Recent Advances in Predicting Drug-Induced Liver Injury (DILI): New Capabilities and SimPops in DILIsym (DS11) and Machine Learning ADMET Predictor Supported Toxicity Parameters

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March 19, 2025

Disclaimer: Simulations Plus QSP Services are developed and provided as an educational tool based on assessment of the current scientific and clinical information, and accepted approaches for drug safety and efficacy. The resultant data, suggestions, and conclusions ("Guidelines") should not be considered inclusive of all proper approaches or methods, and they cannot guarantee any specific outcome, nor establish a standard of care. These Guidelines are not intended to dictate the treatment of any particular patient. Patient care and treatment decisions should always be based on the independent medical judgment of health care providers, given each patient's individual clinical circumstances.

DILIsym Value Proposition by the Numbers

58

Number of biopharmaceutical Sponsors that have engaged DILIsym*

91

Mechanistic projects simulating liver injury based on in vitro data on mechanisms of toxicity

8

Biomarker fitting projects simulating hepatocyte loss consistent with measured transaminase profiles

6

Mechanistic bilirubin projects simulating bilirubin elevations via altered disposition

50+

Known regulatory submissions

8

Marketed drugs in which it has been publicly disclosed that DILIsym contributed to liver safety decisions

* Total DILIsym clients, some with multiple projects / compounds

Executive Summary

- **DILIsym**
  - Mechanistic, mathematical quantitative systems toxicology (QST) model
  - Constructed to support pharmaceutical risk assessment and decision making
- **Clinical Application**
  - DILIsym has been applied to support decisions related to compound DILI risk throughout the clinical development pipeline
    - Projects executed with 55+ companies
  - DILIsym simulation results have been included in numerous communications with regulatory agencies
- **Preclinical Application**
  - Liver Safety+ recently made available
  - Provides efficient evaluation of DILI risk for preclinical compounds
  - Machine learning utilized to generate key parameters based on compound structure, a la QSAR
- **DILIsym support enabled via multiple routes**
  - Simulations Plus services projects
  - Direct license of DILIsym
  - DILI-sim Consortium membership
  - Liver Safety+

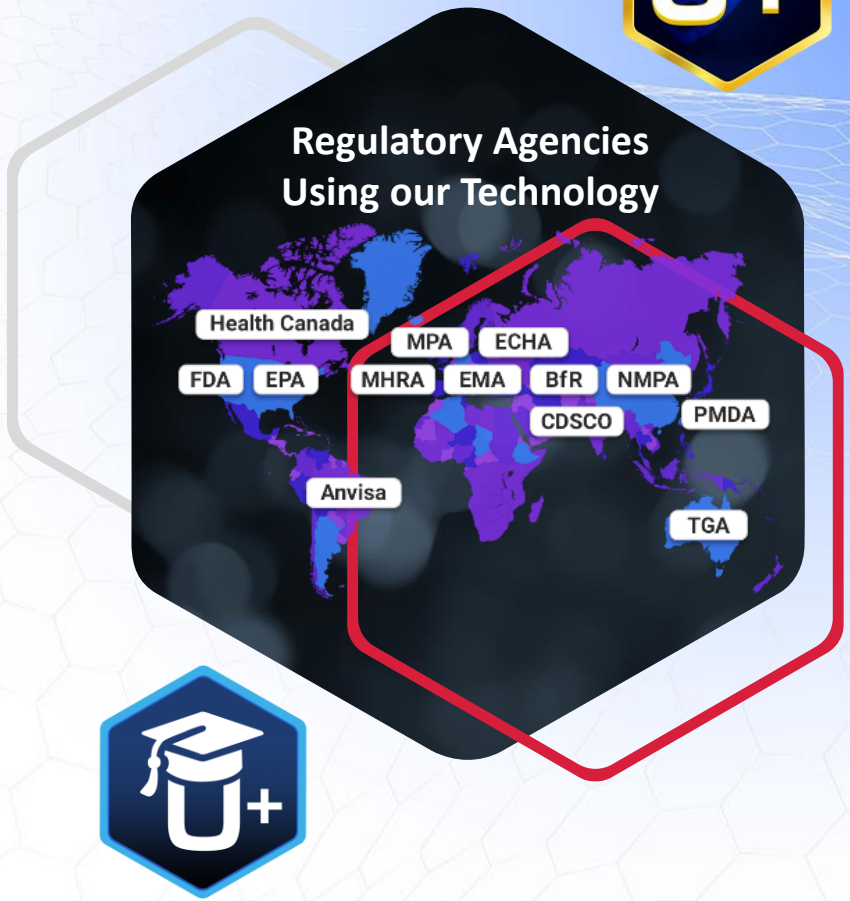
Meeting Agenda

- Introduction to QSP/QST Modeling at Simulations Plus
- DILI-sim Initiative and DILIsym Software
- Clinical DILIsym Applications
- Liver Safety+

Who We Are

NASDAQ: SLP

- **Cheminformatics**
  Software & Services
- **Physiologically Based Pharmacokinetics (PBPK)**
  Software & Services
- **Clinical Pharmacology & Pharmacometrics (CPP)**
  Software & Services
- **Quantitative Systems Pharmacology (QSP)**
  Software & Services
- **Adaptive Learning & Insights (ALI)**
  Services
- **Medical Communications (MC)**
  Services



>280 Pharmaceutical, biotechnology, chemicals, cosmetics, & consumer goods companies in the U.S., Europe, Asia, and South America

250+ Employees Worldwide

>25 yrs. Established In 1996

SimulationsPlus

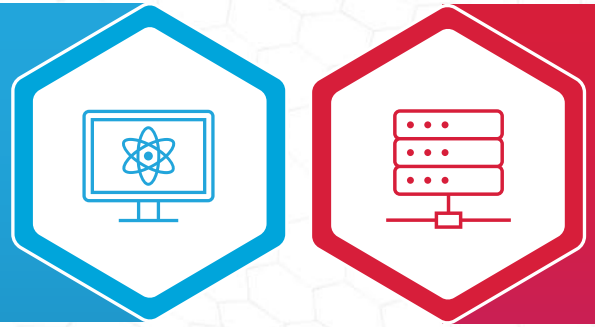
QSP/QST Modeling at Simulations Plus Is A Multi-Disciplinary Effort

- Biochemistry
- Biomarkers
- Clinical
- Computational Capability
- Engineering
- Mathematics
- Pathophysiology
- Pharmacology
- Physiology
- Software Engineering

- Simulations Plus QSP group's technical staff includes 35 modelers
- Expertise in pathophysiological, clinical, and pharmacologic aspects of treating numerous diseases
- Wide array of training and backgrounds coming together to achieve multidisciplinary goals
- Tremendous amount of collective QSP modeling experience including several original pioneers of the discipline
- Superlative communication skills across team
- Emphasis on collaborative approach

Highlights of Simulations Plus QSP Model Development and Use

Developed and used QSP models >20 years
- Helped establish industry-standard methodology for QSP modeling
- Available via licensing and/or services

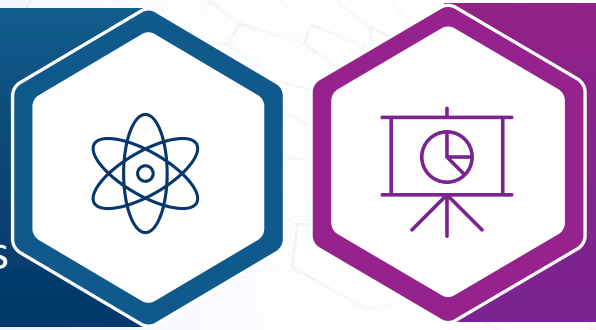


Supported key decisions across pharma pipelines
- Optimization of dosing paradigms for clinical trials
- Go/no-go decisions
- Identification of optimal treatment replacement/addition protocols

Versatile QSP modelers that have used multiple software platforms
- Thales developed in-house as state-of-the-art QSP modeling software

Simulations used to identify primary mechanistic drivers of observed responses to drug administration
- Enabled focus on key mechanisms contributing to efficacy

Simulated >250 compounds in >25 disease and toxicity areas
- Predicted efficacy for monotherapy or various combination treatment paradigms

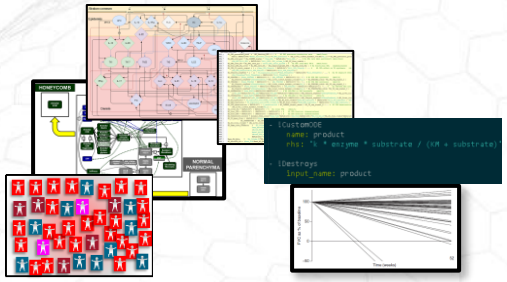


Simulation results included in communications with regulators >60 times to date
- Includes examples related to both toxicity and efficacy

Simulations Plus Has Expertise and Experience to Develop QSP Models to Predict Efficacy Across Therapeutic Areas

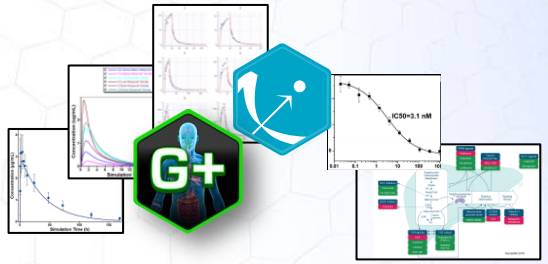
Identify therapeutic area and disease

- Collaborative with project sponsors
- Oncology, fibrosis, neurology, autoimmune, etc.



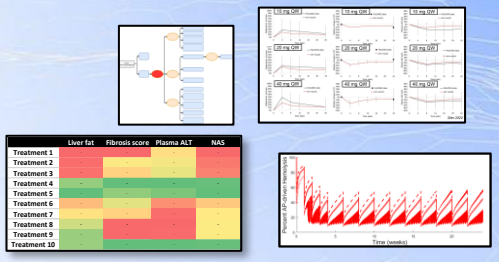
Develop QSP Model

- Summarize key biochemical and clinical data
- Capture key pathophysiological processes and ontology with equations
- Relevant clinical outputs
- Develop SimPops
- Simulate SOC and competitor treatments



Represent novel treatments

- Pharmacokinetics (GastroPlus, Monolix)
- Pharmacodynamics
- Mechanism of action



Predict efficacy of novel treatment

- Optimize clinical trial protocols
- Optimize dosing regimens
- Combination with other treatments
- Efficacy comparison vs. other treatments
- Confirm in vivo drug MoA
- Identify characteristics of responders vs. non-responders

Simulations Plus Has A Growing Library of Existing QSP and QST Models to Address Your Questions

QST: Liver and Kidney Safety

- Drug induced acute kidney injury
- Drug induced liver injury (DILI)

QSP: Metabolic Diseases

- Non-alcoholic fatty liver disease / steatohepatitis (NAFLD/NASH or MASH)
- Obesity

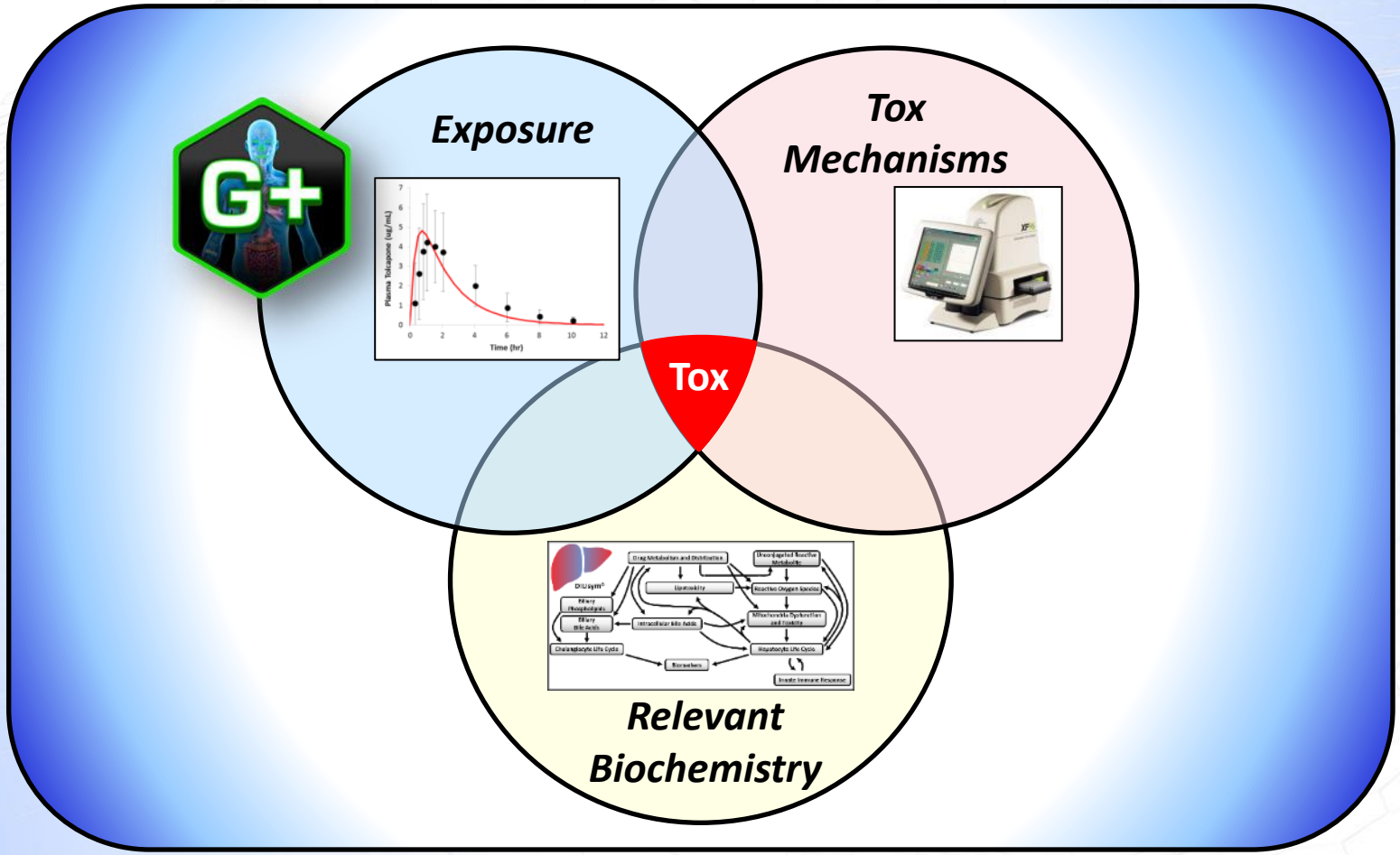
QSP: Immuno-Oncology

- Acute myeloid leukemia (AML)
- Diffuse large B-cell lymphoma (DLBCL)
- Multiple myeloma (MM)
- Myelofibrosis
- Solid tumor (NSCLC, melanoma, prostate cancer, colorectal cancer, ovarian cancer, endometrial cancer)

QSP: Inflammatory, Autoimmune, and Fibrotic Diseases

- Asthma/COPD (*in development*)
- Atopic dermatitis (AD)
- Crohn's disease (CD)
- Dermatomyositis
- Dysregulation of alternative and terminal pathways (AP, TP) of complement
- Idiopathic pulmonary fibrosis (IPF)
- Interstitial lung disease (ILD) associated with systemic sclerosis
- Multiple sclerosis (MS, *in development*)
- Psoriatic arthritis (PSA)
- Psoriasis (PSO)
- Rheumatoid arthritis (RA)
- Systemic lupus erythematosus (SLE including CLE)
- Ulcerative colitis (UC)
- Uric acid disposition in gout
- Wound healing after myocardial infarction (MI)

