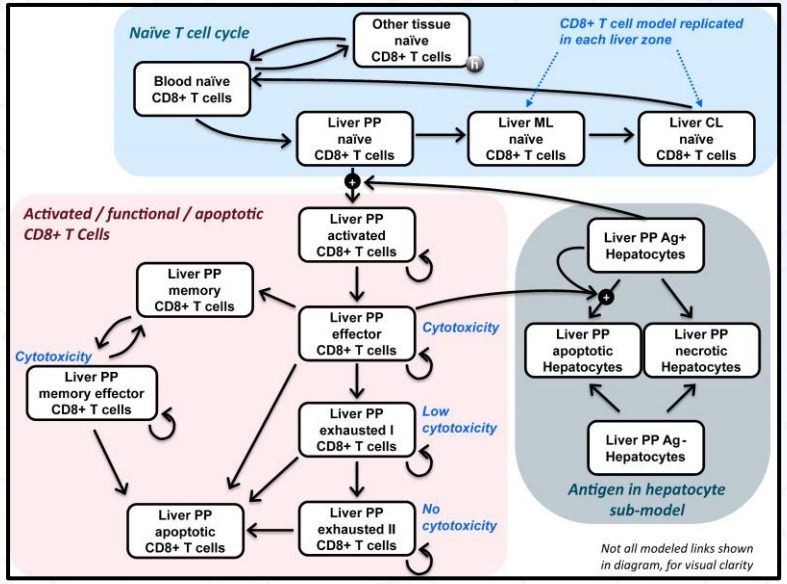
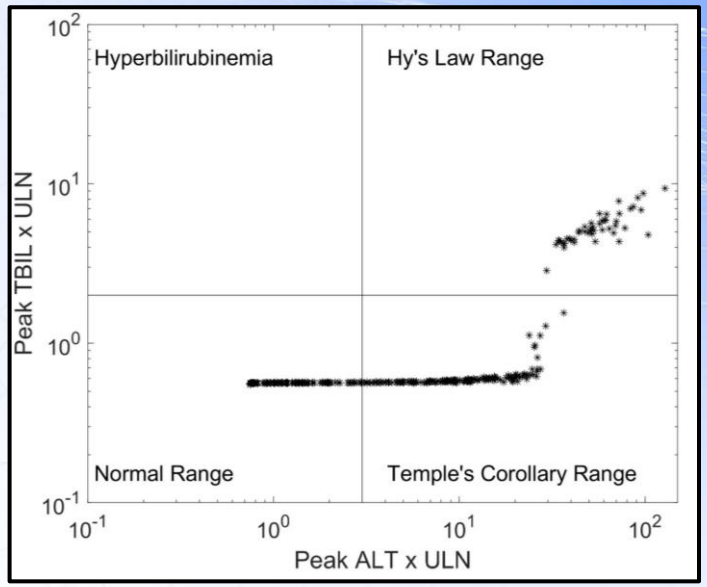
DILIsym 11 Includes a T cell Sub-model for Exploring T cell-mediated DILI

- Submodel includes antigen specific CD8+ T cells and antigen presenting hepatocytes
  - CD8+ T cell life cycle (activation, exhaustion, proliferation, apoptosis,)
  - Presentation of antigen by hepatocytes determined by drug exposure
  - Apoptosis of antigen presenting hepatocytes induced by contact with CD8+ T cells
- Human and mouse T cell SimPops
  - Include variability in parameters governing T cell life cycle, differentiation, cytotoxicity, and dynamics of antigen uptake/clearance by hepatocytes
- Submodel supports exploration of injury profiles and drivers related to T cell mediated DILI, including conditions necessary for initiating T cell responses in novel compounds
  - Explorations with submodel may suggest *in vitro* data that could constrain outcomes and improve representations for novel compounds

