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DILIsym User Training –

Introduction to Quantitative Systems Toxicology (QST) and the DILIsym Software

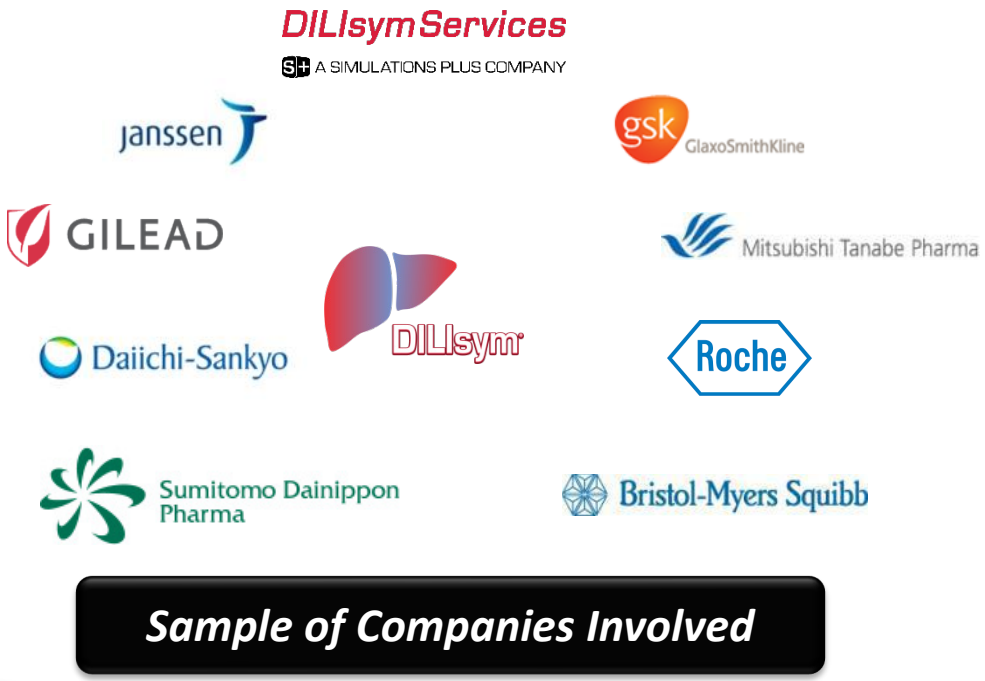
DILIsym Development Team

Goals for the QSP and DILIsym Introduction Training Session

Participants should understand the following general concepts:

- The concepts behind quantitative systems pharmacology/toxicology (QSP/QST)
- Introductory design concepts behind the DILIsym QST software platform
- The typical high-level workflow for using DILIsym for prediction
- Background information on the innate immune sub-model within DILIsym
- DILIsym validation projects done to-date
- Example applications of DILIsym use in pharma

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



Sample of Companies Involved

- *Overall Goals*
  - *Improve patient safety through QST*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *19 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $8 million total invested in project*

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Slide courtesy of CJ Musante

What Are QSP and QST? Some Definitions

"The **integration** of **biological mechanisms** (from **sub-cellular to patient cohorts**) in **quantitative** mechanistic **models** and their application in the **discovery and development** of pharmaco-therapeutics."

UK QSP Network (http://www.qsp-uk.net/themes.html; accessed 2016-07-06)

"The **quantitative** analysis of the **dynamic interactions** between **drug(s)** and a **biological system** to understand the behaviour of the **system as a whole**, as opposed to the behaviour of its individual constituents."

van der Graaf and Benson (2011) Pharm Res 28(7):1460-1464. doi:10.1007/s11095-011-0467-9

"An approach to **translational** medicine that combines **computational and experimental methods** to elucidate, validate and apply new pharmacological concepts to the development and use of **small molecule and biologic drugs**...to determining **mechanisms of action** of new and existing drugs in **preclinical and animal models and in patients**."

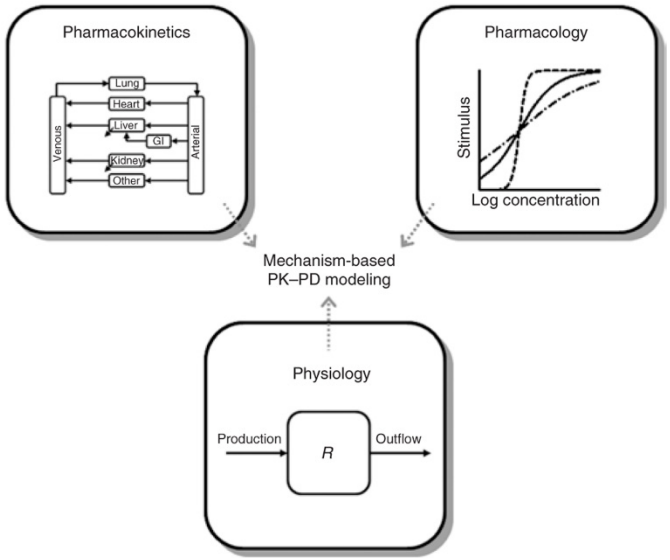
Sorger et al. (2011) NIH QSP Working Group White Paper
https://www.nigms.nih.gov/training/documents/systemspharmawpsorger2011.pdf

Slide courtesy of CJ Musante

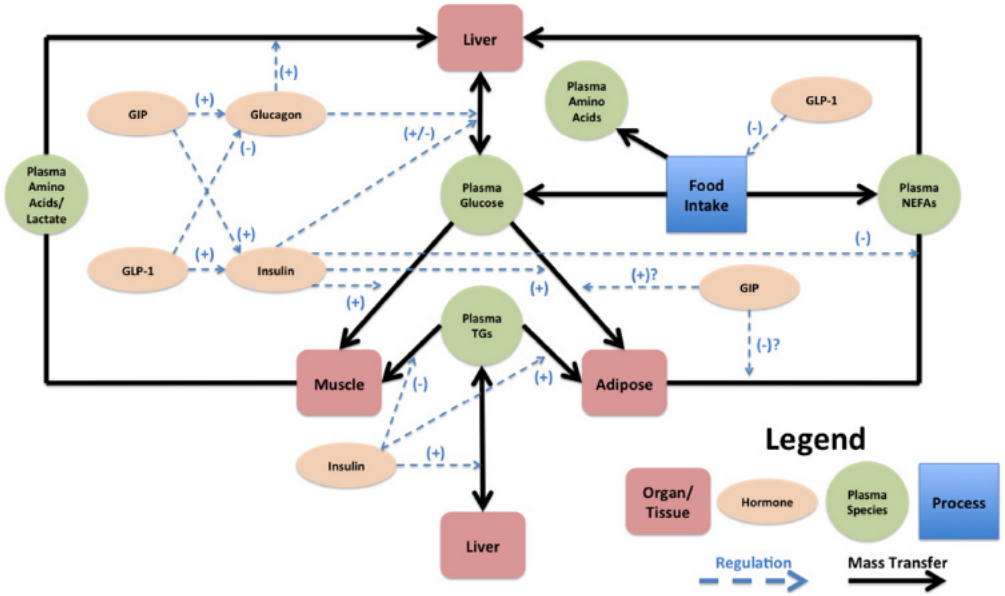
The Intersection of PK, PD, and QSP/QST

Pharmacokinetics (PK): What the body does to a drug
Pharmacodynamics (PD): What a drug does to the body
QSP/QST: PK and PD extended to effects at the systems level (e.g., disease modification)