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



The QST Model DILIsym Provides More Comprehensive Predictions of DILI Risk than Artificial Intelligence Models

	DILIsym (QST model)	Artificial Intelligence models
Primary methodology	Predict DILI in SimPops based on PBPK predictions of hepatocellular drug (parent + metabolites) and primary cellular mechanisms of DILI	Predict DILI based on in vitro signals and correlations with known DILI-causing drugs
Based on compounds that do and do not cause DILI	DILIsym has been used to characterize compounds that do and do not have DILI liabilities, providing a balanced predictiveness	AI models are unlikely to include many (if any) negative controls because they are relying on database of clinical DILI cases
Mechanistic contributions as identified with in vitro assays	Compounds predicted to have DILI risk with DILIsym include contributions from multiple mechanisms, some of which are synergistic	AI models cannot account for synergistic, mechanistic interactions underlying DILI risk
Include liver to plasma ratio within predictions	DILIsym can be used to identify clinically relevant, safe dosing paradigms thanks to the inclusion of hepatocyte drug (parent + metabolites) concentrations in the predictions	AI models cannot account for differences in media and intracellular drug concentrations, where hepatocyte concentrations are frequently much greater than extracellular
Ability to identify susceptible patients	The use of SimPops to account for inter-patient variability and disease status provides ability to identify individuals potentially susceptible to DILI	AI models do not account for inter-patient differences or disease status
User to understand basis for predictions	DILIsym (and most QST models) provides the ability to quantify the contributions from various mechanisms at clinically relevant doses	AI models appear to be a black box to users, with limited to no ability to provide a mechanistic basis for predictions of DILI risk

Meeting Agenda

- Introduction to QSP/QST Modeling at Simulations Plus
- DILI-sim Initiative and DILIsym Software
- Clinical DILIsym Applications
- Liver Safety+

The DILI-sim and RENAsym Consortia are Partnerships Between DILIsym Services and Pharmaceutical Companies to Minimize Organ Injury

Excellent Scientific Advisory Boards

gsk GlaxoSmithKline

janssen

Merck KGaA
Darmstadt, Germany

GILEAD

DILIsym
RENAsym

SERVIER

abbvie

AstraZeneca

Current DILI-sim / RENAsym 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*
- ***At least 30 cases of use for regulatory purposes***
- ***Over 30 publications***

DILIsym on the Non-Clinical DILI Assessment List by FDA

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]

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
icology studies)	
	Liver enzyme, bilirubin elevations
logy	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

Computer or modeling-based (as available and/or upon request)

QST DILISym

SimulationsPlus

Known Use of DILIsym Simulation Results in Sponsor Communications with Regulatory Agencies

50+

Regulatory communications that included DILIsym simulation results

88

Percent of mechanistic liver injury projects

15

Percent of biomarker fitting projects, i.e., investigating underlying hepatocyte loss

7

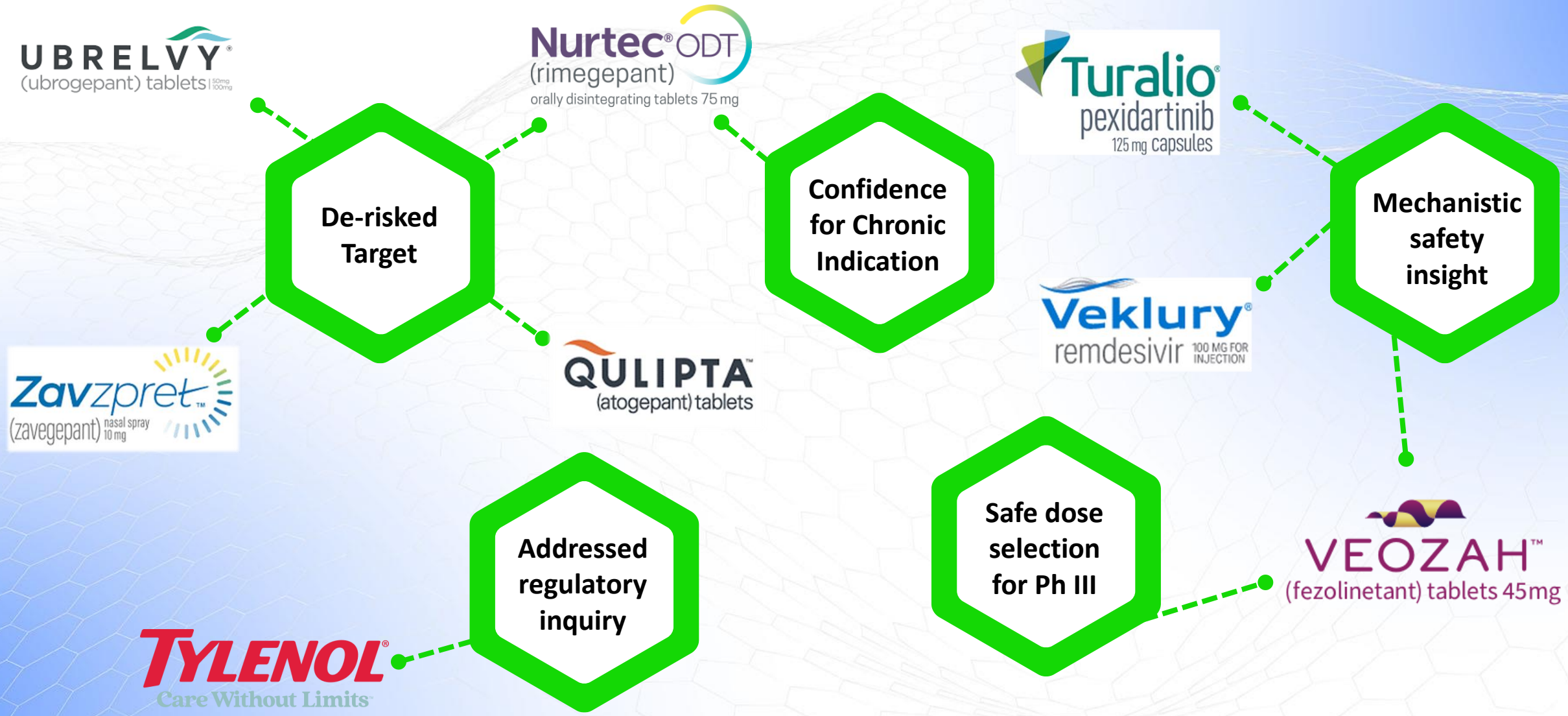
Instances in which DILIsym staff participated in presentation to regulatory agencies

5+

Distinct regulatory agencies

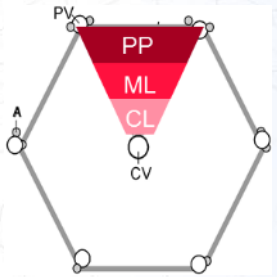
- *Use of simulation results in communications with regulators is generally governed by the sponsor, with imperfect visibility by the DILIsym team*
- *The following reflects our best understanding of their use*

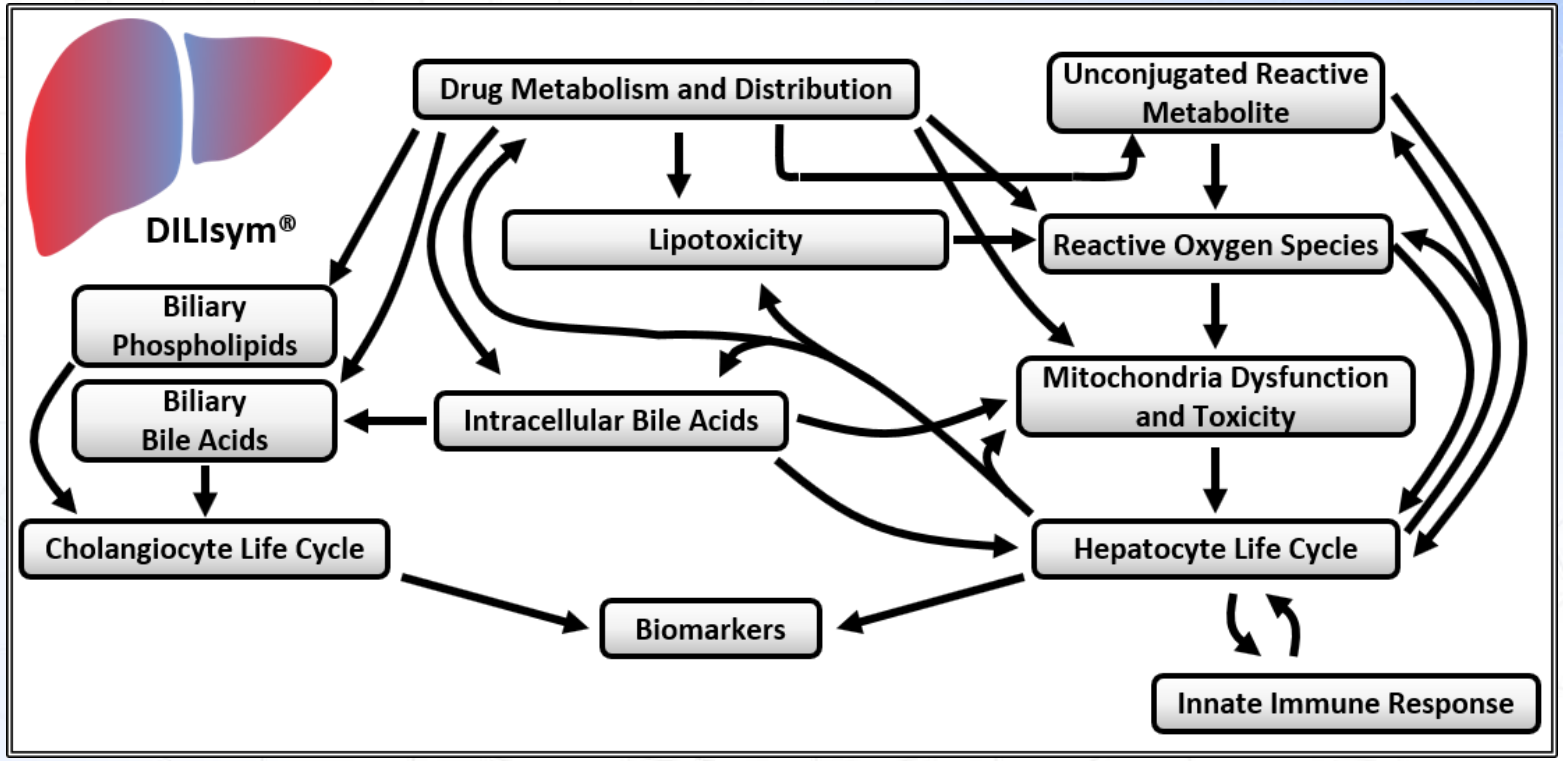
The Proof Is In The Approved Therapies – Liver Safety



DILIsym Software 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
- ~90 detailed representations of validation compounds with >80% success and **zero false positive predictions**
- Single and combination drug therapies





Your ACAT™/PBPK "Foundational" Model

Leverage your model!

Prediction of local and systemic exposure

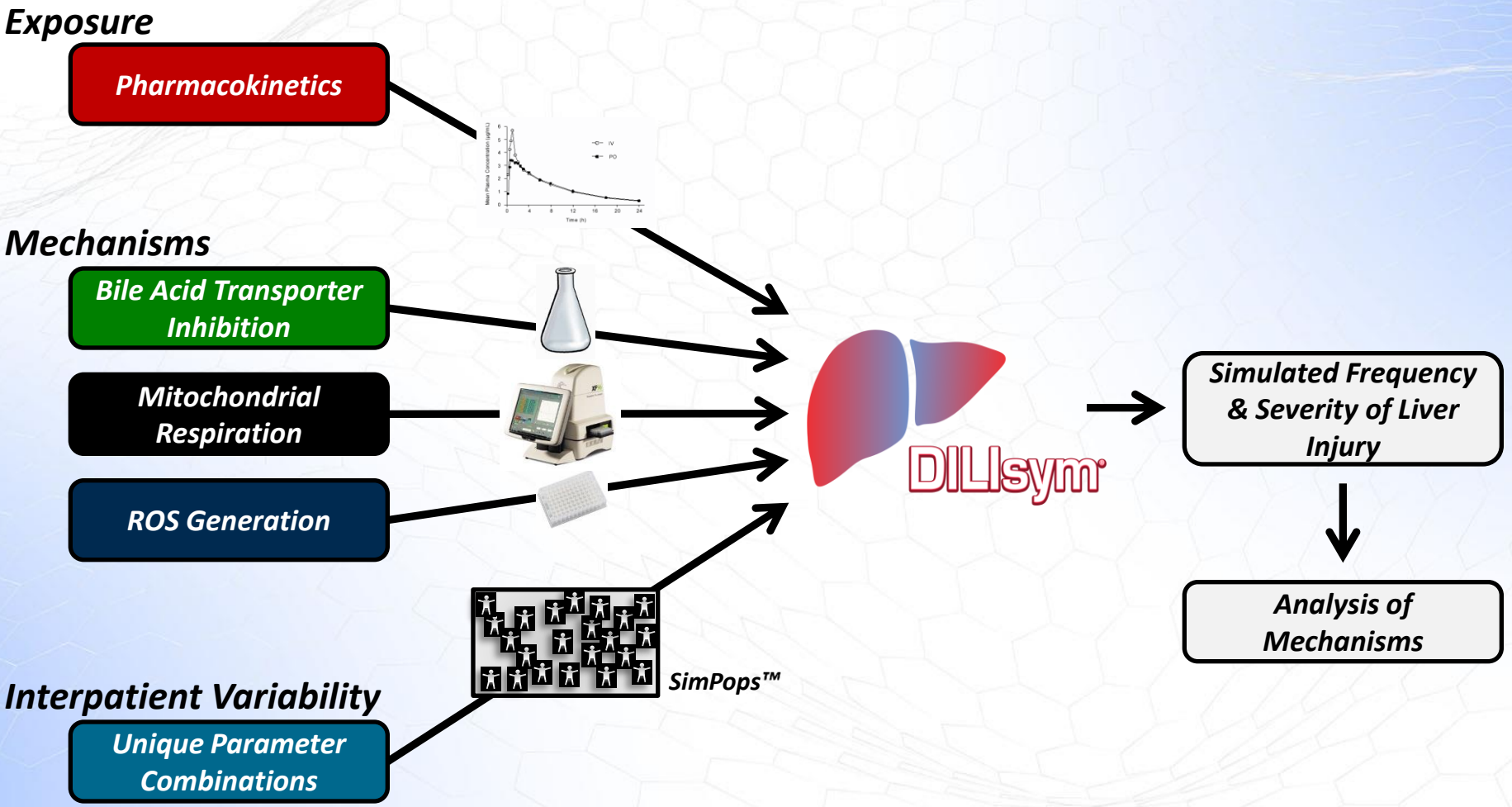
- FIH Predictions
- Drug-Drug Interactions
- Formulation variant investigation/optimization
- IVIVC and virtual BE
- Population predictions
- Pediatric predictions
- Food Effect (ARAs and PPIs)
- Additional dose route predictions
- Expand to other areas of safety such as kidney
- Predict efficacy and outcomes with QSP using PK predictions from G+

DILIsym®

PBPK Model Deliverables:

- GastroPlus® database .mdb (w/input) files
- Simulation output files

DILIsym Integrates Multiple Inputs to Simulate Hepatotoxicity



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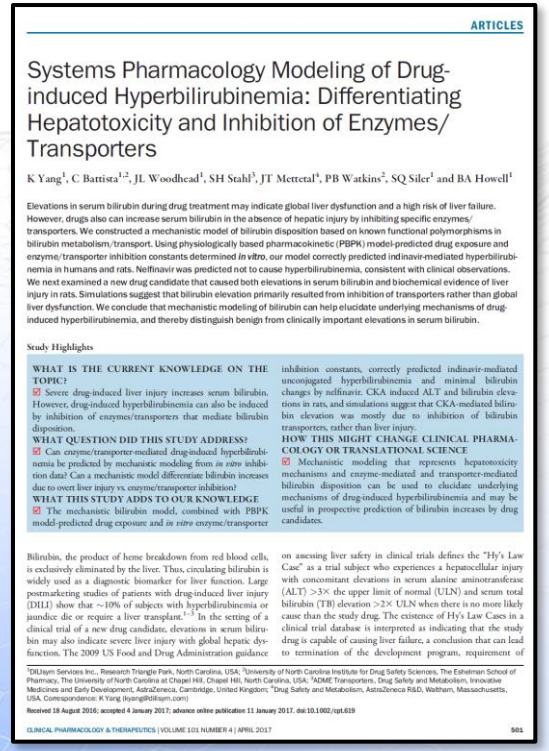
Mechanistic Predictions Utilize *In Vitro* Data on Bilirubin Transporters and Metabolism



ARTICLES

Systems Pharmacology Modeling of Drug-induced Hyperbilirubinemia: Differentiating Hepatotoxicity and Inhibition of Enzymes/Transporters

K Yang[1], C Battista[1,2], JL Woodhead[1], SH Stahl[3], JT Mettetal[4], PB Watkins[2], SQ Siler[1] and BA Howell[1]

Elevations in serum bilirubin during drug treatment may indicate global liver dysfunction and a high risk of liver failure. However, drugs also can increase serum bilirubin in the absence of hepatic injury by inhibiting specific enzymes/transporters. We constructed a mechanistic model of bilirubin disposition based on known functional polymorphisms in bilirubin metabolism/transport. Using physiologically based pharmacokinetic (PBPK) model-predicted drug exposure and enzyme/transporter inhibition constants determined *in vitro*, our model correctly predicted indinavir-mediated hyperbilirubinemia in humans and rats. Nelfinavir was predicted not to cause hyperbilirubinemia, consistent with clinical observations. We next examined a new drug candidate that caused both elevations in serum bilirubin and biochemical evidence of liver injury in rats. Simulations suggest that bilirubin elevation primarily resulted from inhibition of transporters rather than global liver dysfunction. We conclude that mechanistic modeling of bilirubin can help elucidate underlying mechanisms of drug-induced hyperbilirubinemia, and thereby distinguish benign from clinically important elevations in serum bilirubin.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Severe drug-induced liver injury increases serum bilirubin. However, drug-induced hyperbilirubinemia can also be induced by inhibition of enzymes/transporters that mediate bilirubin disposition.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Can enzyme/transporter-mediated drug-induced hyperbilirubinemia be predicted by mechanistic modeling from *in vitro* inhibition data? Can a mechanistic model differentiate bilirubin increases due to overt liver injury vs. enzyme/transporter inhibition?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
☑ The mechanistic bilirubin model, combined with PBPK model-predicted drug exposure and *in vitro* enzyme/transporter inhibition constants, correctly predicted indinavir-mediated unconjugated hyperbilirubinemia and minimal bilirubin changes by nelfinavir. CKA induced ALT and bilirubin elevations in rats, and simulations suggest that CKA-mediated bilirubin elevation was mostly due to inhibition of bilirubin transporters, rather than liver injury.

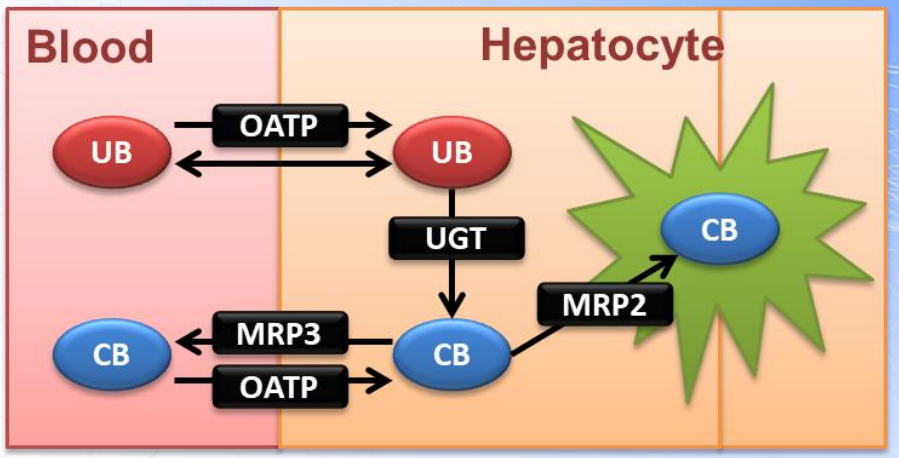
HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
☑ Mechanistic modeling that represents hepatotoxicity mechanisms and enzyme-mediated and transporter-mediated bilirubin disposition can be used to elucidate underlying mechanisms of drug-induced hyperbilirubinemia and may be useful in prospective prediction of bilirubin increases by drug candidates.

Bilirubin, the product of heme breakdown from red blood cells, is exclusively eliminated by the liver. Thus, circulating bilirubin is widely used as a diagnostic biomarker for liver function. Large postmarketing studies of patients with drug-induced liver injury (DILI) show that ~10% of subjects with hyperbilirubinemia or jaundice die or require a liver transplant.[1–3] In the setting of a clinical trial of a new drug candidate, elevations in serum bilirubin may also indicate severe liver injury with global hepatic dysfunction. The 2009 US Food and Drug Administration guidance on assessing liver safety in clinical trials defines the "Hy's Law Case" as a trial subject who experiences a hepatocellular injury with concomitant elevations in serum alanine aminotransferase (ALT) >3× the upper limit of normal (ULN) and serum total bilirubin (TB) elevation >2× ULN when there is no more likely cause than the study drug. The existence of Hy's Law Cases in a clinical trial database is interpreted as indicating that the study drug is capable of causing liver failure, a conclusion that can lead to termination of the development program, requirement of

[1]DILIsym Services Inc., Research Triangle Park, North Carolina, USA; [2]University of North Carolina Institute for Drug Safety Sciences, The Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [3]ADME Transporters, Drug Safety and Metabolism, Innovative Medicines and Early Development, AstraZeneca, Cambridge, United Kingdom; [4]Drug Safety and Metabolism, AstraZeneca R&D, Waltham, Massachusetts, USA. Correspondence: K Yang (kyang@dilisym.com)

Received 18 August 2016; accepted 4 January 2017; advance online publication 11 January 2017. doi:10.1002/cpt.619

- DILIsym represents major transporters and enzymes involved in bilirubin transport and metabolism (shown in figure to right)
- Experimental data may show inhibition potency for bilirubin transporters and enzymes
- Parameter values translated directly from the data will be used as IC_{50} values within DILIsym



Measure	DILIsym parameter value
OATP1B1 IC_{50} (µM)	TBD
OATP1B3 IC_{50} (µM)	TBD
MRP2 IC_{50} (µM)	TBD
MRP3 IC_{50} (µM)	TBD
UGT1A1 IC_{50} (µM)	TBD

SimulationsPlus

Biomarkers of Hepatocellular Function and Death Are Outputs of DILIsym

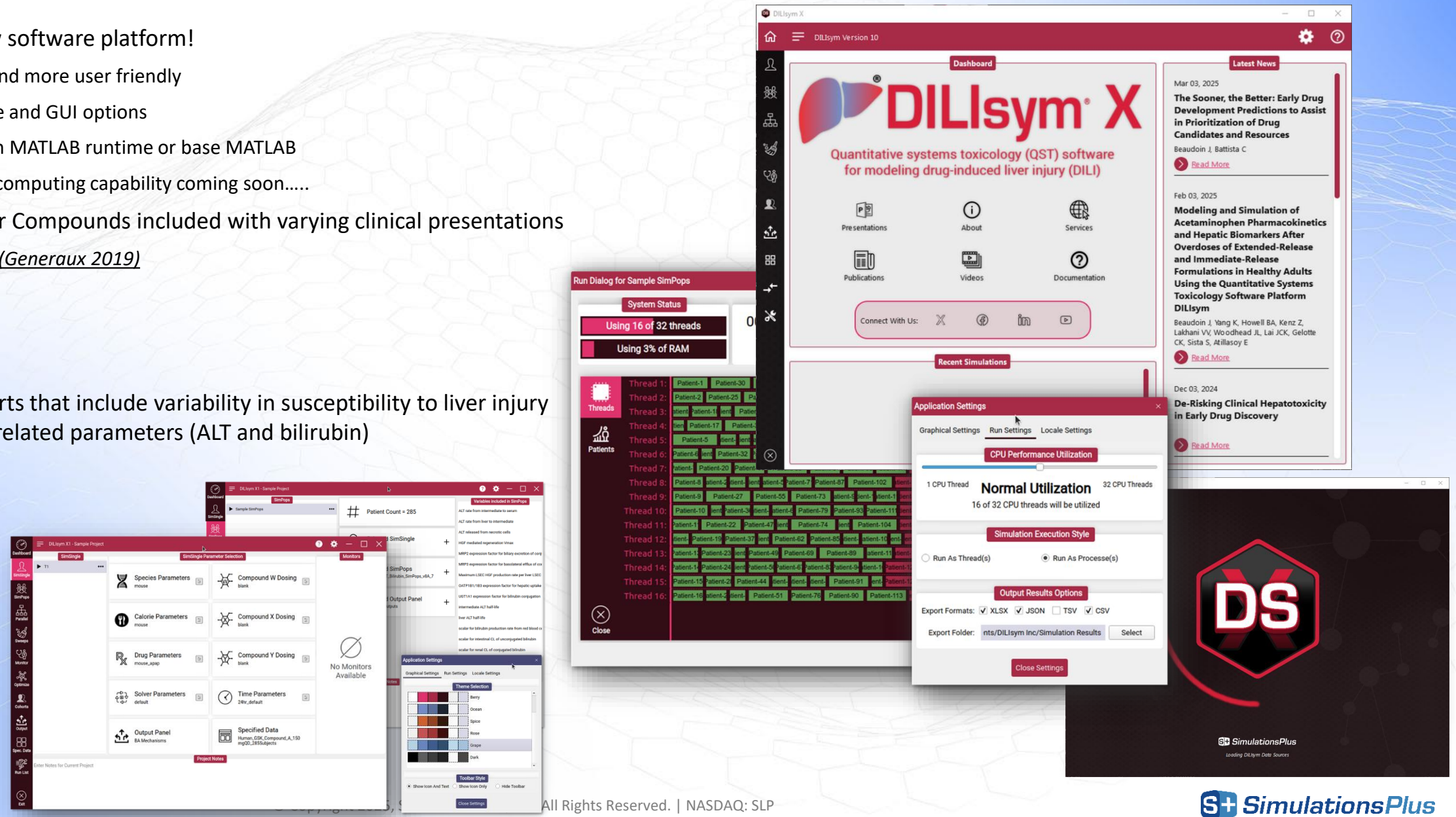
- Clinical biomarkers are outputs of DILIsym
  - Used for validation
  - Used for comparison with clinical and preclinical data
  - Functional, necrotic, and apoptotic indicators
- More biomarkers being added as data are becoming available
  - GLDH most recent addition
- Additional DILIsym outputs include:
  - Fraction of viable hepatocytes
  - Liver ATP
  - Liver glutathione
  - Circulating, liver, and excreted drug and metabolites
  - And more…..

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] (miR122)	**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, , [8]Yang *Tox Sci* 2012, [9]Murayama *Clin Chimica Acta 2008*, [10]Harrill *Clin Pharmacol Ther* 2011, [11]Church *Exp Biol Med* 2017, [12]Yang *Clin Pharmacol Ther* 2017

Highlights of DILIsym® Version X (DSX)

- Completely new software platform!
  - Much faster and more user friendly
  - Command line and GUI options
  - No reliance on MATLAB runtime or base MATLAB
  - Server/cloud computing capability coming soon…..
- 4 NEW exemplar Compounds included with varying clinical presentations
  - PF-04895162 *(Generaux 2019)*
  - Efavirenz
  - Anastrozole
  - Tamoxifen
- 2 New SimCohorts that include variability in susceptibility to liver injury and biomarker-related parameters (ALT and bilirubin)



SimulationsPlus

Coming Soon: DILIsym 11

DILIsym 11 Will Be Released in Q2 2025 with *Key Scientific Updates*

Mechanistic updates

- Adaptive immune exploration infrastructure
- NRF2-mediated adaptation to ROS
- NEW pediatrics representations
  - Four age groups representing infants, preschool children, school-aged children, and adolescents
- Cholestatic liver injury / bile acid updates

Exemplar compound updates

- 7 NEW exemplar compounds and updates
  - Immune exemplar: amodiaquine
  - MDR3 exemplars: chlorpheniramine, itraconazole, loratadine, verapamil
  - APAP immediate- and extended-release formulations

SimPops updates

- 7 NEW SimPops
  - Post-menopausal women
  - Pediatrics (4 age groups)
  - Adaptation (e.g., NRF2, biogenesis)

Troglitazone (TGZ) Hepatotoxicity Characterized by Largely Modest Transaminase Elevations and Delayed Presentation

- Analysis of N=17/291 (5.8%) of T2D patients on TGZ with transaminase elevations >1.5x ULN demonstrates delayed time to peak
  - N=15 patients with peak ALT, AST between 1.5x – 5x ULN
  - N=2 patients progressed to severe liver injury
  - N=6/291 (2.1%) with >3x ULN
- Another analysis of N=2510 patients receiving TGZ indicated ALT elevations >3x ULN in N=48 (1.9%) of patients
  - Onset was typically delayed (i.e., >1 mo)
  - Most patients w/ALT >3x ULN did not have symptoms of liver dysfunction
  - N=3/475 (0.6%) of placebo-controlled patients
  - Watkins 1998, PMID 9518284