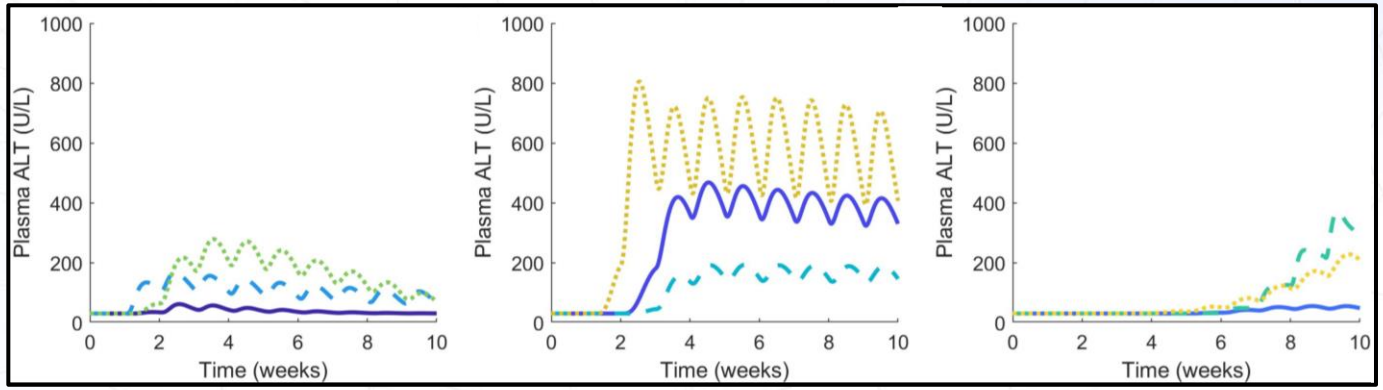
T cell sub-model diagram



eDISH for Human T Cell SimPops treated with amodiaquine 600 mg QW



Variability in injury dynamics during AQ 600 mg QW



**Please see our manuscript for more information*

Potential Reasons for DILIsym False Negative Predictions: Population-specific Susceptibility

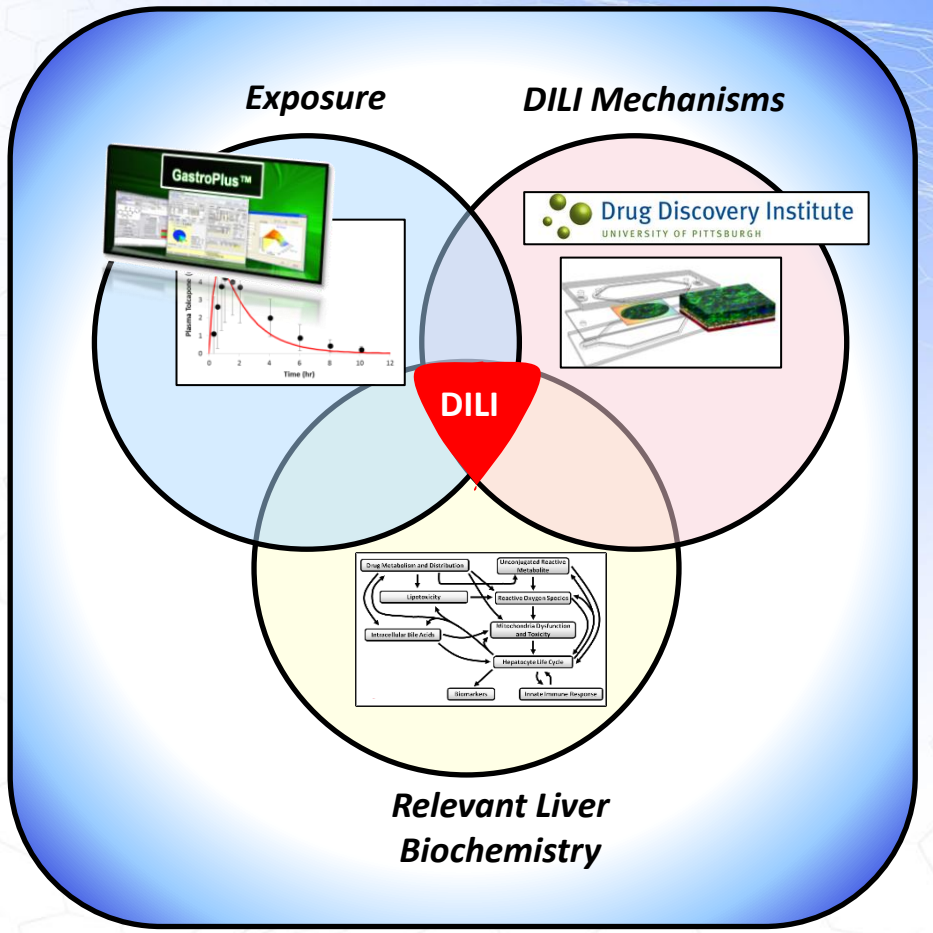
- DILIsym represents simulated populations (SimPops) representing inter-individual variability in DILI mechanisms
  - Individuals with normal liver conditions
  - MASLD/MASH
  - T2D
- Recently released DILIsym 11 represents new SimPops
  - Pediatrics
  - PMW
- Custom SimPops can be developed for intended patient groups
  - Infection, inflammation, hepatocellular carcinoma
  - Dependent upon availability of data describing how disease affects hepatic function