PK and PD



QSP



Mager and Jusko 2008

Rieger and Musante 2016

http://www.merckmanuals.com

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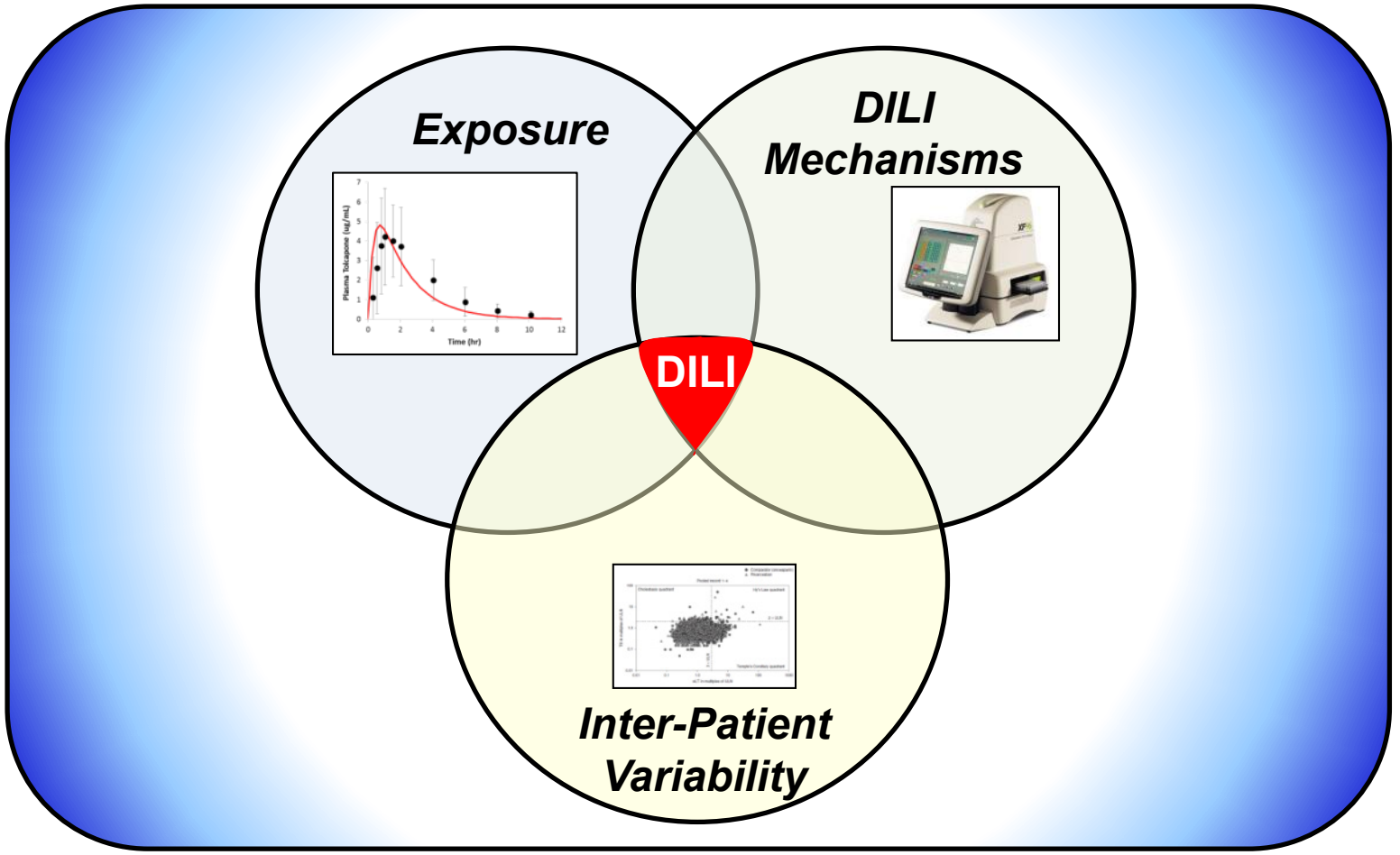
DILIsym Predicts DILI via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



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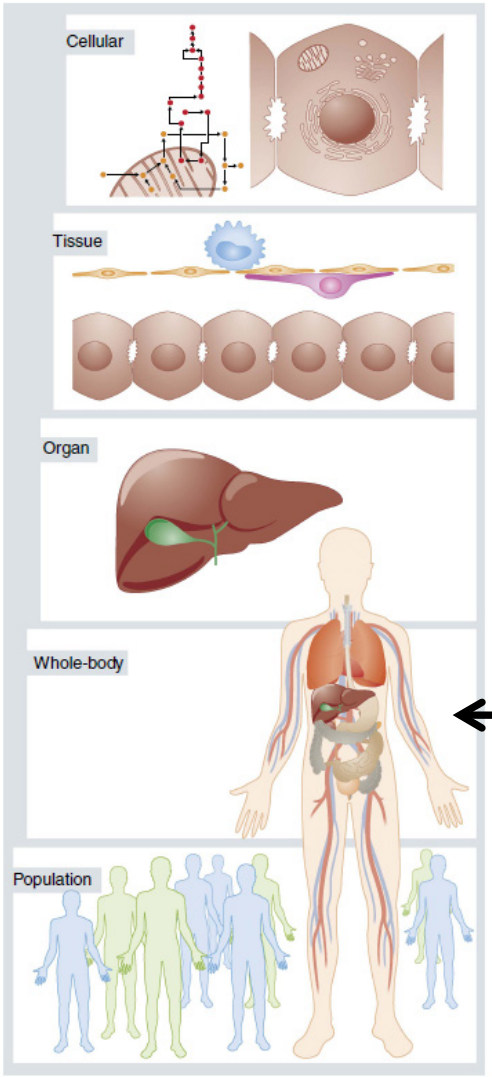
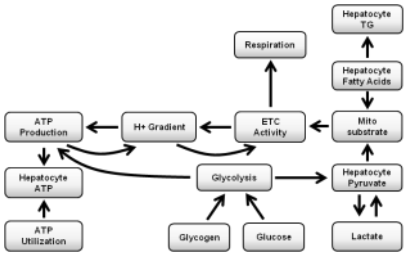
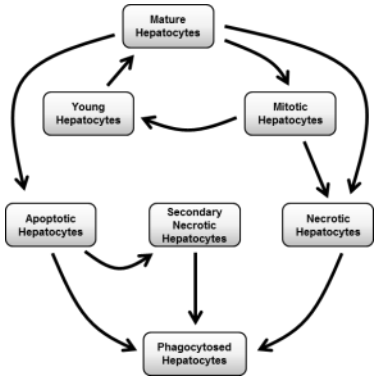
DILIsym: Quantitative Systems Toxicology



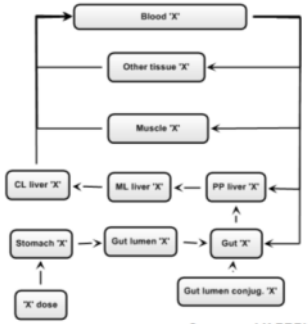
Mitochondrial dysfunction



Cellular life-cycle

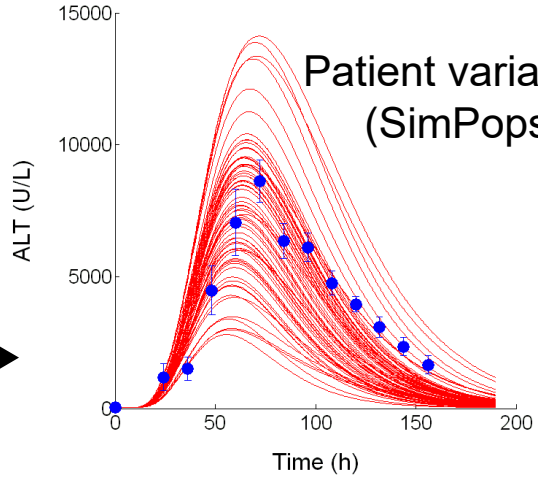


Drug distribution & metabolism



Compound X PBPK

Patient variability (SimPops)