Table 2. Clinical Outcomes for Subjects with Elevated Liver Enzymes

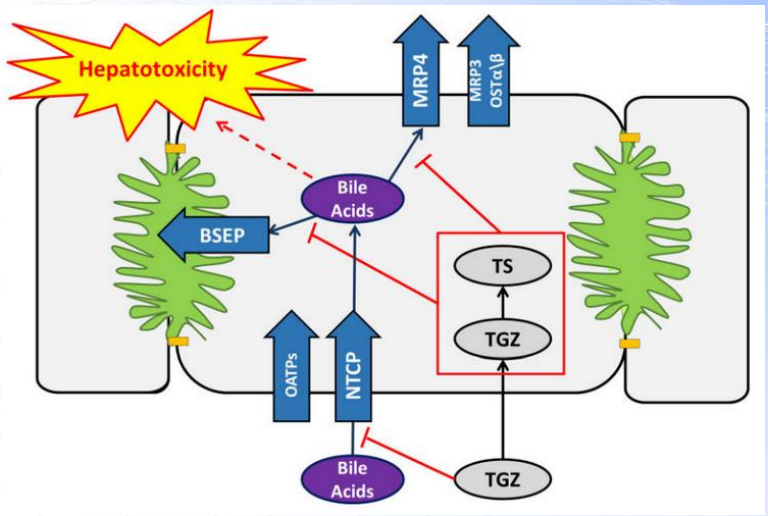
Sex	Age (yrs)	Time to Peak (mo)	Concurrent Statin[a]	Peak AST, ALT (IU/L)	Troglitazone Withdrawn	LFT Returned to WNL[b]
M	62	23.8		AST 89	Yes	Yes
M	54	3.8	Simvastatin	AST 167	Yes	Died[c]
F	73	4.7	Atorvastatin	ALT 239	Yes	Yes
F	54	28.5		AST 74	No	Yes
F	76	17.7		AST 64	Yes	Yes
F	67	20.6	Atorvastatin	AST 72	No	Yes
F	63	19.3		AST 66	No	Yes
M	50	2.2		AST 67	No	Yes
M	73	17.2	Atorvastatin	AST 91	No	Yes
F	60	19.6	Atorvastatin	ALT 2882	Yes	Yes
F	59	18.4	Atorvastatin	AST 99	No	Died[d]
F	52	14.5		AST 64	Yes	No[e]
F	75	11.5	Atorvastatin	AST 147	No	Yes
F	64	7.4		ALT 171	Yes	Yes
F	64	4.2		AST 65	No	Yes
M	69	3.7	Fluvastatin	AST 5915	Yes	Yes
F	43	8.8		AST 65	Yes	Unknown[f]

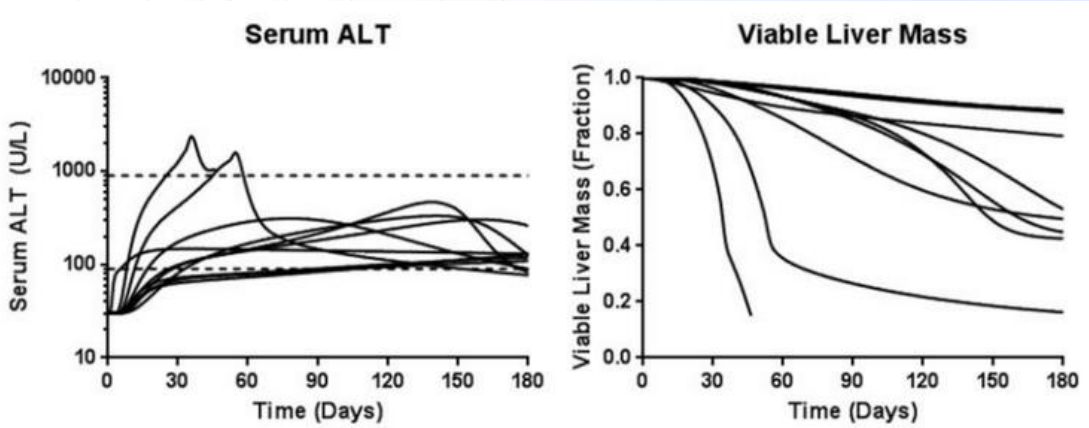
AST = aspartate aminotransferase; ALT = alanine aminotransferase; WNL = within normal limits.
[a]Potentially hepatotoxic concurrent agents.
[b]ALT and AST returned to ≤ 1.5 times upper limit of normal.
[c]Patient died from sepsis secondary to a testicular abscess.
[d]Patient died from ventricular fibrillation secondary to renal failure and hyperkalemia.
[e]Patient's AST remains mildly elevated at a most recent value of 61 IU/L.
[f]Patient was lost to follow-up.

St Peter 2001, PMID 11213855

TGZ Delayed Presentation can be Explained via Drug-Intrinsic Bile Acid Toxicity

- TGZ- and TGZ-sulfate-mediated inhibition of bile acid transport has been previously represented in DILIsym (Yang 2014)
- At common clinical doses (200-600 mg QD), simulated ALT >3x ULN was 0.3-5.1% consistent with clinical data (Watkins 1998)
  - A few Hy's law cases simulated consistent with relatively infrequent severe liver injury
- Simulation results suggested that delayed ALT elevations could be due to drug-intrinsic toxicity, independent of an adaptive immune response
- Nevertheless, the availability of TGZ in DILIsym provides an opportunity to evaluate liver injury assuming the presence of reactive T cells



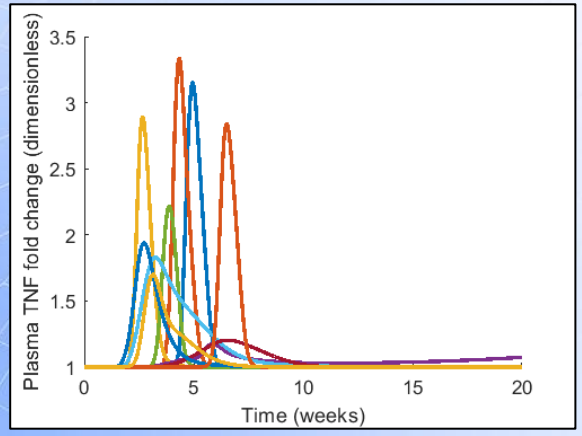


Yang 2014, PMID 25068506

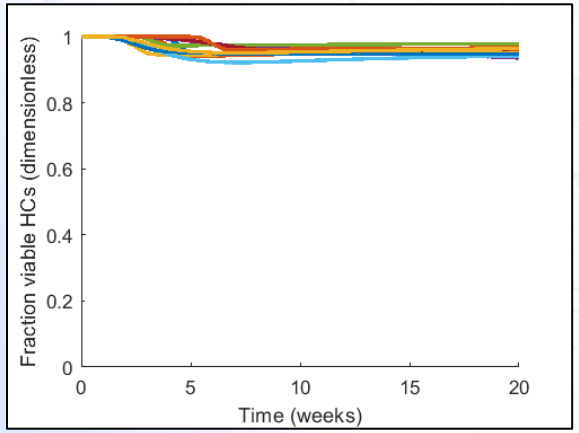
SimCohorts Identified with Relatively Mild and Transient Bile Acid Mediated ALT Elevations Following TGZ Treatment

- Simulated TGZ 300 mg QD in NHV SimPops
  - Simulated without adaptive immune sub-model
- SimCohorts (N=10) identified largely characterized by modest and transient TNF-α elevations, modest injury, and transient ALT elevations
- Mild liver injury accompanied by inflammation pre-disposes these individuals to adaptive immune responses within the liver

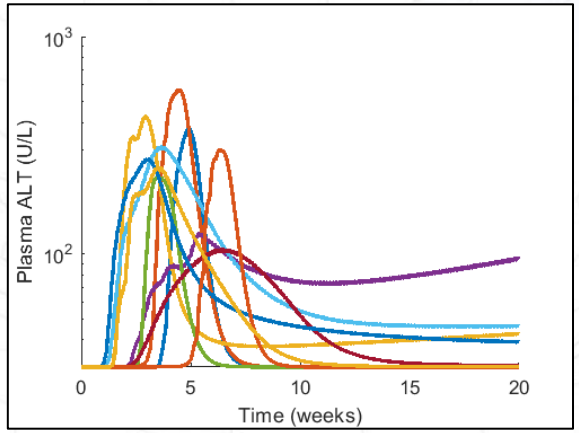
Plasma TNF



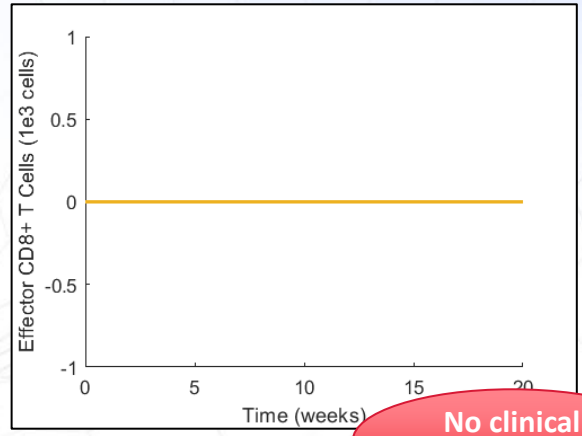
Frac Viable HCs



Plasma ALT



Effector CD8+ T Cells

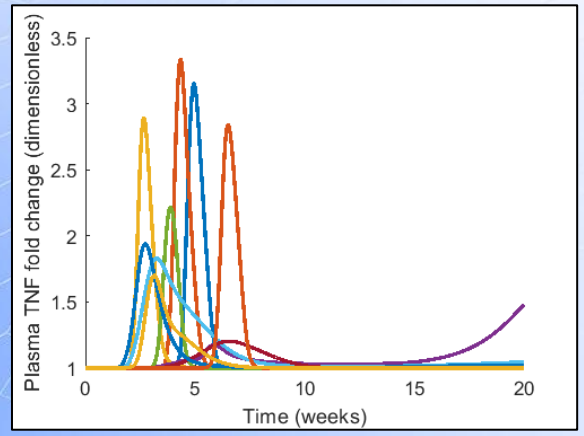


No clinical monitoring

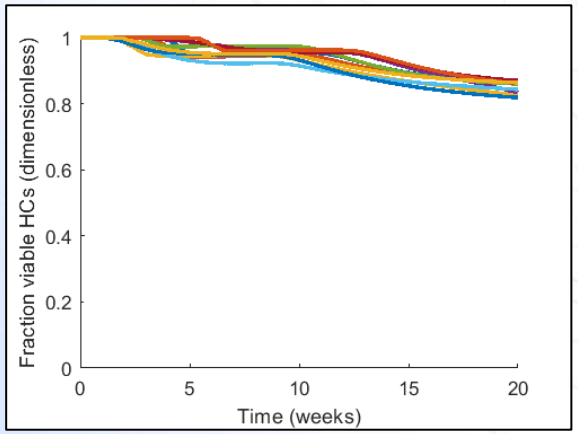
Initial Overlay of T cell Responses Drives Progression in Liver Injury and Additional Delayed ALT Elevations

- SimCohorts simulated with same TGZ dosing scheme with adaptive immune sub-model
  - Identical CD8+ T cell dynamics parameters for all individuals; parameters designed to give moderate contribution from adaptive immune response
- Individuals with early mild liver injury can exhibit delayed adaptive immune responses leading to further liver injury
  - Balance of CD8+ T cell dynamics and antigen presentation determine if an individual will progress to severe liver injury
  - For this parameter set, adaptive immune related cytotoxicity balances with T cell exhaustion and hepatocyte proliferation
- Different questions can be assessed with different simulation approaches
  - Multiple drug-intrinsic responses with single T cell parameterization can be used to understand how drug-intrinsic and body mass characteristics can impact potential adaptive immune responses
  - Single drug-intrinsic response simulated with T cell SimPops can be used to evaluate potential magnitude and dynamics of an adaptive immune response

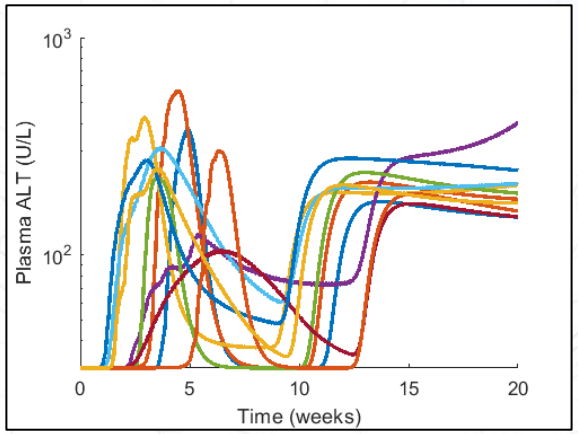
Plasma TNF



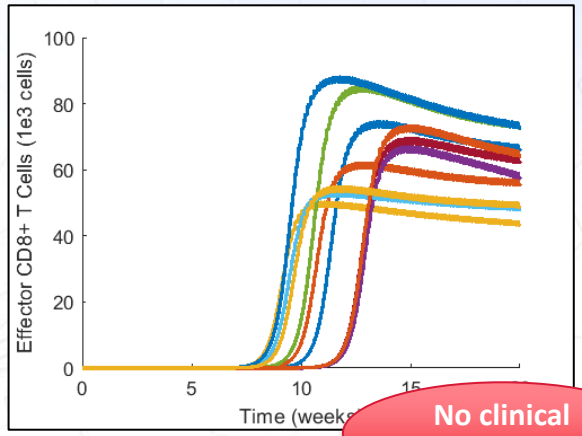
Frac Viable HCs



Plasma ALT



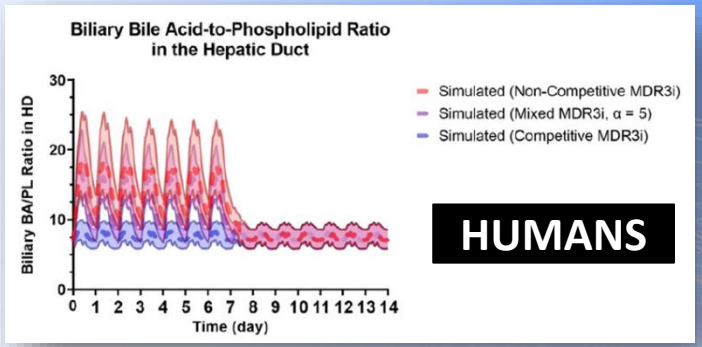
Effector CD8+ T Cells

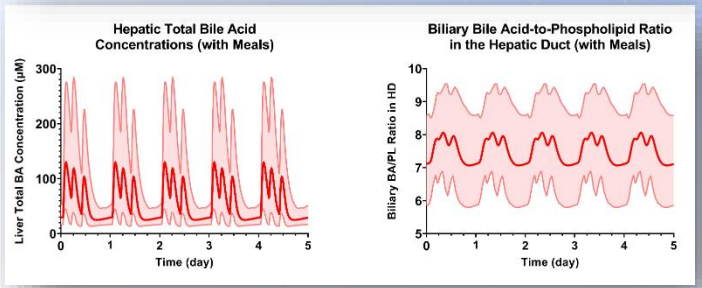


No clinical monitoring

New Bile Acid and Phospholipid Submodels and SimPops Included in DILIsym 11

- Bile acid (BA) and phospholipid (PL) submodels have been updated with aspects relevant to cholestatic liver injury:
  - (1) Cholehepatic shunting of BAs
  - (2) Biliary HCO_3^- secretion and its impact on:
    - Bile flow
    - BA shunting
    - Cholangiocyte toxicity
  - (3) Different modes of MDR3* inhibition
  - (4) Non-MDR3-mediated PL efflux
  - (5) Cholangiocyte regeneration
- New SimPops that represents variability in both BA toxicity and cholestasis mechanisms has been developed
- Open access manuscript describing the extended BA and PL submodels within DILIsym has recently been published



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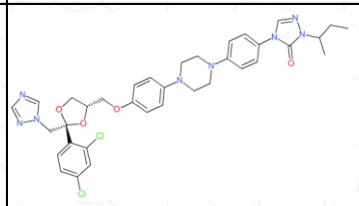
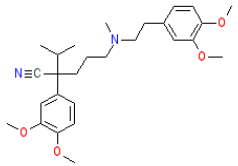
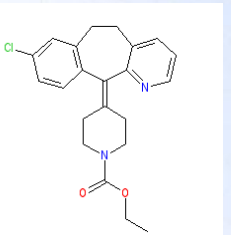
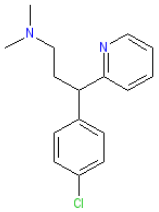
OPEN ACCESS

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*]