DILIsym has been extensively used to evaluate *small molecule*-mediated hepatotoxicity

Can we predict *biologics*-mediated hepatotoxicity?

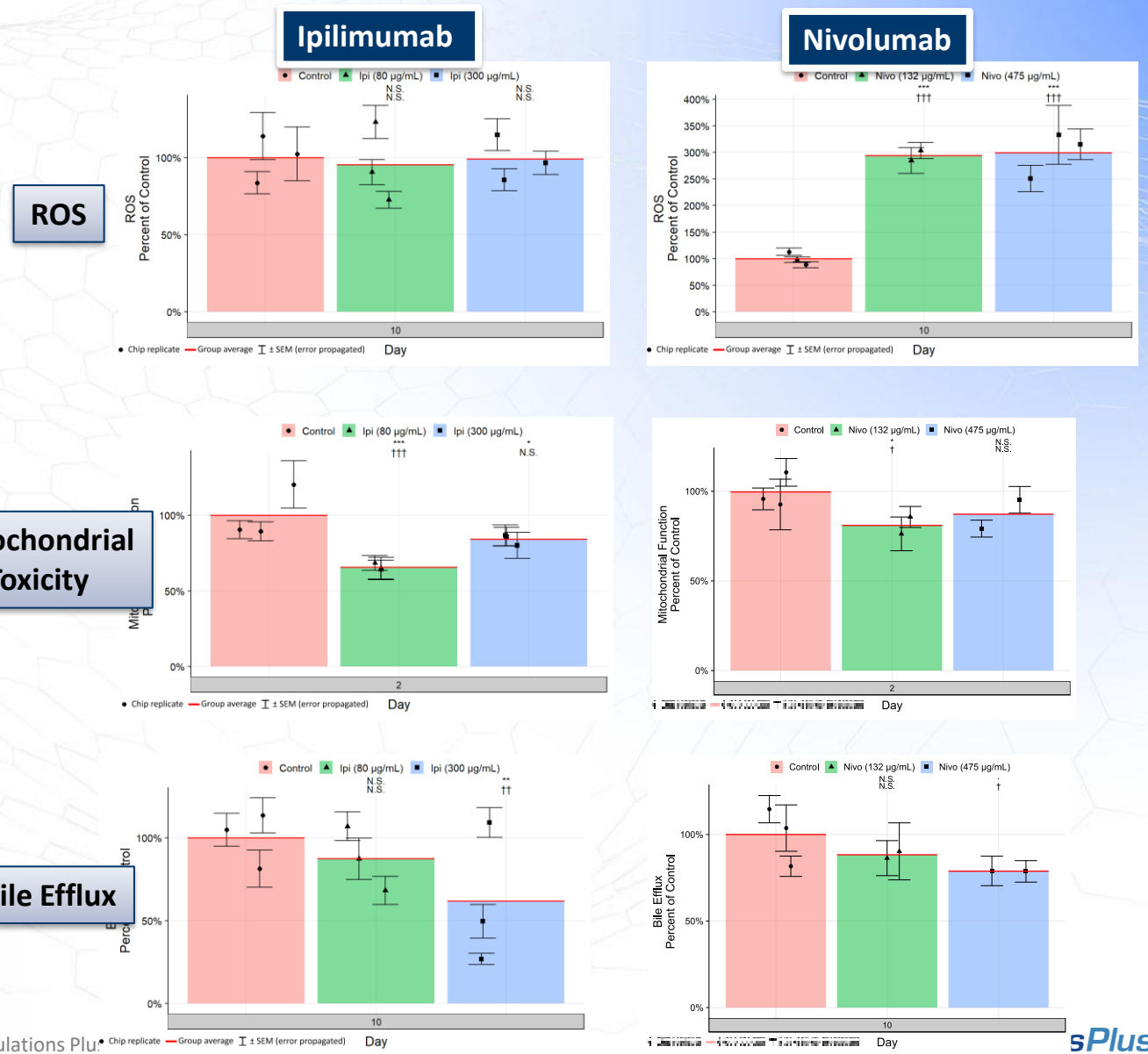
BIOLOGXsym is Being Developed Leveraging Mechanistic Data from In Vitro Human Liver Microphysiology System