Kuepfer 2010, Molecular Systems Biology

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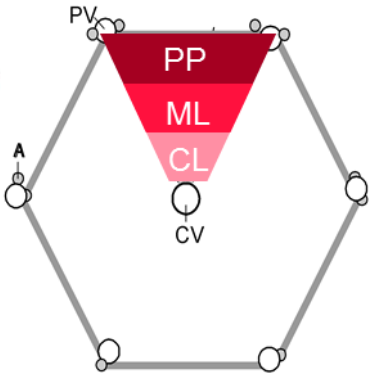
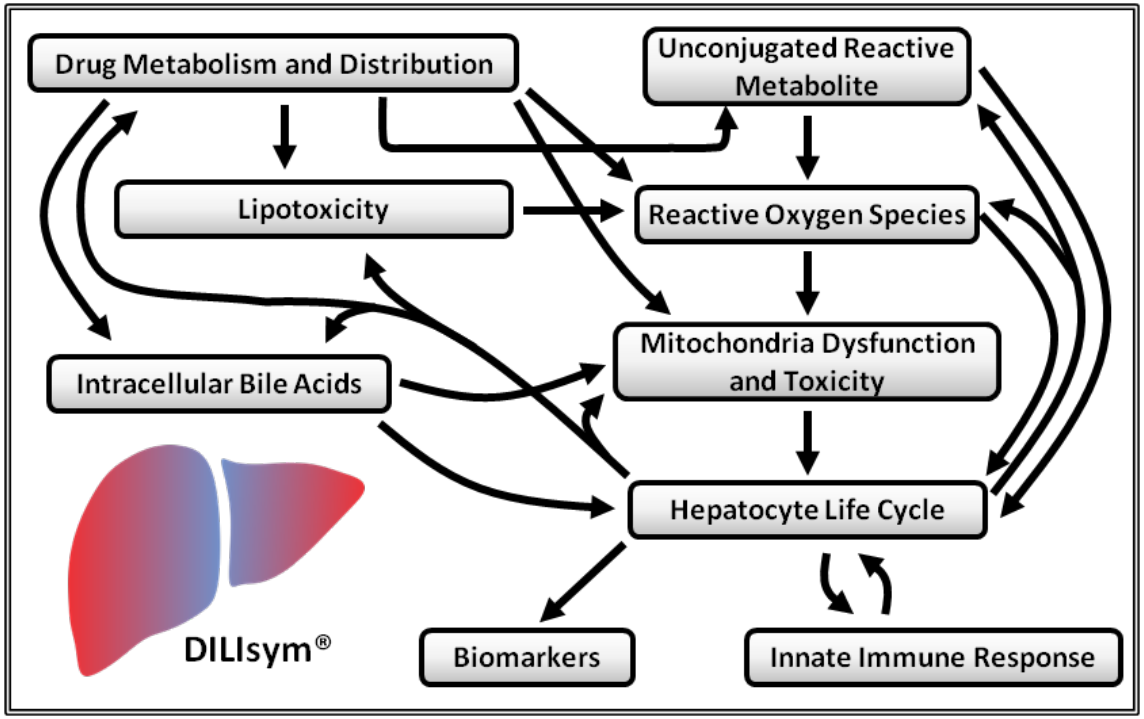
DILIsym Overview

- **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**
  - Pharmacokinetics
  - Dosing (IP, IV, Oral)
  - Transporter Inhibition
  - Drug metabolism
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Bile acid mediated toxicity
  - Steatosis and lipotoxicity
  - Cellular energy balance
  - Hepatocyte apoptosis and necrosis, and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers



- **Over 30 detailed representations of optimization or validation compounds**
- **Single and combination drug therapies**



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DILIsym Utilizes Various Data Types to Inform Decisions

Exposure Data

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*

Modeling & Simulation

Simulations and Assays inform:

- **Prediction of DILI risk**
- **Participating DILI mechanisms**
- **Characteristics of patients at risk for DILI**
- **Drug dosing paradigms**
- **DILI monitoring strategies**

In vitro Mechanistic DILI Data

Assays performed to determine quantitative aspects of DILI mechanisms

- **Oxidative stress**
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity**
  - *ETC inhibition*
  - *Uncoupling*
- **Bile acid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

Clinical Data

- **Dosing Protocols, fasting/fed state, meal times**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

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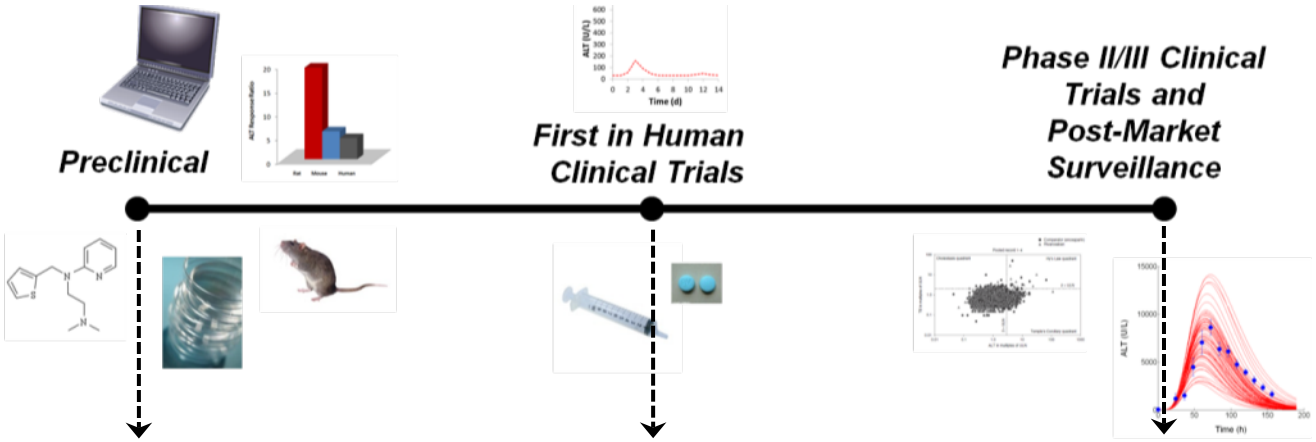
Applications of DILIsym Along the Drug Development Pipeline

Predictions of hepatotoxicity for humans and preclinical animal models



• Mechanism exploration • Rank candidates for DILI potential • Extrapolation from animal and *in vitro* findings to humans	• Dose optimization (risk versus presumed benefit) • Infer magnitude of injury based on measured biomarkers • Extrapolation from healthy volunteers to patient groups • Guide incorporation of emerging biomarker measurements in clinical trials • Analysis of mechanisms underlying observed liver signals	• Inform choice and timing of biomarker measurement • Aid identification of risk factors leading to personalized medicine approaches • Analysis of mechanisms underlying observed liver signals

Biomarkers of Hepatocellular Function and Death Are Outputs of DILIsym

- 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
- Additional DILIsym outputs include:
  - Fraction of viable hepatocytes
  - Liver ATP
  - Liver glutathione
  - Circulating, liver, and excreted drug and metabolites

Marker	Category
Alanine aminotransferase (ALT)[1,2,3,4,5]	Necrosis
Bilirubin (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

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?
  - Secondary question: was the general magnitude of injury over-predicted (O), under-predicted (U), or correctly predicted (C), based on severity and frequency of injury?

Drug	Human	Rat	Mouse	Dog
Entacapone (Clean)	C	--	--	--
Tolcapone (DILI)	C	--	--	--
Methapyrilene (Clean)	C	C	C	--
Troglitazone (DILI)	O	C	--	--
Pioglitazone (Clean)	C	--	--	--
AMG009 (DILI)	C	C	--	--
Compound A (DILI)	U	--	--	--
Bosentan (DILI)	O	C	--	--
Telmisartan (Clean)	C	--	--	--
Tolvaptan (DILI)	O	--	--	--
Compound B (DILI)	O	--	--	--
Compound C (DILI)	O	C	--	--
Compound E (DILI)	U	--	--	--
AMAP (N/A)	--	--	U	--
Compound F (DILI)	C	--	--	--
AMG 853 (Clean)	C	--	--	--
Compound G (DILI)	O	--	--	--
Solithromycin (DILI)	C	--	--	--
CKA (Clean/Some DILI)	U	U	--	--
Compound H (Clean)	C	--	--	--
Erythromycin (DILI)	C	--	--	--
Clarithromycin (DILI)	C	--	--	--
Compound N (DILI)	O	--	--	--
Compound O (DILI)	C	--	--	--
Compound P (DILI)	U	--	--	--
Telithromycin (DILI)	U	--	--	--
Azithromycin (DILI)	U	--	--	--
MK-0536 (DILI)	U	--	--	--
TAK-875 (DILI)	C	--	--	--
Metformin (clean)	C	--	--	--
Phenformin (lactic acidosis)	C	--	--	--
Compound Q sc. 1 (DILI)	U	--	--	--
Compound Q sc. 2 (DILI)	U	--	--	--
Lixivaptan (clean)	C	--	--	--
Compound R (DILI)	U	--	--	--
Compound S (DILI)	O	--	--	--
Sitaxsentan (DILI)	C	--	--	--
Ambrisentan (clean)	C	--	--	--
Didanosine (clean)	C	--	--	--
Compound L (DILI)	U	--	--	--