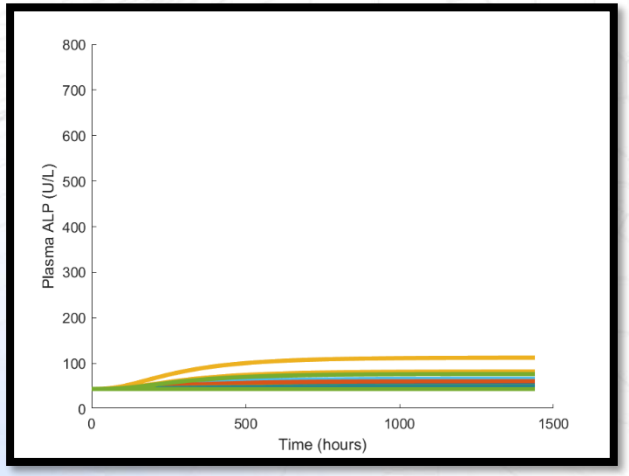
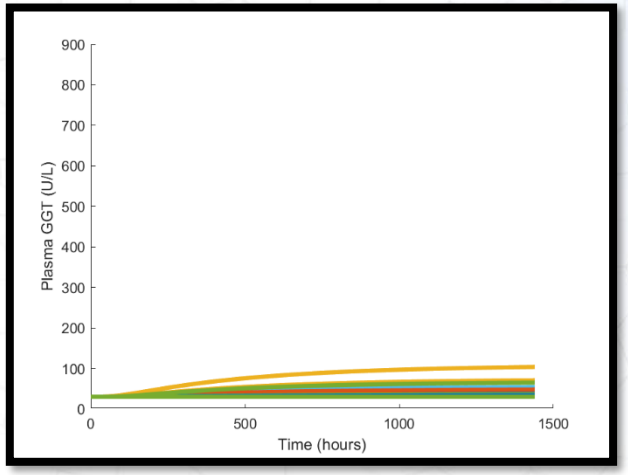
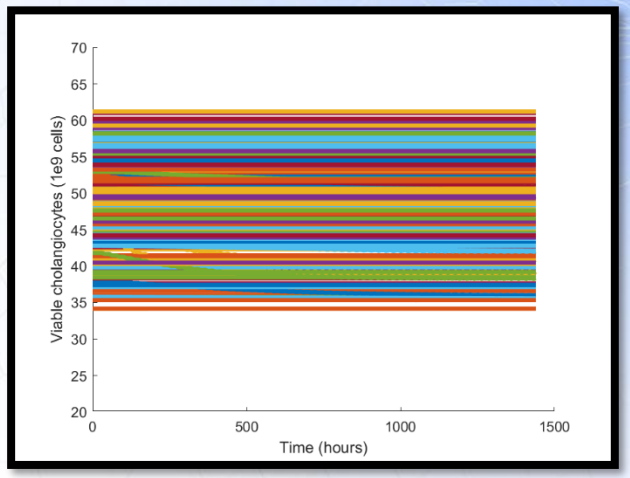
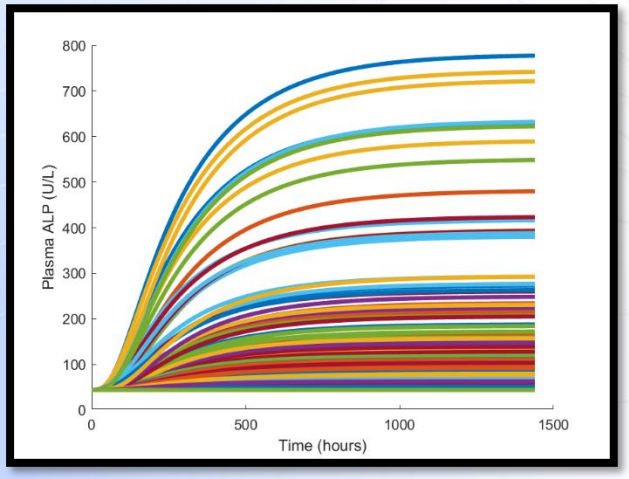
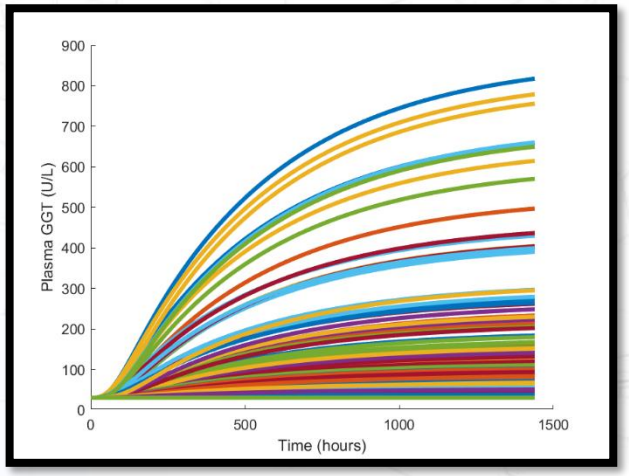
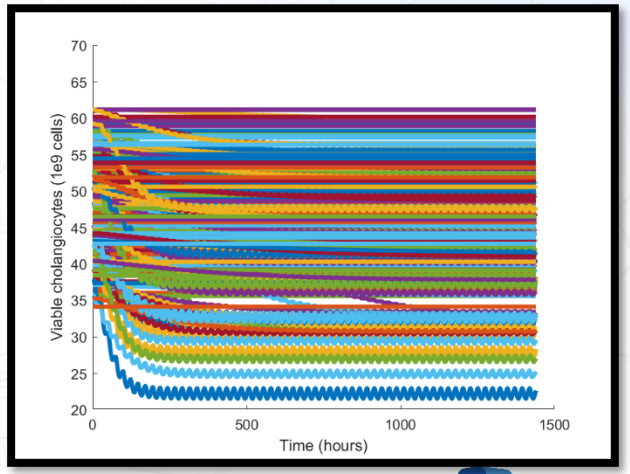
MDR3: Multidrug Resistance Protein 3, a PL floppase on the canalicular membrane of hepatocytes often implicated in cholestatic hepatotoxicity

Properties of Selected MDR3 Inhibitors With and Without Cholestatic DILI Liability

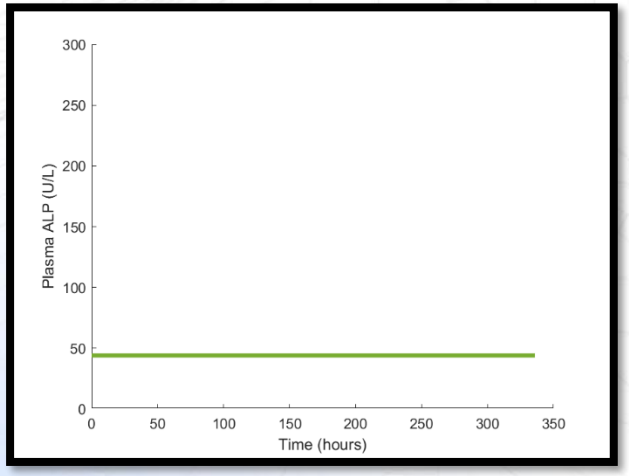
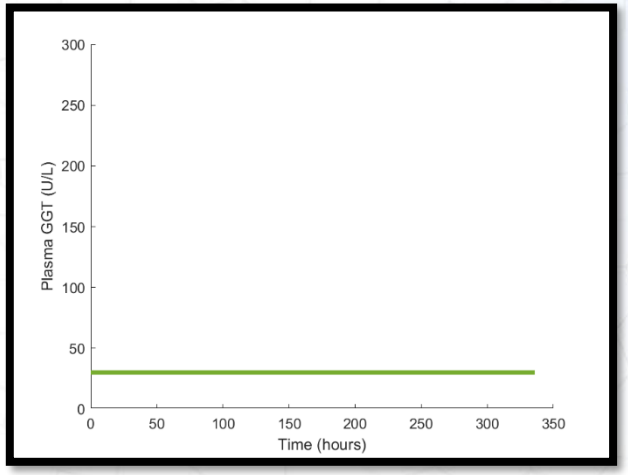
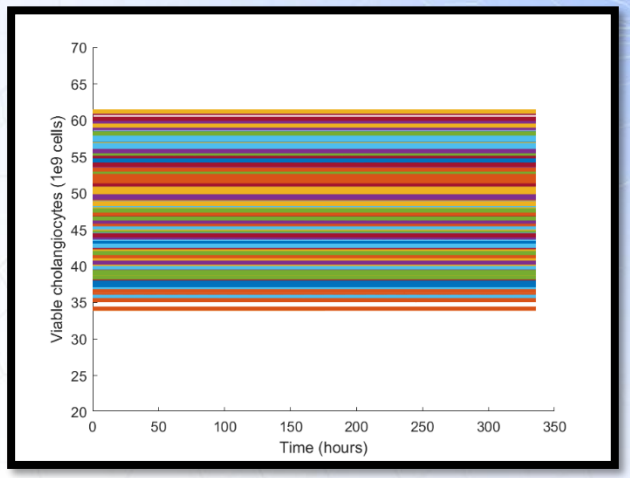
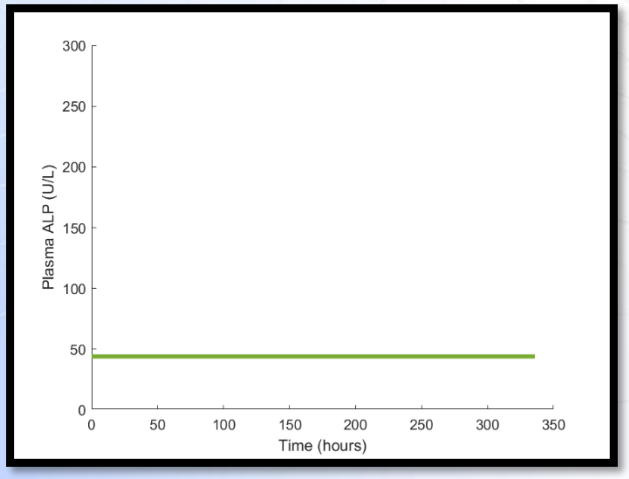
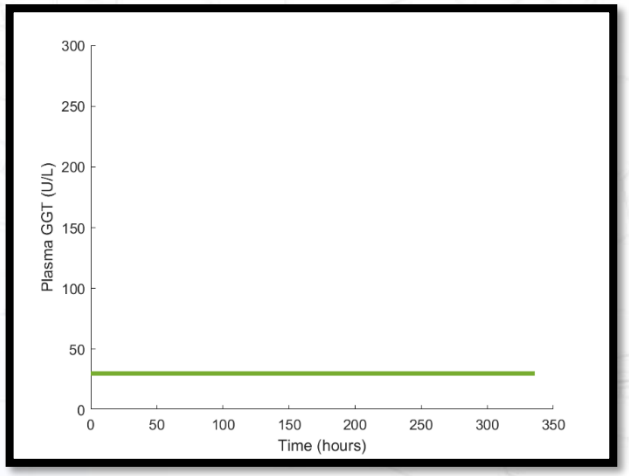
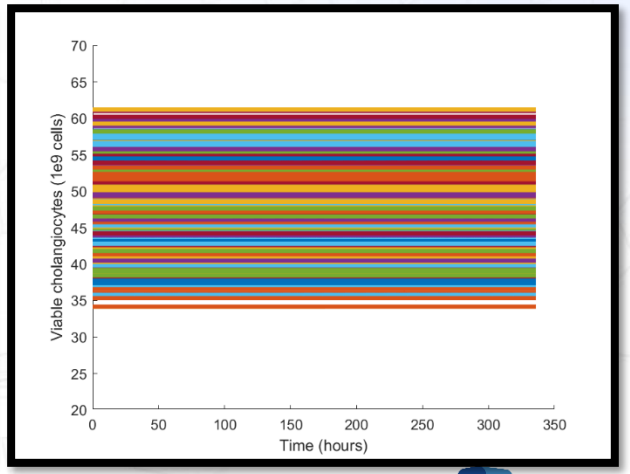
Parameter	Itraconazole	Verapamil	Loratadine	Chlorpheniramine
Chemical structure				
Molecular weight	705.65	454.61	382.89	274.8
B/P	0.58	1.10	0.78	0.9
fu_p (%)	0.03	7.3	3.0	29.1
Predicted $K_{p,liver}$ (used in the final model)	5.94	16.59	6.06	8.17
Experimental $K_{p,liver}$ previously reported in the literature	2 – 3 (Sporanox® package insert); 3 (mouse)	7 – 9 (human); 7 (rat); 10 (mouse)	NR	3.5 (rabbit)
BSEP IC50 (uM)	3	178.9	29	N/A
MDR3 IC50 (uM)	2.1 or 0.17	6.3	3	15
Dosing regimen used in DILIsym simulations	200 mg BID	222 mg/d	10 mg/d	4 mg QID
Clinically reported cholestatic DILI liability?	Yes (Most DILI concern)	Yes (Less DILI concern)	No	No

- In the compound-specific PBPK models developed in GastroPlus, only the parent compound has been explicitly represented
- In the DILIsym hepatotoxicity simulations, MDR3 and BSEP inhibition was attributed to parent compound only
- Mode of BSEP inhibition was assumed to be mixed (α=5), while both mixed and non-competitive inhibition of MDR3 were simulated
- Cholestatic pattern of liver injury is characterized by injury to cholangiocytes
  - ALP > 2x ULN in combination with a major elevation of GGT and ALT/ALP (fold ULN) < 2; ALP normal range: 44-147 U/L
  - According to the FDA Guidance for Industry, **ALP > 1.5x ULN** should be reported during premarketing clinical evaluation of DILI

Verapamil (222 mg/d) Simulations with Non-Competitive MDR3 Inhibition Predicted Cholestatic Liver Injury in the New Healthy SimPops

MDR3 Ki: 6.3 uM	Plasma ALP (U/L)	Plasma GGT (U/L)	Total Viable Cholangiocytes (1e9 cells)
Mixed Inhibition (α = 5) (ALP > 1.5x ULN: 0%)			
Non-competitive Inhibition (ALP > 1.5x ULN: 9.5%)			

Loratadine (10 mg/d) Simulations in the New Healthy SimPops Did Not Predict Cholestatic Liver Injury

MDR3 Ki: 3 uM	Plasma ALP (U/L)	Plasma GGT (U/L)	Total Viable Cholangiocytes (1e9 cells)
Mixed Inhibition (α = 5) (ALP > 1.5x ULN: 0%)			
Non-competitive Inhibition (ALP > 1.5x ULN: 0%)			

Meeting Agenda

- Introduction to QSP/QST Modeling at Simulations Plus
- DILI-sim Initiative and DILIsym Software
- Clinical DILIsym Applications
- Liver Safety+

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TOXICOLOGICAL SCIENCES, 00(0), 2022, 1–9

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Advance Access Publication Date: 12 May 2022
Research article

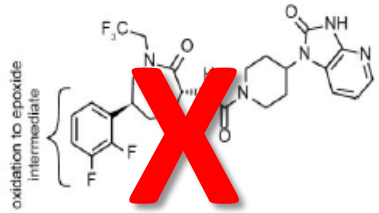
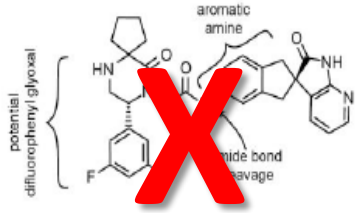
Comparing the Liver Safety Profiles of 4 Next-Generation CGRP Receptor Antagonists to the Hepatotoxic CGRP Inhibitor Telcagepant Using Quantitative Systems Toxicology Modeling

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

[*]DILIsym Services, Inc., A Simulations Plus Company, Research Triangle Park, North Carolina 27706, USA; [†]Institute for Drug Safety Sciences, UNC-Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, USA; and [‡]Biohaven Pharmaceuticals, Inc., New Haven, Connecticut 06510, USA

[1]To whom correspondence should be addressed at DILIsym Services, Inc., A Simulations Plus Company, 6 Davis Drive, Research Triangle Park, NC 27709, USA. E-mail: jeff.woodhead@simulations-plus.com.