- BIOLOGXsym is a mechanistic, mathematical model which is being developed to identify biologics-induced liver injury liabilities in new biologic drug candidates and predict clinical liver injury outcomes
  - Collaborative efforts between Simulations Plus and University of Pittsburgh Drug Discovery Institute (UPDDI) were made to leverage data from mechanistic experiments in a human liver biomimetic (LAMPS)
  - Represents mechanistic pathways specific to biologics such as receptor-mediated indirect responses and target-mediated effects
- Initial development supported by NIH Small Business Innovation Research (SBIR) grant phase 1 & 2
  - Liver biochemistry, mechanisms, and simulated populations (SimPops) developed
  - Seven exemplar compounds including immune checkpoint inhibitors tested



LAMPS Assays Show Hepatocyte Stress Signals for Ipilimumab and Nivolumab

- LAMPS experimental outputs demonstrate early hepatocyte stress signals and mechanisms for ipilimumab and nivolumab
- Ipilimumab significantly decreased mitochondrial function and bile efflux
- Nivolumab significantly increased ROS and decreased mitochondrial function and bile efflux
- Bevacizumab (negative control) did not show any significant mechanistic signals

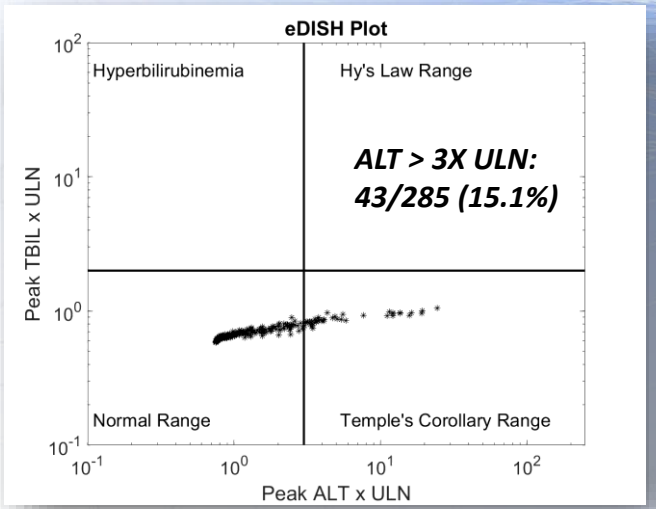


Unpublished Data

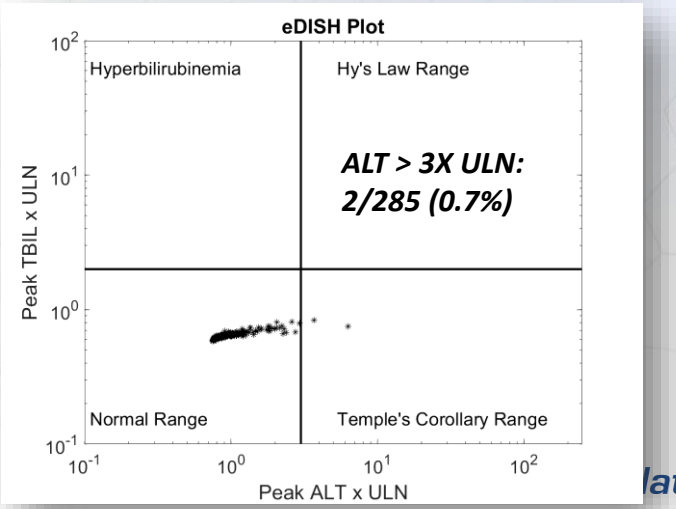
BIOLOGXsym Simulations Leveraging LAMPS Data Predicted Modest Hepatocyte Stress Signals by Ipilimumab and Nivolumab

- Ipilimumab simulations with a SimPops representing normal liver conditions (n=285) predicted modest hepatocyte stress and ALT elevations based on intrinsic toxicity mechanisms informed by LAMPS data
  - Ipilimumab clinical exposure was simulated by PBPK modeling
  - Ipilimumab-mediated mitochondrial dysfunction parameters were optimized to the LAMPS data
- Nivolumab simulations with a SimPops representing normal liver conditions (n=285) predicted mild hepatocyte stress and ALT elevations based on intrinsic toxicity mechanisms informed by LAMPS data
  - Nivolumab clinical exposure was simulated by PBPK modeling
  - Nivolumab -mediated mitochondrial dysfunction and oxidative stress parameters were optimized to the LAMPS data

Ipilimumab (10 mg/kg IV every 3 weeks)