Color Key – Accuracy of DILIsym	
Good	
Bad	

83% (33/40) generally predicted well

HUMAN **MICE** **RATS** **DOGS**

Clinical, Preclinical Data and Simulation Results

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Many Publications Include DILIsym Design Information and Application Examples

1. Jeffrey L. Woodhead, et al., “The Role of Quantitative Systems Pharmacology in Predicting Adaptive Immune Mediated Idiosyncratic DILI”, Drug Metabolism and Pharmacokinetics, (2016) Under final review.
2. Jeffrey L. Woodhead, et al., “Application of a mechanistic model to evaluate putative mechanisms of tolvaptan drug-induced liver injury and identify patient susceptibility factors,” Toxicological Sciences, (2016) DOI: 10.1093/toxsci/kfw193.
3. Jeffrey L. Woodhead, et al., “Safety Margin and Optimization of Dosing Protocol for [Compound X] using Quantitative Systems Pharmacology Modeling,” In preparation.
4. Lisl K.M. Shoda, et al., “Representing Innate Immunity in DILIsym,” Gene Regulation and Systems Biology, (2016) Under review.
5. Kyunghee Yang, et al., “Quantitative Systems Pharmacology Modeling with DILIsym Predicts the Clinically Observed Differences in Hepatotoxicity Between Two Potent BSEP Inhibitors: AMG009 and AMG853,” In preparation.
6. Kyunghee Yang, et al., “Mechanistic modeling predicts drug-induced hyperbilirubinemia that involves inhibition of enzymes and transporters,” Clinical Pharmacology and Therapeutics, (2016) Under review.
7. Christina Battista, et al., “Using DILIsym to investigate observed species differences in CKA-mediated hepatotoxicity,” In preparation.
8. Kyunghee Yang, et al., “Sandwich-Cultured Hepatocytes as a Tool to Study Drug Disposition and Drug-Induced Liver Injury,” Journal of Pharmaceutical Sciences, 105 (2016) 443-459.
9. Diane M. Longo, et al., “Elucidating Differences in the Hepatotoxic Potential of Tolcapone and Entacapone With DILIsym, a Mechanistic Model of Drug-Induced Liver Injury,” CPT: Pharmacometrics and Systems Pharmacology, 1 (2016) e31, DOI: 10.1002/psp4.12053.
10. Brett A. Howell, et al., “A Mechanistic Model of Drug-Induced Liver Injury Aids the Interpretation of Elevated Liver Transaminase Levels in a Phase I Clinical Trial,” CPT: Pharmacometrics and Systems Pharmacology, 3 (2014) e98, DOI: 10.1038/psp.2013.74.
11. Yuching Yang, et al., “MITOsym: A Mechanistic, Mathematical Model of Hepatocellular Respiration and Bioenergetics,” Pharmaceutical Research, (2014) DOI: 10.1007/s11095-014-1591-0.
12. Jeffrey L. Woodhead, et al., “Exploring BSEP inhibition-mediated toxicity with a mechanistic model of drug-induced liver injury,” Frontiers in Pharmacology, (2014) DOI: 10.3389/fphar.2014.00240.
13. Jeffrey L. Woodhead, et al., “Mechanistic Modeling Reveals the Critical Knowledge Gaps in Bile Acid-Mediated DILI,” CPT: Pharmacometrics and Systems Pharmacology, 3 (2014) e123, DOI: 10.1038/psp.2014.21.
14. Kyunghee Yang, et al., “Systems Pharmacology Modeling Predicts Delayed Presentation and Species Differences in Bile Acid-Mediated Troglitazone Hepatotoxicity,” Clinical Pharmacology and Therapeutics, 96 (2014) 5: 589-598.
15. Lisl K. M. Shoda, et al., “Linking physiology to toxicity using DILIsym®, a mechanistic mathematical model of drug-induced liver injury,” Biopharmaceutics and Drug Disposition, (2013) DOI: 10.1002/bdd.1878.
16. Brett A. Howell, et al., “In vitro to in vivo extrapolation and species response comparisons for drug-induced liver injury (DILI) using DILIsym™, a mechanistic, mathematical model of DILI,” Journal of Pharmacokinetics and Pharmacodynamics, 39 (2012) 527-541.
17. Jeffrey L. Woodhead, et al., “An Analysis of N-Acetylcysteine Treatment for Acetaminophen Overdose Using a Systems Model of Drug-Induced Liver Injury,” Journal of Pharmacology and Experimental Therapeutics, 342 (2012) 529-540.
18. Sudin Bhattacharya, et al., "Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches," Frontiers in Physiology, 3 (2012) 462: 1-18.

Known DILIsym Applications Submitted to or Intended for Regulatory Agencies

N	Agency	Context	Scenario	Simulation Type	Presented/ Submitted By
1	FDA	Simulation results included in formal, written correspondence to agency	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
2	FDA	Simulation results included in formal, written correspondence to agency	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
3	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor and DSS
4	BARDA*	Simulation results presented to sponsor group at BARDA	Sponsor responding to concerns over liver safety signals	Mechanistic liver injury (predictive)	DSS and Sponsor
5	FDA and Japanese FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor addressing concerns over liver safety in NDA submission	Mechanistic liver injury (predictive)	Sponsor and DSS
6	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor repurposing compound that failed due to hepatotoxicity in IND submission	Mechanistic liver injury (predictive)	Sponsor and DILIsym Services
7	FDA	Simulation results included in formal, written correspondence to agency and presented during meeting	Sponsor addressing concerns over liver signals from other drug in same class with same indication	Mechanistic liver injury (predictive)	Sponsor
8	FDA	Simulation results included in formal, written correspondence to agency	Sponsor addressing concerns over liver safety in NDA submission	Mechanistic liver injury (predictive)	Sponsor
9	FDA	Simulation results included in formal, written correspondence to agency and discussed during call with FDA	Sponsor responding to concerns over liver safety signals	Hepatocyte loss (biomarker fitting)	Sponsor
10	FDA and global regulators	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver safety signals	Hepatocyte loss (biomarker fitting) Mechanistic liver injury (predictive)	Sponsor
11	FDA	Sponsor intended to submit simulation results	Sponsor addressing concerns over liver signals from other drug in same class with same indication	Mechanistic liver injury (predictive)	Sponsor
12	FDA	Sponsor intended to submit simulation results	Sponsor reformulating existing compound on the market	Mechanistic liver injury (predictive)	Sponsor
13	FDA	Sponsor intended to submit simulation results and present at meeting	Sponsor addressing concerns over liver safety signals	Mechanistic bilirubin (predictive)	Sponsor

*Not a direct regulatory agency, but affiliated closely with NIH and FDA

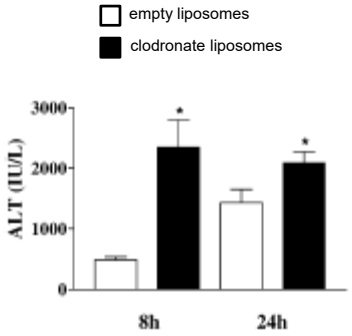
**Several additional sponsors have declared intent to include results in regulatory communications in the future

***Additional drug development teams have implied that regulators have informally requested or recommended DILIsym simulations

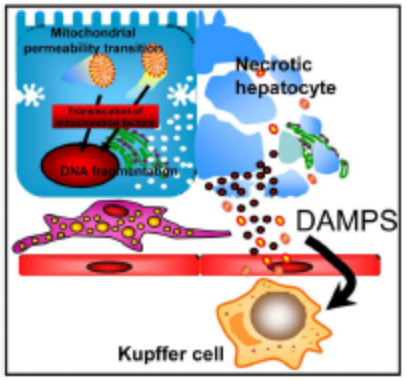
Innate Immune Cells Implicated in DILI and/or Recovery