Calcitonin Gene-related Peptide (CGRP) Receptor Antagonists for Treatment of Migraines

Parameter	Telcagepant[a]	MK-3207[b]
Structure[d]		
Potency IC_{50}[e]	2.2 nM	0.12 nM
Pivotal conventional nonclinical toxicology study liver findings	3M rat: <3 × ALT/AST with no liver histopathology at 15× exposure margin 6M rat: no liver safety signal at 7x margin 9M NHP: no liver safety signal at 7× margin 6M mouse: <2 × ALT/AST with no liver histopathology at 14× margin	6M rat: no liver safety signal at 25× exposure margin 9M NHP: no liver safety signal at 4× margin 6M mouse: no liver safety signal at 12× margin 1M dog: slight periportal vacuolation with <4 × ALT/AST associated with excessive body weight loss at 17x margin

Next-in-class Compounds

- Ubrogepant ?
- Rimegepant ?
- Atogepant ?
- Zavegepant ?

CGRP Receptor Antagonist Project Objectives

- Project undertaken when no large Phase 3 clinical trials had been reported for next-in-class compounds
  - Next-in-class representations were **purely predictive**
- Replicate the clinically observed toxicity for telcagepant
- Determine potential safety/toxicity of novel compounds rimegepant and zavegepant compared to telcagepant
  - Rimegepant has clinical exposure data; zavegepant has not been tested in humans
- Determine potential safety/toxicity of competitor compounds ubrogepant and atogepant
  - No clinical data available for either; representation based entirely on IVIVE

Data Used for CGRP Antagonist Compound Projects

PBPK Modeling

- **Compound Properties**
  - Tissue partition coefficients
- **Tissue penetration studies**
  - *Liver to blood ratio*
- **Pharmacokinetic data**
  - *Absorption, extra-hepatic clearance, metabolites*
- ***in vitro* data**
  - *Metabolite synthesis, active uptake*

Data Collected for Quantitative DILI Mechanism Info

- **Oxidative stress** *(high content imaging)*
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity** *(XF Analyzer)*
  - *ETC inhibition*
  - *Uncoupling*
- **Bile acid / phospholipid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP, MDR3*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

- **Telcagepant**: published clinical PK data
- **Rimegepant**: internal clinical PK data available
- **Zavegepant**: ADMET Predictor informed by animal data and intra-nasal dosing route
- **Atogepant**: pure IVIVE using ADMET Predictor
- **Ubrogepant**: pure IVIVE using ADMET Predictor

- Full in vitro data set collected for all five compounds

CGRP Antagonist Compound Simulation Results

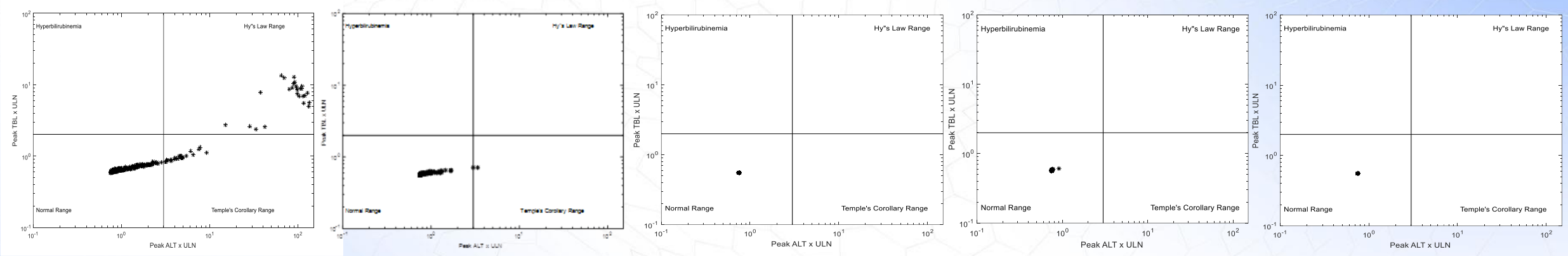
Telcagepant; 140 mg BID, 12 weeks, high ETCi

Rimegepant; 75 mg QD, alternate day dosing, 14 total doses over 28 days

Zavegepant; 20 mg IN or 750 mg PO or 7.5 mg IV, 25 straight days

Atogepant; 60 mg BID, 12 weeks

Ubrogepant; 100 mg QD, 25 straight days



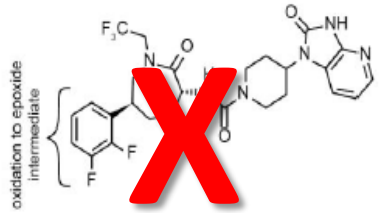
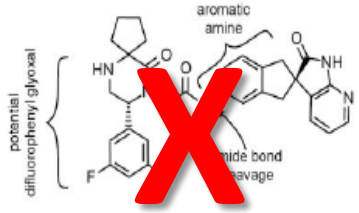
- Telcagepant toxicity correctly predicted by DILIsym
- Rimegepant predicted to be safe at clinical doses, with mild ALT elevations occurring only in extreme dosing conditions
- Zavegepant, atogepant, and ubrogepant all predicted to be safe with substantial safety margins

CGRP Antagonist Compound Simulation Results

- Mechanistic results predict difference between telcagepant and other CGRP antagonist compounds
  - Telcagepant toxicity predicted to be due to mixed-mode bile acid transporter inhibition and mitochondrial ETC inhibition
  - Other compounds' signals generally due to other mechanisms

Compound	Oral Dosing Protocol	Simulated* ALT > 3X ULN	Observed ALT > 3X ULN in Clinic
Telcagepant – Original ETC	140 mg BID, 12 weeks	17.5% (50/285)	1.9% (5/263)
	280 mg BID, 12 weeks	76.1% (217/285)	3.2% (8/265)
Telcagepant – Alternate ETC	140 mg BID, 12 weeks	0.0% (0/285)	1.9% (5/263)
	280 mg BID, 12 weeks	7.72% (22/285)	3.2% (8/265)
Rimegepant	75 mg QD, alternate day dosing, 14 total doses	0.35% (1/285)	--
	75 mg QD, 5 days on, 1 day off, 25 total doses	0.7% (2/285)	--
	75 mg QD, daily dosing for 25 days, 25 total doses	1% (3/285)	--
Zavegepant	750 mg oral QD, 25 days, 25 total doses	0.0% (0/285)	
	7.5 mg IV QD, 25 days, 25 total doses	0.0% (0/285)	
Atogepant	60 mg BID, 12 weeks	0% (0/285)	
	300 mg BID, 12 weeks	0.3% (1/285)	
	600 mg BID, 12 weeks	10.2% (29/285)	
Ubrogepant	100 mg QD, 25 days	0% (0/285)	
	500 mg QD, 25 days	1.4% (4/285)	
	1000 mg QD, 25 days	11.6% (33/285)	

Calcitonin Gene-related Peptide (CGRP) Antagonists for Treatment of Migraines

Parameter	Telcagepant[a]	MK-3207[b]
Structure[d]		
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Next-in-class Compounds

- Ubrogepant ✓
- Rimegepant ✓
- Atogepant ✓
- Zavegepant ✓

FDA Approval Achieved for Rimegepant, Ubrogepant, Atogepant, and Zavegepant

FDA APPROVES BIOHAVEN'S NURTEC® ODT (RIMEGEPANT) FOR PREVENTION: NOW THE FIRST AND ONLY MIGRAINE MEDICATION FOR BOTH ACUTE AND PREVENTIVE TREATMENT

Nurtec® ODT (rimegepant) orally disintegrating tablets 75 mg

Allergan Receives U.S. FDA Approval for UBRELVY™ for the Acute Treatment of Migraine with or without Aura in Adults

UBRELVY® (ubrogepant) tablets 50mg 100mg

FDA Approves QULIPTA™ (atogepant), the First and Only Oral CGRP Receptor Antagonist Specifically Developed for the Preventive Treatment of Migraine

QULIPTA™ (atogepant) tablets

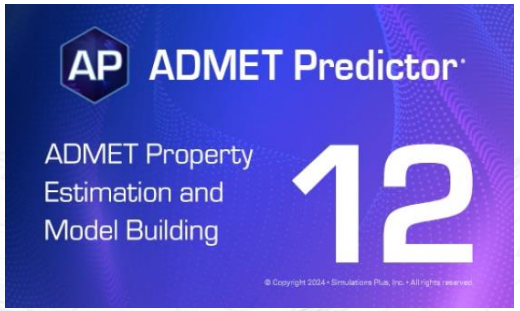
Pfizer's ZAVZPRET™ (zavegepant) Migraine Nasal Spray Receives FDA Approval

Zavzpret™ (zavegepant) nasal spray 10 mg

Meeting Agenda

- Introduction to QSP/QST Modeling at Simulations Plus
- DILI-sim Initiative and DILIsym Software
- Clinical DILIsym Applications
- Liver Safety+

Combination of QST and AI Provide Efficient, Understandable Assessment of Compound DILI Risk



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Liver Safety+

AI

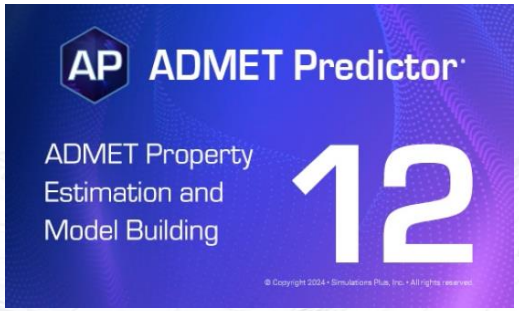
Predict mechanistic DILIsym Input Parameters from compound structure

QST

Predict mechanistic DILIsym Input Parameters from compound structure

Efficient DILI risk assessment, readily applied to preclinical compound screening

Simulations Plus Has Developed a Roadmap to Derive an Early Assessment of Hepatotoxic Risk



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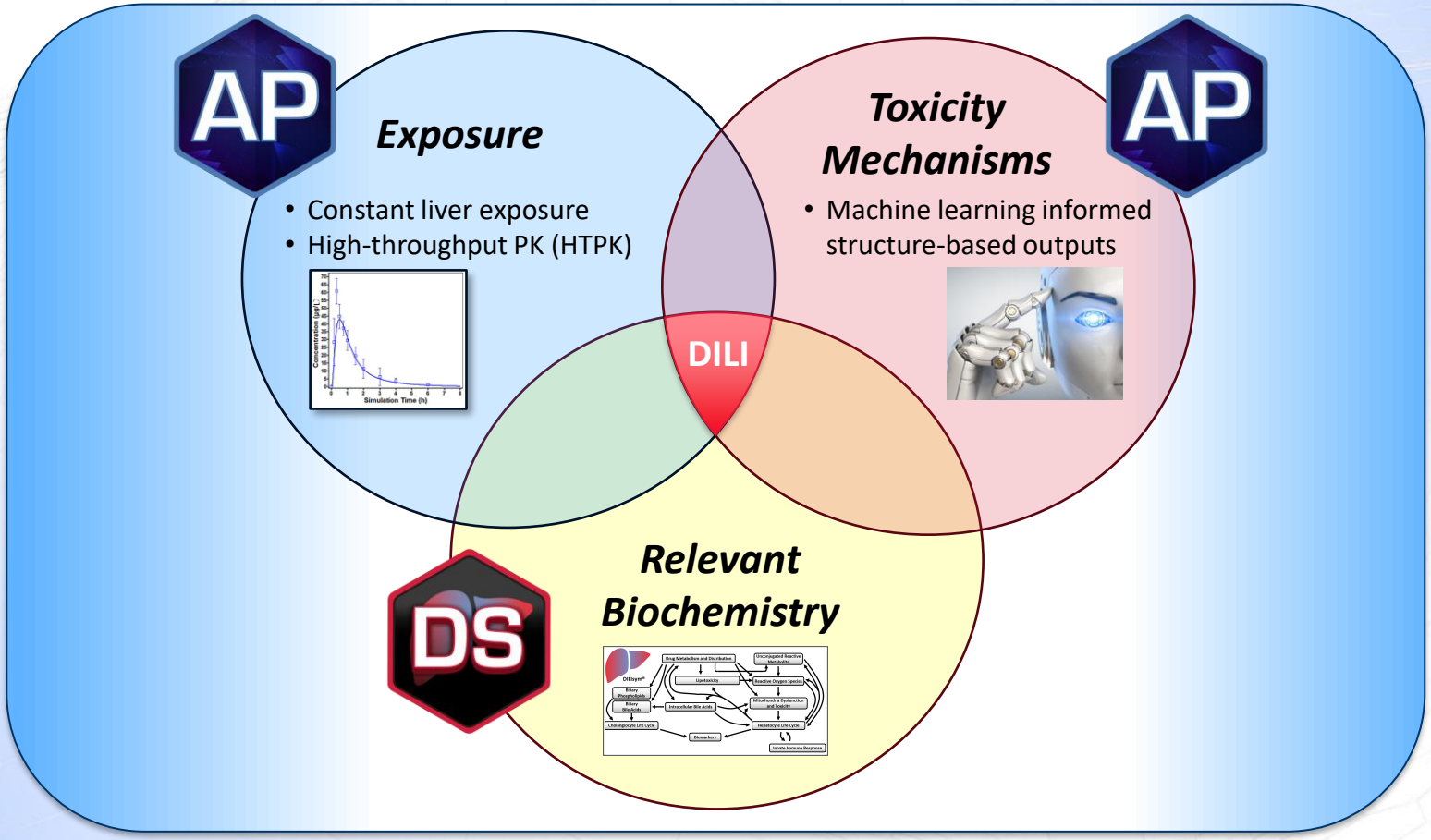
Liver Safety+

- New module in ADMET Predictor 12 generates outputs that can be used to inform inputs for DILIsym
  - Permissive of liver safety assessment during **early** drug discovery efforts!
  - Predictions of the current offering are qualitative
    - Yes/no toxicity mechanism classifications
    - Rank ordering of a compound's toxicity assessment with other in-class compounds
  - Accuracy and use of outputs will improve iteratively, as more data become available to inform predictions
- Workflow permissive for early discovery applications
  - No need for data from typical DILIsym *in vitro* assays
  - Leverages ADMET Predictor informed structure-based compound properties
  - Applies ADMET Predictor Machine Learning from a library of DILI/clean compounds
  - Use of constant liver exposure based on molar concentrations OR use of ADMET Predictor High-Throughput PK (HTPK) results
  - Integration of the above in the DILIsym *in vivo* context for early insights into liver liabilities

Liver Safety+ Prediction Package Tailored for Early Discovery Data



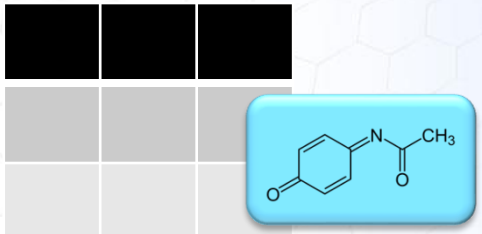
APD Module Applies Machine Learning to Bridge from Compound Structure to DILIsym