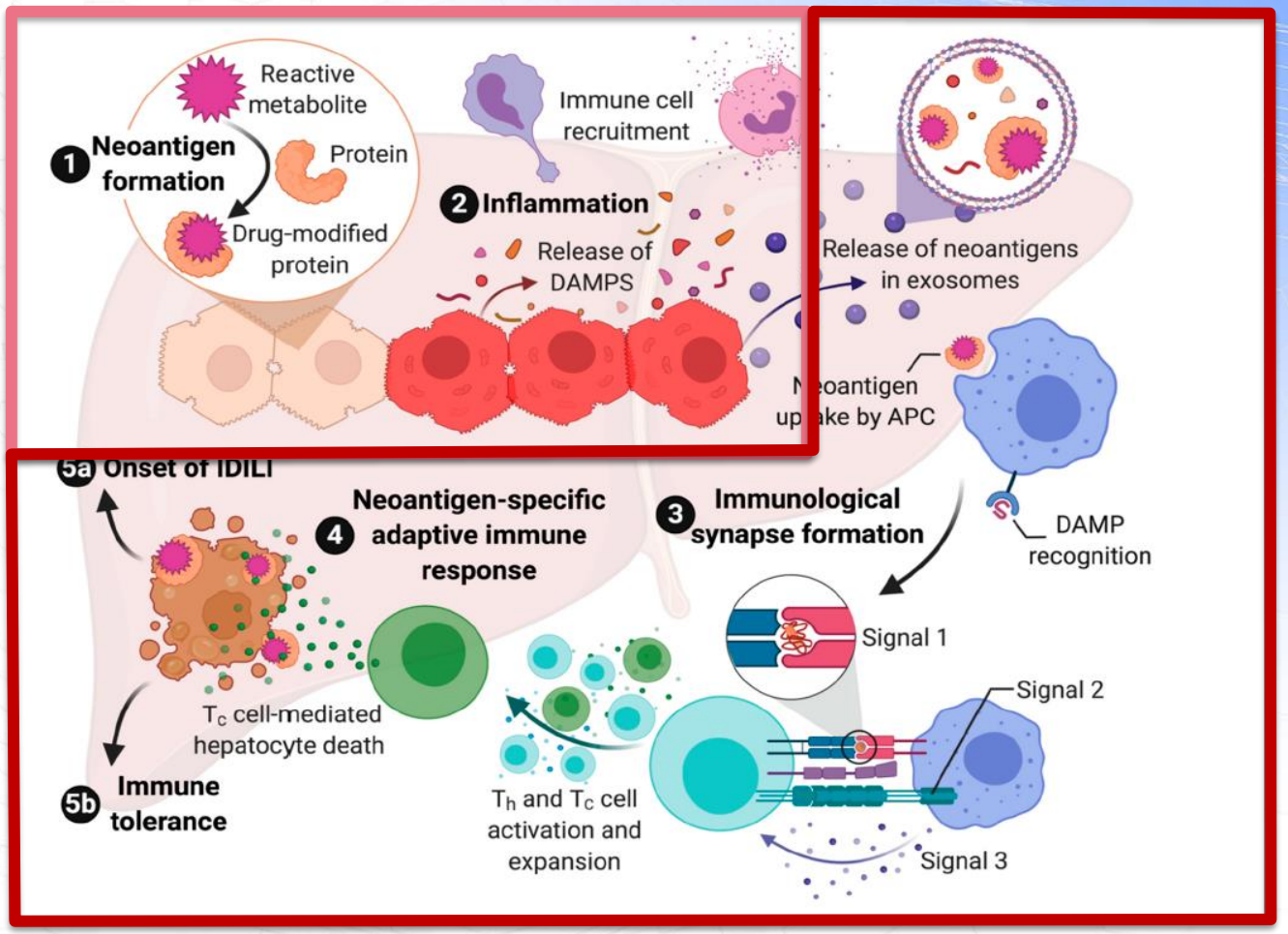


Nivolumab (480 mg IV every 4 weeks)



BIOLOGXsym Simulations Leveraging LAMPS Data Predicted Modest Hepatocyte Stress Signals by Ipilimumab and Nivolumab