MICE

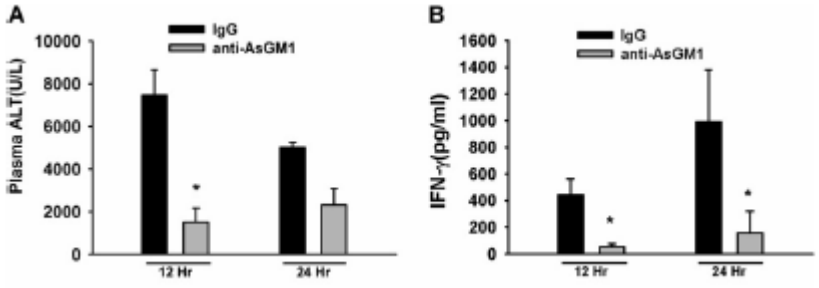
- Immune cell types in DILI
  - APAP (macrophages, LSECs, DCs, PMNs, NK cells, NKT cells)
    - Ju 2002, Campion 2008, Fisher 2013, You 2013, McCuskey 2005, Kato 2011, Connolly 2010, Marques 2015, Huebener 2015, Liu 2004, Liu 2006, Masson 2008, Ishida 2006
  - Halothane (PMNs, NK cells, NKT cells)
    - You 2006, Dugan 2011, Cheng 2010
  - Amodiaquine (NK cells)
    - Metushi 2015
  - Isoniazid (NK cells)
    - Mak 2015
- Interpretation of cell type manipulation studies often challenging
- Initial focus on APAP
  - Macrophages, including Kupffer cells
  - LSECs



Ju et al. 2002



Jaeschke 2015



Dugan et al. 2011

Preclinical Data

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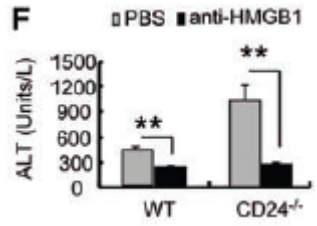
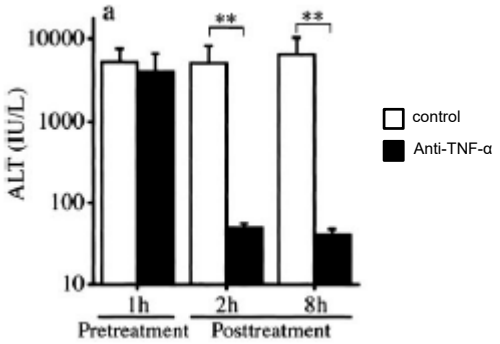
Mediators Derived from Immune Cells Implicated in DILI and/or Recovery

MICE

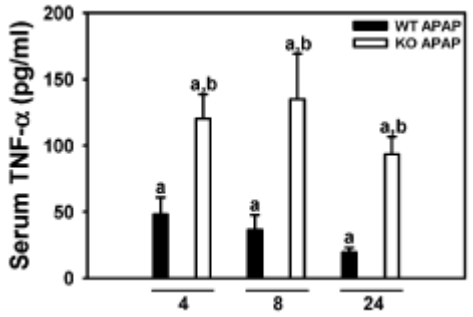
- Functional role in DILI generally defined by addition of exogenous or blockade of endogenous mediator
  - *e.g.,* anti-HMGB1, anti-TNF-α, exogenous HGF
- Mechanistic attributes generally defined by *in vitro* studies
  - May also drive required inclusion
- Exposure profile generally defined by plasma measurements
- Initial focus on APAP
  - HMGB1, TNF-α, IL-10, (VEGF), HGF



Chen et al. 2009



Ishida et al. 2004



Yee et al. 2007

Preclinical Data

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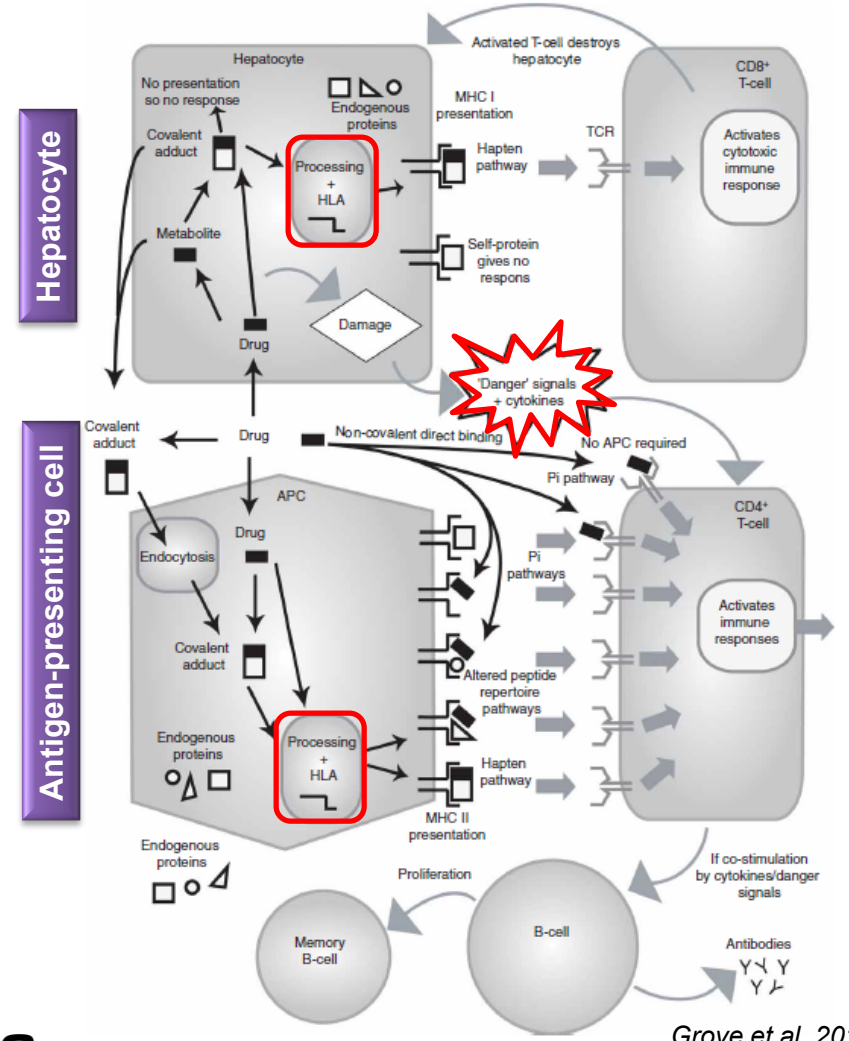
Evidence for T Cells Indirectly Supports Activation of Innate Immune Cells

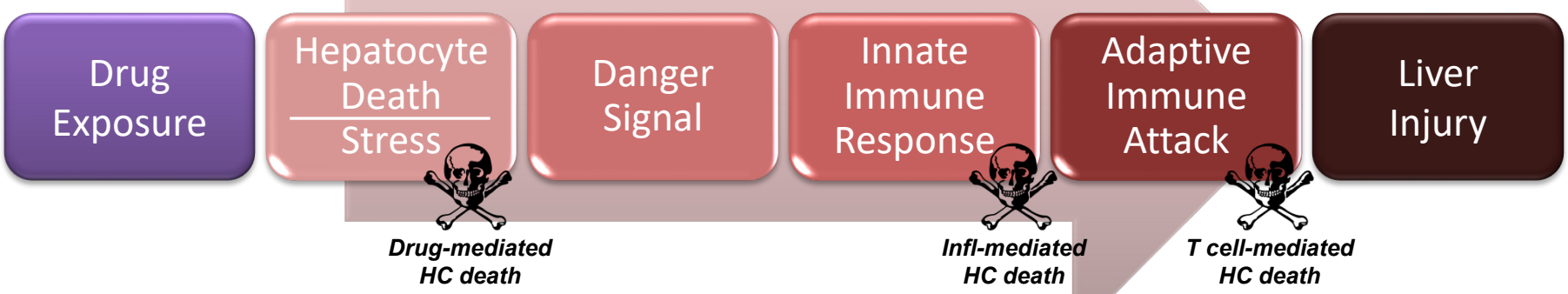
- Genome wide association studies (GWAS) have identified associations between human leukocyte antigen (HLA) alleles and some DILI compounds
- While these associations implicate an adaptive immune response, they also implicate activation of innate immune cells
  - Innate immune cells, particularly dendritic cells, are professional antigen-presenting cells, typically needed for initiation of T cell response
  - Activating signals (*e.g.*, DAMPs, cytokines) required for upregulation of key molecules involved in antigen presentation
  - Cytokines provide “signal 3” in T cell differentiation, shaping the character of resultant response