Compound Library In Vitro Assay Data

- Mitochondrial respiration (Seahorse assay)
- Oxidative stress (High content screening)
- Bile acid efflux transporter inhibition (Inside-out vesicles)
- Phospholipid transporter inhibition (Transfected cells)

Filtering, Automated Fitting, Translation

Databases



Machine Learning Algorithms

- Mitochondrial dysfunction
- Oxidative stress
- Bile acid efflux transporter inhibition
- Phospholipid transporter inhibition

ADMET Predictor 12
DILIsym module

DILIsym

Novel compound

SimulationsPlus

APD Module Outputs Include Values for Four Key Mechanisms of Hepatotoxicity

- APD module provides classifications (yes/no) and key parameter values for each of the four main mechanisms of toxicity represented in DILIsym
- Outputs are evaluated for potential toxicity
- If outputs suggest toxicity, user can move to identifying parameter values for DILIsym simulations
- Details on each of the APD module outputs and machine learning model construction are available in the ADMET Predictor 12 Manual, and will be summarized in the next section

Toxicity Mechanism	APD classification[§] output	APD MEC[†] output	APD AC_{50}[‡] output	APD IC_{50}[‖] output
Mitochondrial dysfunction	✓	✓	✓	—
Reactive oxygen species	✓	✓	✓	—
BSEP inhibition	✓	—	—	✓
MRP3/MRP4 inhibition	✓	—	—	—
MDR3 inhibition	✓	—	—	✓

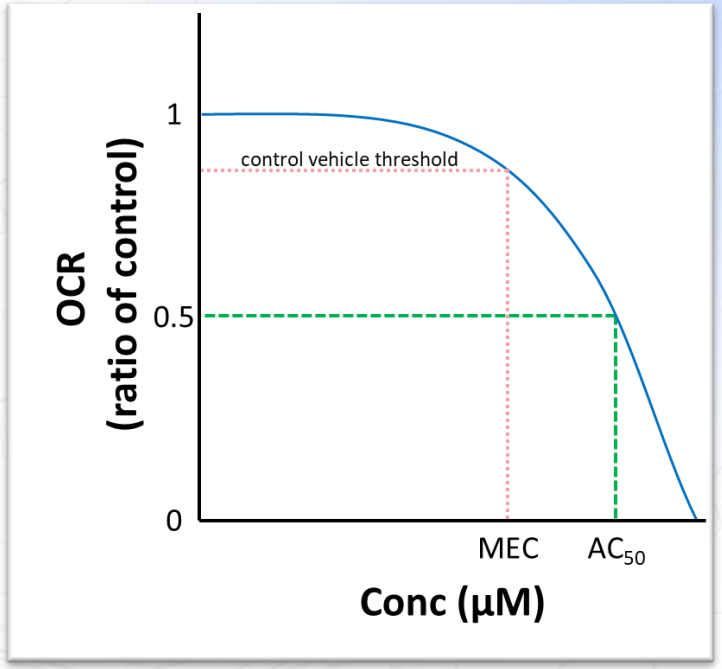
§ yes/no prediction for *in vitro* signals
† minimum effective concentration (MEC) that significantly crosses vehicle control threshold
‡ concentration at which 50% maximum effect is observed
‖ concentration at which 50% inhibition is observed

The ADP Module Contains Three Mitochondrial Dysfunction Models

- Mito_Tox
  - Classification model that predicts Yes or No for mitochondrial toxicity based on the Seahorse assay
  - Based on dataset containing 204 molecules with a large percentage (86%) of experimental positives
- Mito_MEC
  - Predict the minimum effective concentration (MEC) that significantly crosses the control vehicle threshold
- Mito_AC50
  - Predicts the concentration at which 50% maximum effect is observed

Model	Set	Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
Mito_Tox	Training	25	154	179	155	86.6%	85.7%	92.0%
	Test	4	21	25	20	80.0%	81.0%	75.0%

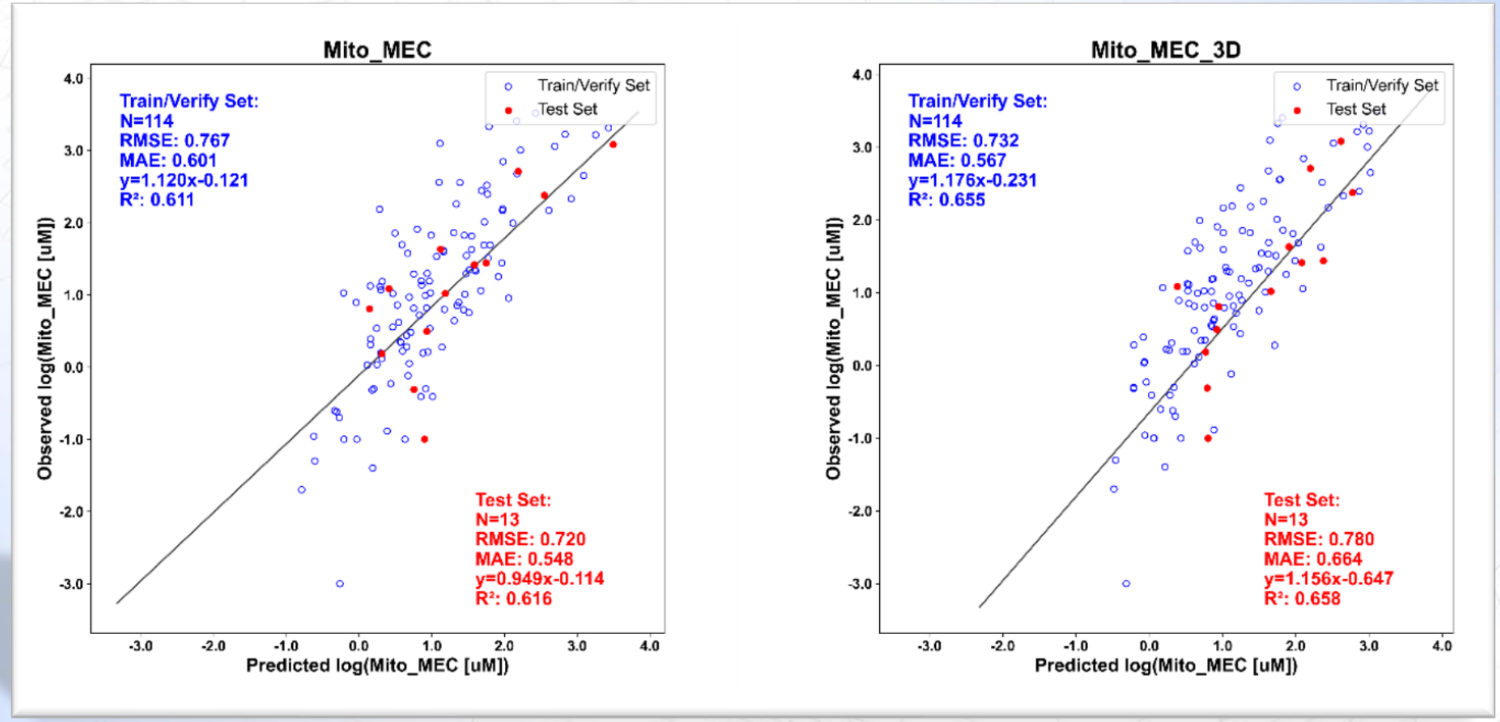
The single mispredicted negative from the test set is fenclozic acid, a compound that was withdrawn from the market due to jaundice



ETC inhibition with a complete knockdown of OCR at high concentrations

Mitochondrial Dysfunction Models With 2D and 2D+3D Descriptors Were Created: Mito_MEC

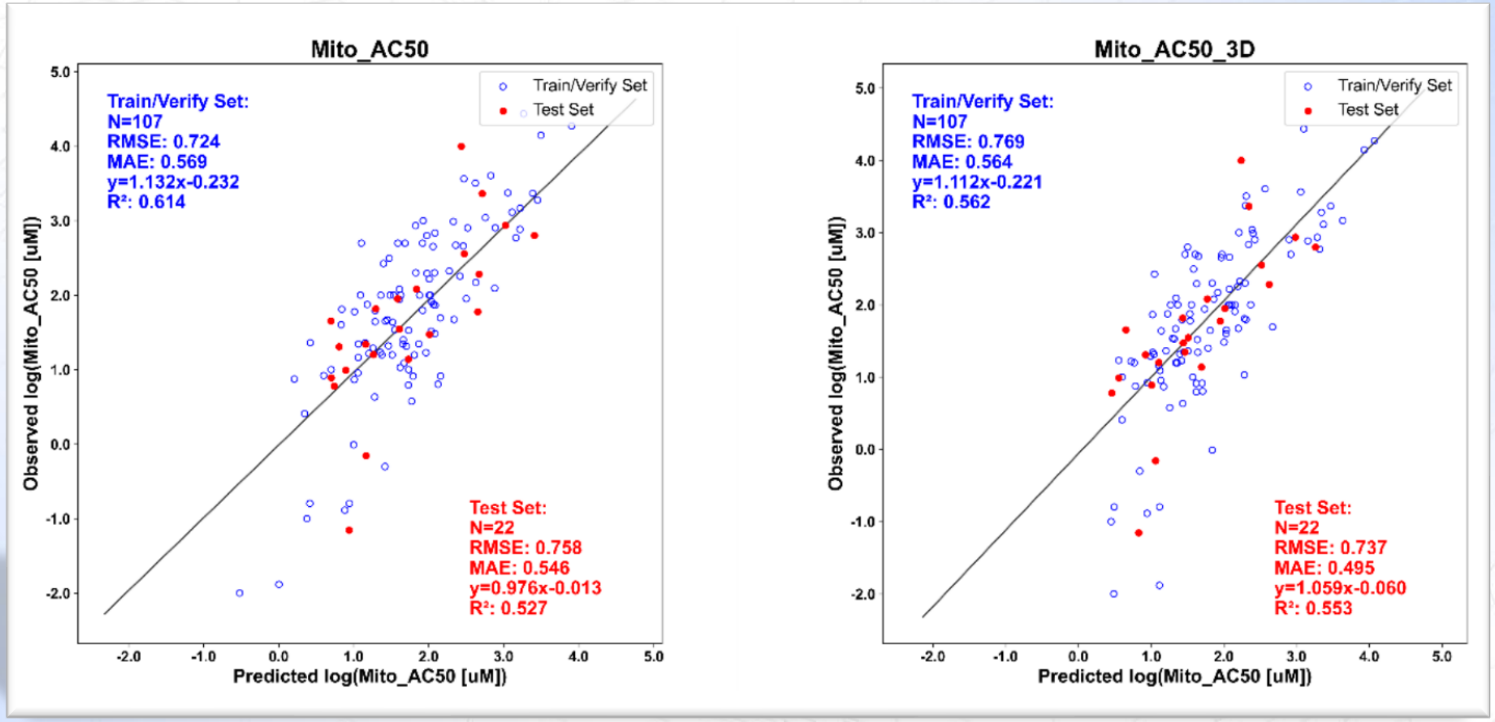
- The Mito_MEC dataset contains 127 compounds with 13 (~10%) in the test set
- The most active compound is rotenone, with an observed MEC value of 0.001 µM



Plots show the log of the experimental Mito_MEC value in micromolar units (µM) versus the log of the predicted value

Mitochondrial Dysfunction Models With 2D and 2D+3D Descriptors Were Created: Mito_AC50

- The Mito_AC50 dataset contains 129 compounds with 22 (~17%) in the test set
- The two most active compounds are antimycin A (Mito_AC50=0.01 µM) and rotenone (Mito_AC50=0.013 µM)



Plots show the log of the experimental Mito_AC50 value in micromolar units (µM) versus the log of the predicted value

The ADP Module Contains Three Reactive Oxygen Species Models

- ROS_Tox
  - Classification model that predicts Yes or No for reactive oxygen species formation
  - Based on dataset containing 243 molecules with 25 (~10%) in the test set
- ROS_MEC
  - Predict the minimum effective concentration (MEC) that significantly crosses the control vehicle threshold
- ROS_AC50
  - Predicts the concentration at which 50% maximum effect is observed

Model	Set	Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
ROS_Tox	Training	70	148	218	172	78.9%	80.4%	75.7%
	Test	6	19	25	22	79.8%	81.4%	76.3%

The ADP Module Utilizes the Existing BSEP Models in ADMET Predictor and Contains a New MRP3 Model for Bile Acid Transporter Inhibition

- BSEP_Inh
  - Classification model that predicts Yes or No for inhibition of the bile salt export pump (BSEP), a bile acid transporter on the canalicular membrane of hepatocytes
  - Based on dataset containing 615 compounds (Morgan et al. 2013), of which 127 inhibit BSEP below 60 µM
- BSEP_IC50
  - Regression model, using 155 compounds with half-maximal inhibitory concentration (IC_{50}) values below 133 µM, that predicts BSEP IC_{50} value
  - Test set consisted of 24 (~15%) compounds
- MRP3_Inh
  - Classification model that predicts Yes or No for inhibition of the multidrug resistance-associated protein 3 (MRP3), a bile acid transporter on the basolateral membrane of hepatocytes
  - Based on dataset containing 107 compounds (Köck et al. 2014, Ali et al. 2017), of which 43 inhibit MRP3 below 100 µM

Model	Set	Negatives	Positives	Total	Correct	Concordance	Sensitivity	Specificity
MRP3_Inh	Training	54	36	90	87	96.7%	94.4%	98.1%
	Test	10	7	17	15	88.2%	85.7%	90.0%

APD Module Predictions Are Used to Set Up Active Toxicity Mechanisms in DILIsym

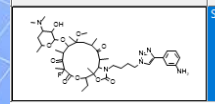
Machine Learning Algorithms

- Mitochondrial dysfunction
- Oxidative stress
- Bile acid efflux transporter inhibition
- Phospholipid transporter inhibition

ADMET Predictor 12
DILIsym module

Novel Compound (Solithromycin)

DILIsym

Structure	Identifier	Geometry	3D Quality	AP_FWeight	BSEP_Inh	BSEP_IC50	MDR3_IC50	MDR3_Inh	Mito_AC50	Mito_MEC	Mito_Tox	MRP3_Inh	ROS_AC50	ROS_MEC	ROS_Tox
	Solithromycin	3D	1.000	845.028	Yes (83%)	8.860	0.677	No	71.064	5.243	Yes (99%)	Yes (93%)	50.259	7.298	Yes (89%)

DILI Mechanism Selector for Solithromycin (Solithromycin_1nM)

Select Molecule: CompY

Select Mechanism: incRNSROSproduction1

Customized Variables

Filter By Name...