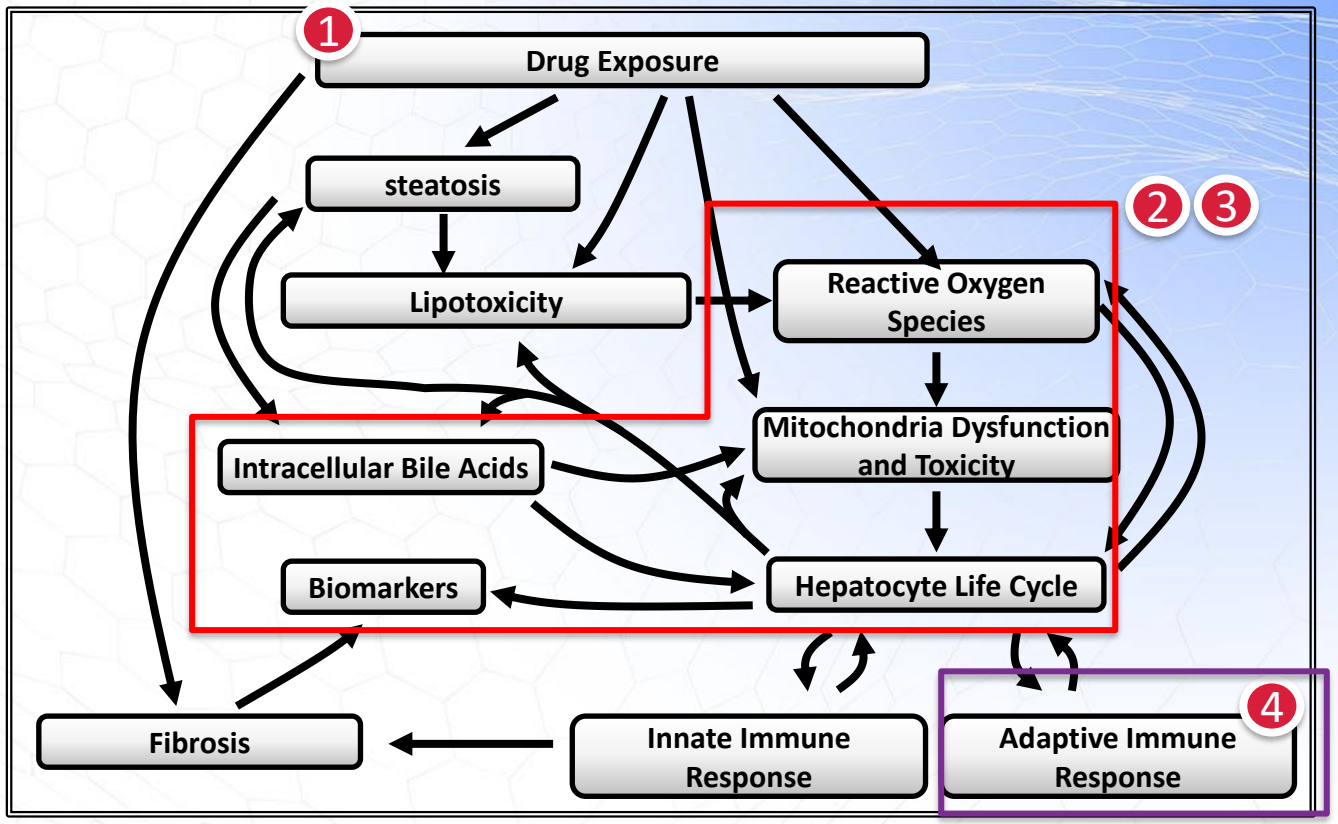
- LAMPS data was incorporated in BIOLOGXsym to represent hepatocyte stress signals, which set the stage for a potential adaptive immune attack by altering the liver micro-environment to be less tolerogenic and more inflammatory
  - ***Hypothesis: immune checkpoint inhibitors can induce low-level hepatocyte stress (e.g., indirect effects via Kupffer cells that express PD-1 and CTLA-4 and/or off-target effects) and sensitize liver to T cell effects***
  - LAMPS provides mechanistic insights underlying hepatocyte stress/liver sensitization