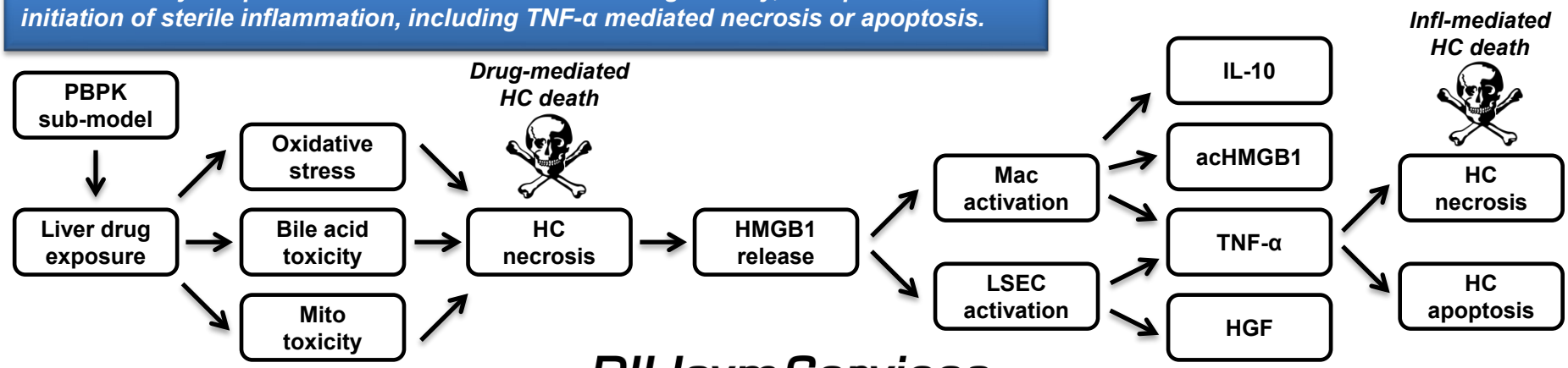
Grove et al. 2014

Intrinsic Drug Toxicity and Subsequent DAMP Release Drive Innate Immune Activation

Theory on sequence of events driving potential contributors to liver injury, including intrinsic drug toxicity, sterile inflammation, and adaptive immune attack.



Current DILIsym representation allows for intrinsic drug toxicity, with potential initiation of sterile inflammation, including TNF-α mediated necrosis or apoptosis.



DILIsym Services

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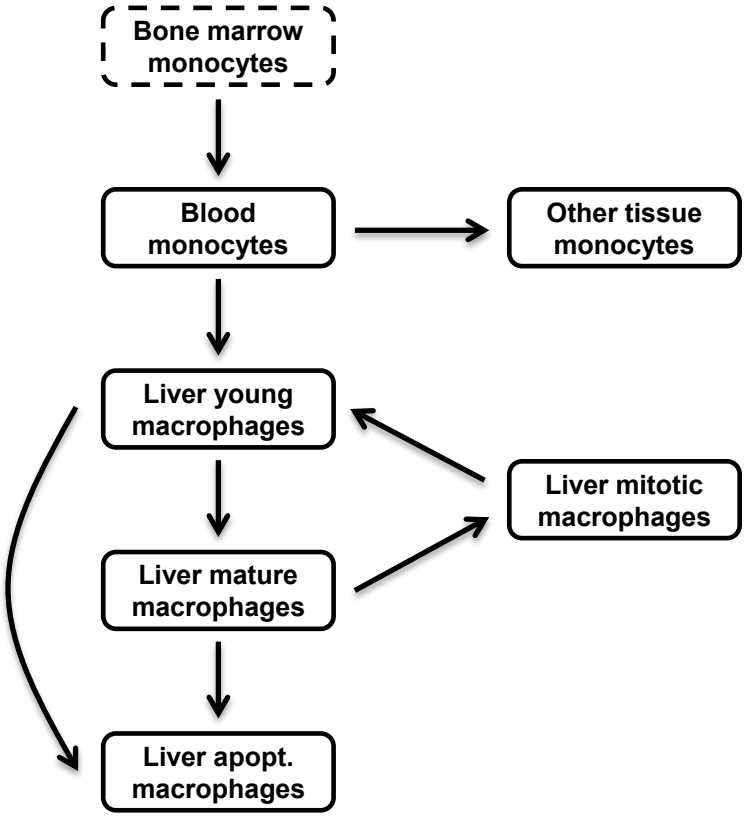
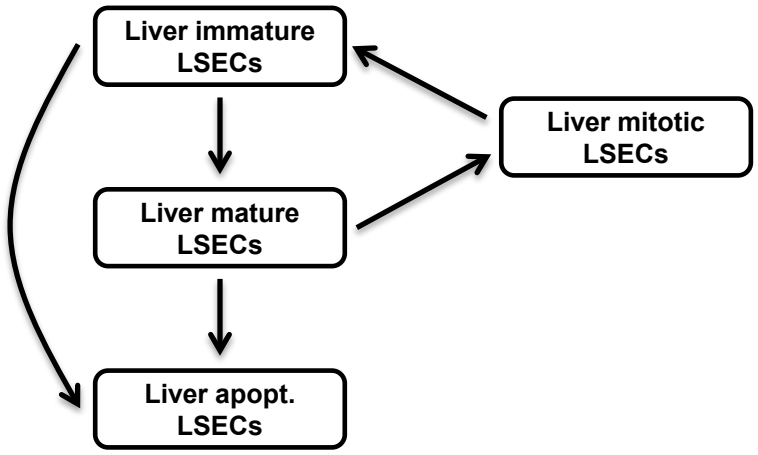
Macrophage and LSEC Cellular Life Cycles

Liver macrophage population maintained by recruitment of blood monocytes from blood and local proliferation



Shoda et al. 2017. Gene Regulation and Systems Biology

Liver LSEC population maintained by local proliferation



HUMANS

RATS

MICE

TABLE 1.—Endothelial cell labeling index of liver and adipose tissue in humans, rats, and mice.[a]

		Human (n = 10)	Rat (n = 10)	Mouse (n = 10)
Liver	(Male)	0.25 ± 0.13	0.36 ± 0.10[*]	1.14 ± 0.34[**,##]
	(Female)	0.20 ± 0.10	0.28 ± 0.08[*]	0.84 ± 0.28[**,##]
Brown Fat	(Male)	ND[b]	0.39 ± 0.09	0.80 ± 0.31[##]
	(Female)	ND[b]	0.40 ± 0.10	0.75 ± 0.41[##]
White Fat	(Male)	0.11 ± 0.09	0.42 ± 0.23[**]	0.89 ± 0.25[**,##]
	(Female)	0.15 ± 0.08	0.44 ± 0.13[**]	0.59 ± 0.33[**,#]

[a]Mean ± SD.
[b]ND: not determined.
[*]$p < 0.05$, [**]$p < 0.01$ as compared to human.
[#]$p < 0.05$, [##]$p < 0.01$ as compared to rat.