Molecule / Mechanism	Value	Units
CompY_Mech_inhBAtransport		
Compound Y NTCP inhibition constant	1.000000e+10	umol/L
Compound Y NTCP alpha constant for inhibition	1.000000e+10	dimensionless
Compound Y NTCP switch	1.000000e+00	dimensionless
Compound Y BSEP inhibition constant	8.86	umol/L
Compound Y BSEP alpha constant for inhibition	5	dimensionless
Compound Y BSEP switch	0	dimensionless
Compound Y basolateral inhibition constant	1.000000e+10	umol/L
Compound Y basolateral alpha constant for inhibition	1.000000e+10	dimensionless
Compound Y basolateral switch	1.000000e+00	dimensionless
CompY_Mech_inhETC3		
Coefficient for ETC inhibition 3	0.040746	umol/L
Max inhibitory effect for ETC inhibition 3	0.39355	dimensionless
CompY_Mech_inhETC1		
Coefficient for ETC inhibition 1	2379.481	umol/L
CompY_Mech_incRNSROSproduction4		
Liver RNS-ROS production rate Vmax 4	5.8195	1/hour
Liver RNS-ROS production rate Km 4	9.1224	umol/L
Liver RNS-ROS production rate Hill 4	4.5496	dimensionless
CompY_Mech_incRNSROSproduction1		
Liver RNS-ROS production rate constant 1	0.053744	mL/nmol/hour

Clear | Save with Custom | Save As New | Save As New with Custom | Cancel

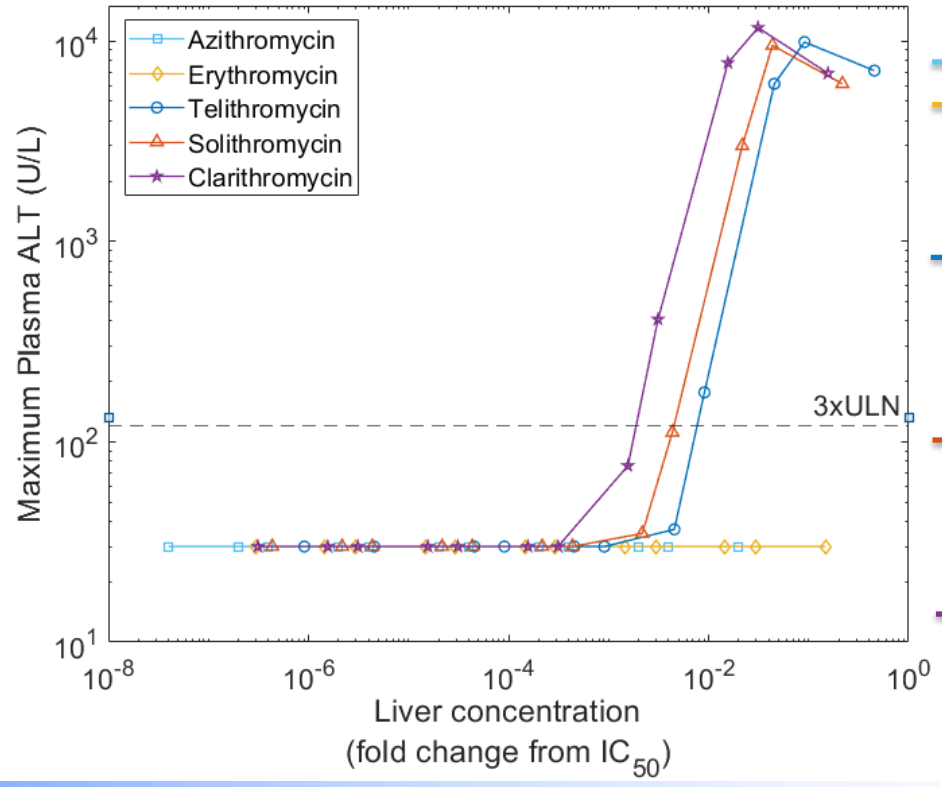
Multiple Options for Liver Exposure in DILI Toxicity Ranking Process

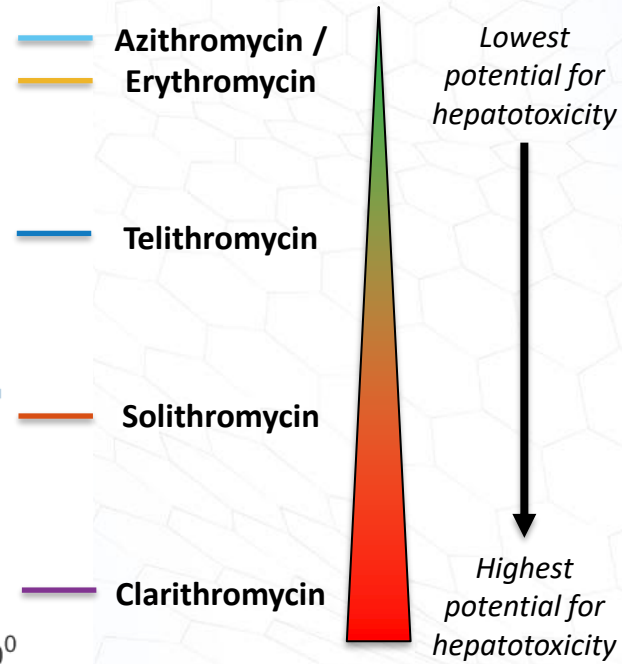
- APD module is designed to provide insight into DILI toxicity rankings at any stage in the drug development pipeline
- Based on where a compound is in the drug development pipeline, different information about exposure in humans is available
  - Compounds further along in the pipeline likely have more information available to define exposure
  - Compounds very early on in development may have minimal data to inform exposure

- Potential options for liver exposure to drive hepatotoxicity mechanisms in DILIsym:
  1. Constant liver exposure based on molar concentrations
     - *DILIsym simulations to be performed at a range of constant liver concentrations*
     - *For rank-ordering hepatotoxicity risk of multiple in-class compounds using the "constant liver exposure" approach, liver concentrations need to be normalized using a relevant metric which provides consideration to compound-specific efficacy ranges*
  2. Assume or estimate liver profiles from preclinical PK data
  3. Estimate liver exposure from ADMET Predictor HTPK using predicted C_{max} and liver partition coefficient from user-specified doses
  4. Predict liver exposure from GastroPlus PBPK model

APD Module Outputs Reproduce Clinical and Previous DILIsym Simulation Toxicity Rankings: Macrolide Antibiotics

ML Tox Model Predictions





Clinical Data & Previous DILIsym Simulation Results

Table III Results in the v4A_1 SimPops for Each of the Five Macrolides in DILIsym v5A Compared to Reported Clinical data. Observed Data are from the Literature (3,10,31)

Compound	Protocol	Peak ALT >3X ULN	
		Observed	Simulated**
Solithromycin	Oral (CE01–300)	5.4%[a] (22/411)	3.9% (11/285)
	IV-to-Oral (CE01–301)	9.1%[b] (38/417)	6.0% (17/285)
Clarithromycin	500 mg BID 7 days	1–2%	2.8% (8/285)
Erythromycin	500 mg QID 10 days	1–2%	2.8% (8/285)
Telithromycin	800 mg QD 10 days	~0.5%	0%
Azithromycin	500 mg QD day 1 250 mg QD days 2–5	1.2%	0%

Upper limit of normal (ULN) in DILIsym is 40 U/L
[a] (9); 2.8% among patients with normal baseline ALT
[b] (8); 6.6% among patients with normal baseline ALT


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

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

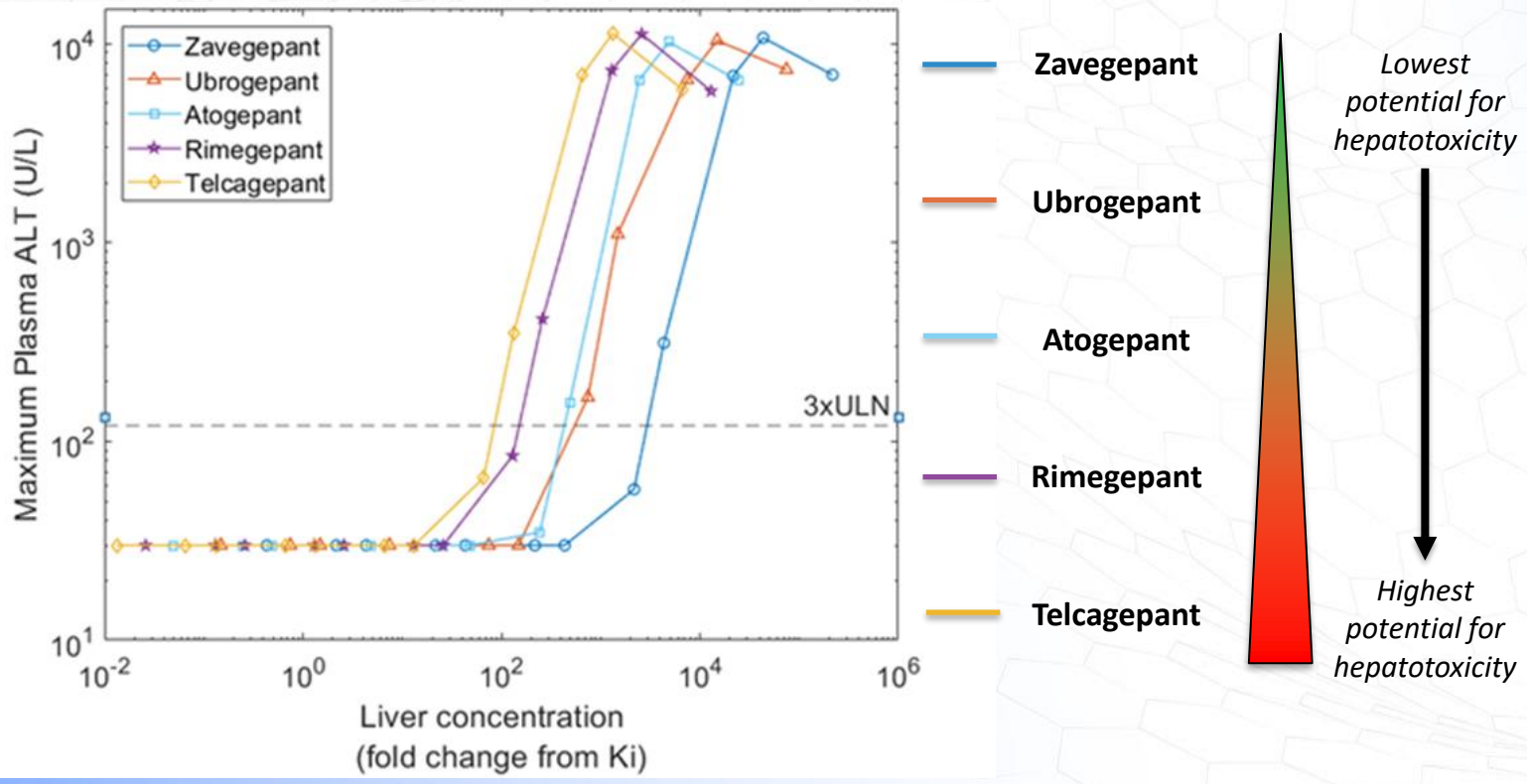
Jeffrey L. Woodhead[1] · Kyunghee Yang[1] · David Oldach[2] · Chris MacLauchlin[2] · Prabhavathi Fernandes[2] · Paul B. Watkins[3] · Scott Q. Siler[1] · Brett A. Howell[1]

- Liver concentrations were normalized to OATP1B1 IC_{50} values for macrolide antibiotics

SimulationsPlus

APD Module Outputs Reproduce Clinical and Previous DILIsym Simulation Toxicity Rankings: CGRPR Antagonists

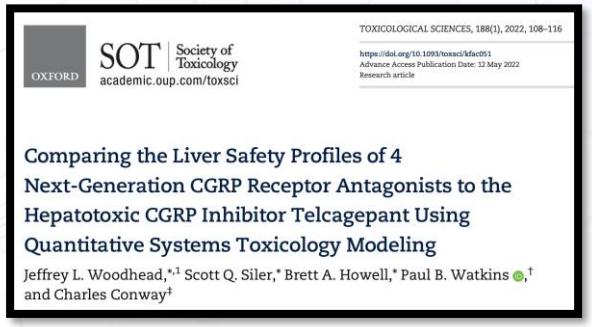
ML Tox Model Predictions



Clinical Data & Previous DILIsym Simulation Results

Table 2. Simulated ALT Elevations in the v4A_1 SimPops for Each of the CGRP Compounds

Compound	Oral Dosing Protocol	Simulated ALT > 3X ULN[a]	Observed ALT > 3X ULN in Clinic
Telcagepant—High ETC	140 mg BID, 12 weeks	17.5% (50/285)	1.9% (5/263)
	280 mg BID, 12 weeks	76.1% (217/285)	3.2% (8/265)
Telcagepant—Low ETC	140 mg BID, 12 weeks	0.0% (0/285)	1.9% (5/263)
	280 mg BID, 12 weeks	7.72% (22/285)	3.2% (8/265)
Rimegepant	75 mg QD, alternate day dosing, 14 total doses	0.35% (1/285)	—
	75 mg QD, 5 days on, 1 day off, 25 total doses	0.7% (2/285)	—
	75 mg QD, daily dosing for 25 days, 25 total doses	1% (3/285)	—
Zavegepant	750 mg oral QD, 25 days, 25 total doses	0.0% (0/285)	
	75 mg oral QD, 25 days, 25 total doses	0.0% (0/285)	
	20 mg IN QD, 25 days, 25 total doses	0.0% (0/285)	
	2 mg IN QD, 25 days, 25 total doses	0.0% (0/285)	
	0.75 mg IV QD, 25 days, 25 total doses	0.0% (0/285)	
	7.5 mg IV QD, 25 days, 25 total doses	0.0% (0/285)	
Atogepant	60 mg BID, 12 weeks	0% (0/285)	
	120 mg BID, 12 weeks	0% (0/285)	
	300 mg BID, 12 weeks	0.3% (1/285)	
	600 mg BID, 12 weeks	10.2% (29/285)	
Ubrogepant	100 mg QD, 15 days	0% (0/285)	
	200 mg QD, 15 days	0% (0/285)	
	500 mg QD, 15 days	1.1% (3/285)	
	1000 mg QD, 15 days	11.6% (33/285)	
	100 mg QD, 25 days	0% (0/285)	
	200 mg QD, 25 days	0% (0/285)	
	500 mg QD, 25 days	1.4% (4/285)	
	1000 mg QD, 25 days	11.6% (33/285)	



- Liver concentration were normalized to CGRP receptor Ki values for CGRP receptor antagonists

Workflow: APD Module Enables Efficient Assessment of Hepatotoxic Rankings for In-Class Compounds at Any Stage of Drug Development!

Determine relevant group of compounds to be assessed and gather SMILES or chemical structure for each

Run APD module and export predictions for mito, ROS, BA and PL transporter inhibition toxicity

Use assumptions previously discussed to optimize mito and ROS toxicity parameters for DILIsym use

Extract known EC_{50}, IC_{50}, or Ki values for each compound to determine range of concentrations to be tested

Set up DILIsym SimSingles for each compound at each concentration (with specified liver concentration) with toxicity parameters determined by APD module

Run SimCohorts simulations

Normalize liver concentrations to relevant metric (e.g., EC_{50}) available for all compounds and analyze toxicity biomarker results

Simulations Plus Liver Safety+ Package Contains All Necessary Software for Toxicity Ranking Predictions!

Executive Summary

- **DILIsym**
  - Mechanistic, mathematical quantitative systems toxicology (QST) model
  - Constructed to support pharmaceutical risk assessment and decision making
- **Clinical Application**
  - DILIsym has been applied to support decisions related to compound DILI risk throughout the clinical development pipeline
    - Projects executed with 55+ companies
  - DILIsym simulation results have been included in numerous communications with regulatory agencies
- **Preclinical Application**
  - Liver Safety+ recently made available
  - Provides efficient evaluation of DILI risk for preclinical compounds
  - Machine learning utilized to generate key parameters based on compound structure, a la QSAR
- **DILIsym support enabled via multiple routes**
  - Simulations Plus services projects
  - Direct license of DILIsym
  - DILI-sim Consortium membership
  - Liver Safety+