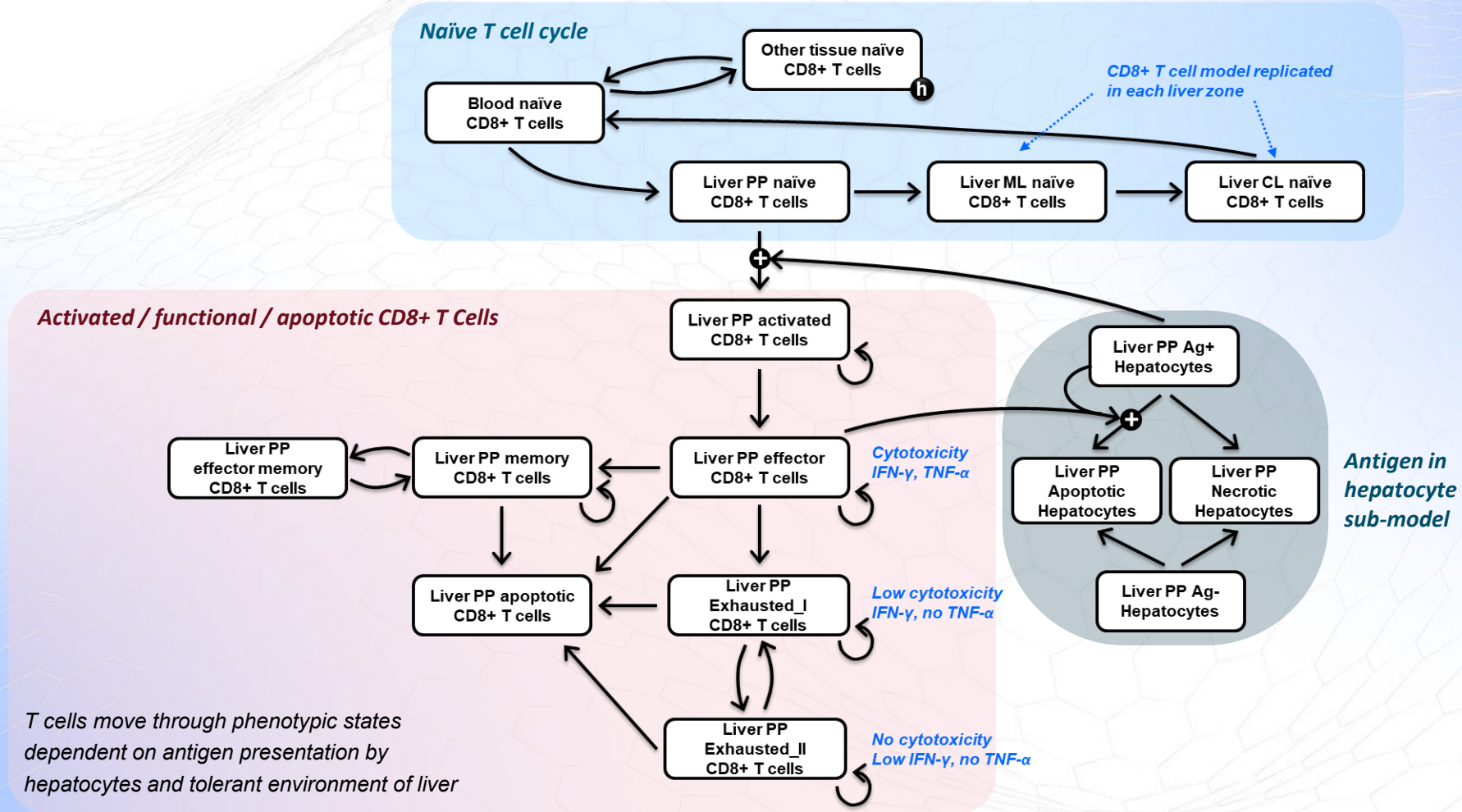
Uetrecht et al. (2021) Int J Mol Sci

A Staged Approach for QST Modeling of Immune Checkpoint Inhibitor-Mediated Hepatotoxicity

1. Develop and validate PBPK models of ipilimumab and nivolumab
   - Estimate plasma and liver concentrations of ipilimumab and nivolumab
2. Identify direct hepatocyte stress mechanisms from LAMPS assays
3. Simulate hepatic responses based on direct hepatocyte stress signals
   - Does not include target-mediated effects yet
4. Simulate hepatic responses combining direct hepatocyte stress mechanisms and target-mediated mechanisms for adaptive immune systems
   - Ipi or nivo amplifies CD8+ T cell response
   - Ipi increases effector CD8+ T cell prolif, mediator production, cytotoxicity
   - Nivo increases exhausted CD8+ T cell prolif, mediator production, cytotoxicity



CD8+ T Cell Representation Is Being Developed in BIOLOGXsym to Investigate Requirements for T cell Cytotoxicity to Explain ICI Hepatitis



Not all modeled links shown in diagram, for visual clarity

Every method has limitations

Understanding those limitations allows for proper interpretation of results

Data Needs to Support Mechanistic Toxicity Modeling in DILIsym –Typically Gathered

Mechanistic Data Description*	
Ki or IC_{50} of Compound X inhibition of human BSEP, human MRP3, human MRP4, human MDR3, and human NTCP	IC_{50} assays or Ki measurements
EC_{50} of Compound X effects on mitochondrial electron transport chain inhibition and/or proton gradient uncoupling	Cellular OCR assays (HepG2)
EC_{50} of Compound X effects on reactive oxygen species production (parent)	Cellular ROS assays (HepG2)
EC_{50} of Compound X effects on reactive oxygen species production (parent + metabolites)	Cellular ROS assays (HepaRG spheroids)

**Raw data typically used by Simulations Plus Services team to define DILIsym input parameters*