Ohnishi et al 2007

Measured EC proliferation rates across species

Preclinical Data

DILIsym Services

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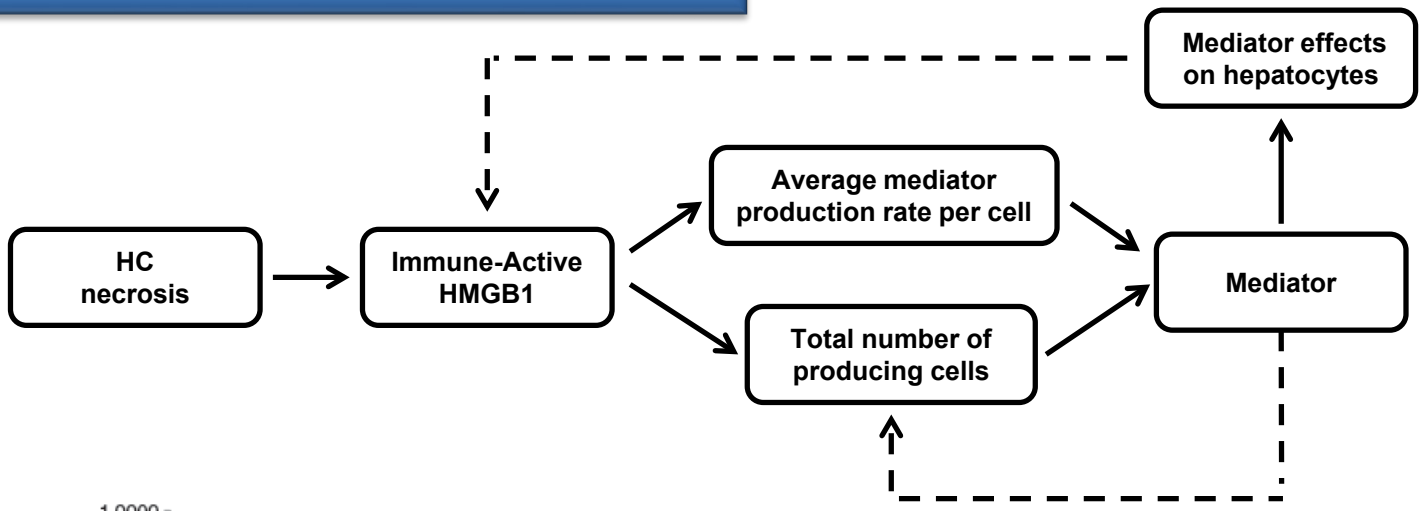
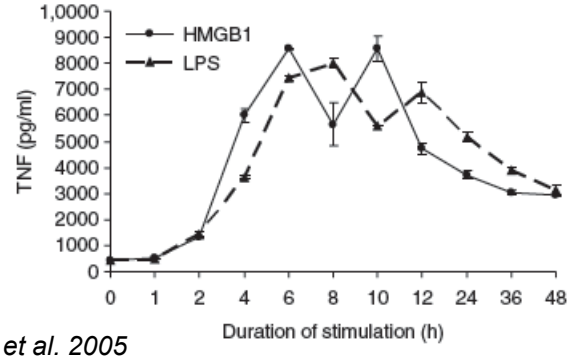
Regulation of Mediator Production

Mediators reflect production rate and cell numbers and include both feed forward and feedback loops



RATS



Kokkola et al. 2005

HMGB1 stimulates macrophage TNF-α production

Preclinical Data

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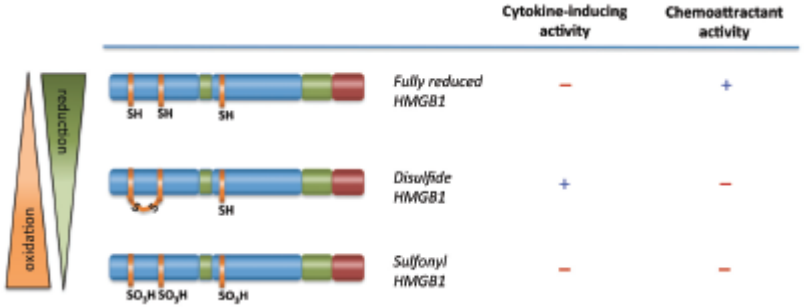
HMGB1: Function Follows Form

- Alternate re-dox forms of HMGB1 have been associated with distinct functions
  - Fully reduced or disulfide HMGB1 associated with pro-inflammatory functions
  - Oxidized HMGB1 generated during apoptotic cell death
- Macrophage activation results in alternate cellular localization and processing
  - Putative biomarker for macrophage involvement



Antoine et al. 2014

	Grouped by outcome	
	Survival	Death or required liver transplant
Number	51	27
Age (IQR-yr)	36 (28-43)	44 (30-57)
Gender (M/F)	22/29	9/18
ALT activity (IQR-U/L)	4005.1 (2595.1-7280.8)	3334.0 (1777.4-6226.3)
Prothrombin time (IQR-sec)	40.5 (23.0-69.3)	47.5 (31.0-80.0)
Creatinine (IQR-μmol/L)	85.5 (61.0-188.8)	201.0 (142.3-263.3)†
Number with encephalopathy grade 3-4	7/51	26/27
Necrosis related K18 (IQR-U/L)	23,383.8 (8171.7-55,931.4)	64,151.0 (20,070.1-110,381.5)††
Apoptosis related K18 (IQR-U/L)	2391.0 (790.5-6739.3)	3339.0 (2377.4-8523.4)†
Apoptosis based on K18 (IQR-%)	18.1 (5.6-26.1)	5.6 (2.5-18.4)†
Total HMGB1 (IQR-ng/ml)	8.9 (4.5-15.2)	15.9 (8.2-40.1)††
Acetylated HMGB1 (IQR-ng/ml)	0.06 (0.02-0.38)	4.0 (0.9-6.2)**

Antoine et al. 2012

HUMAN

Clinical Data

DILIsym Services

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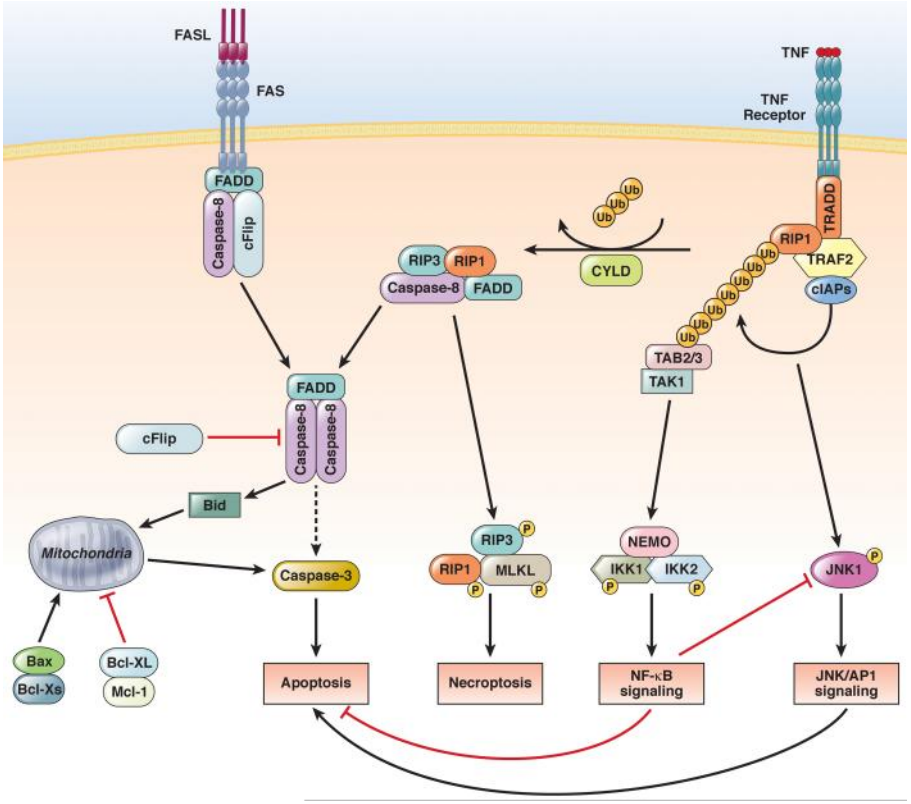
TNF-α Pleiotropic Activity Includes Survival, Proliferation, Apoptosis, and Necrosis

- Alternate signaling pathways characterized
  - Includes newly described pathway of programmed necrosis, “necro-apoptosis” or “necroptosis”
- Liver-specific data, *e.g.*,
  - Proliferation in partial hepatectomy
  - Survival following TNF-α pre-treatment
  - Cell death following LPS or TNF-α and D-galactosamine
- Modeling challenge
  - Hepatocytes must respond dynamically to TNF-α in simulations
  - How will the hepatocyte response be determined?



Luedde et al. 2014

Survival/Proliferation | Apoptosis | Necro-apoptosis

DILIsym Services

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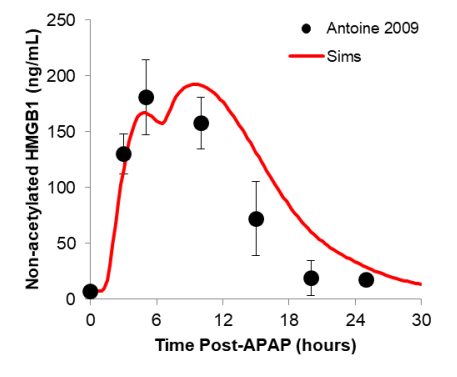
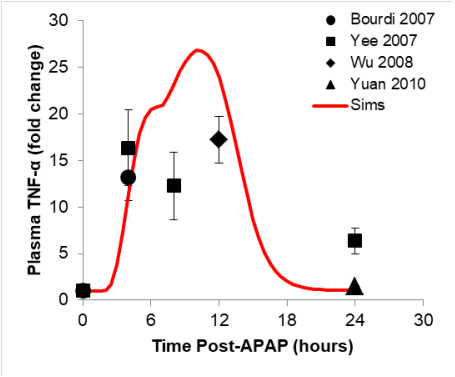
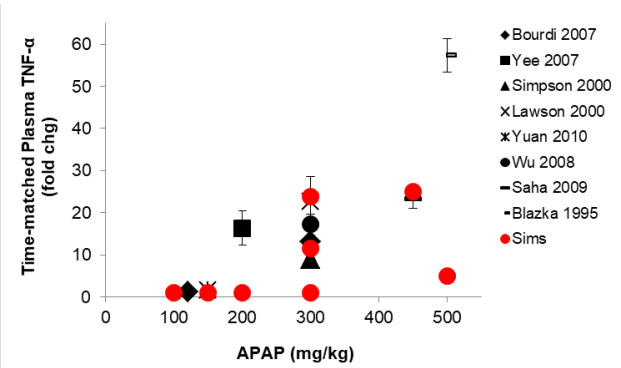
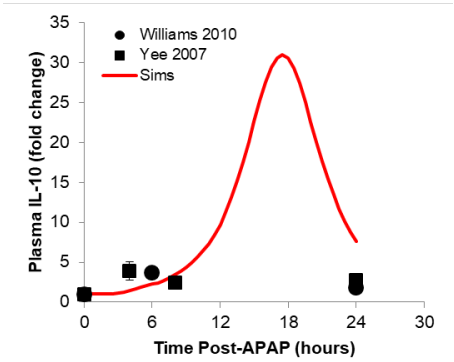
Mouse Simulations Consistent with Preponderance of Data

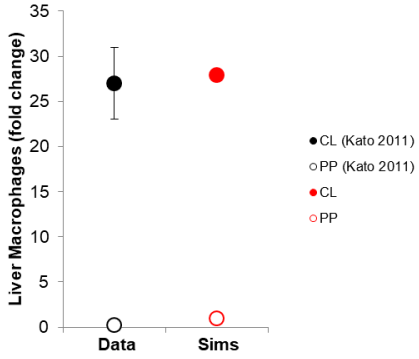
- APAP-centric
- Time- and dose-dependent data comparisons applied wherever possible

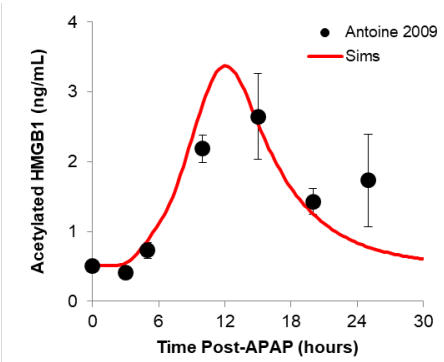












Preclinical Data & Simulation Results

DILIsym Services

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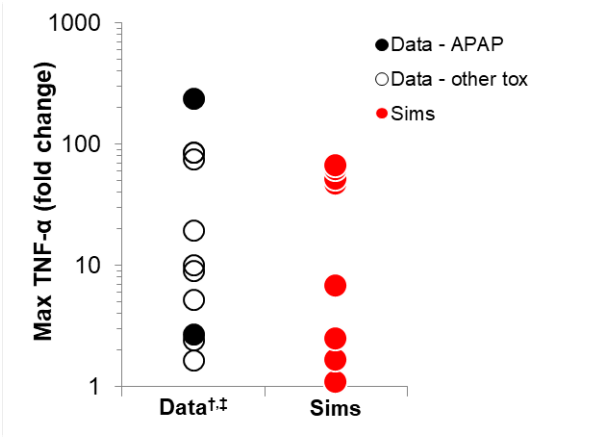
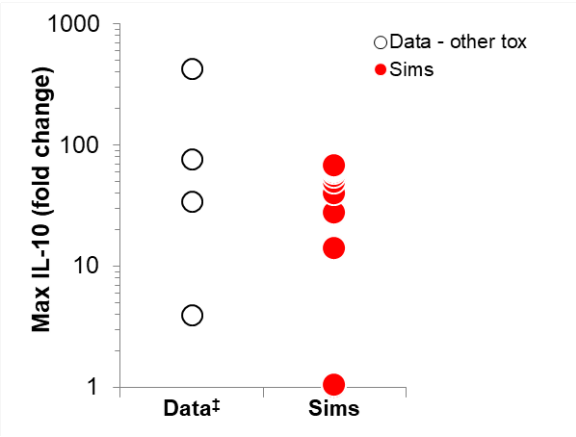
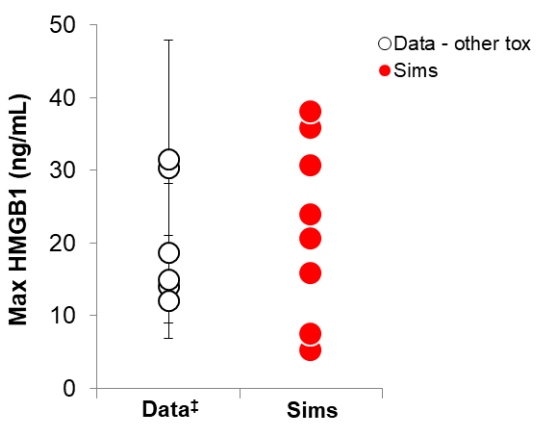
Rat Simulations Consistent with Preponderance of Data

- Minimal APAP data set necessitates use of other liver toxicant/injury models
- Focus on ability to approximate data range using alternate APAP doses







† *APAP: Arafa 2009, Merrick 2006*

‡ *Other tox: Bautista 2010, Chen 2008, Matsuhashi 2005, Nakamoto 2003, DeCicco 1998, Hagiwara 2008, Koga 2012*

‡ *Other tox: Nakamoto 2003, Swain 1999, Hagiwara 2008, Kono 2006*

‡ *Other tox: Takano 2010, Hagiwara 2008, Koga 2012, Oishi 2012, Liu 2010*

Preclinical Data & Simulation Results

DILIsym Services

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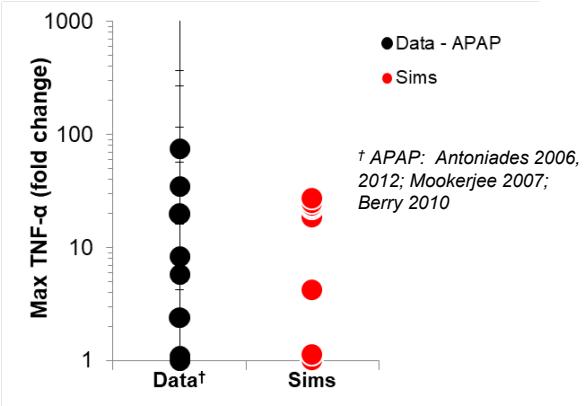
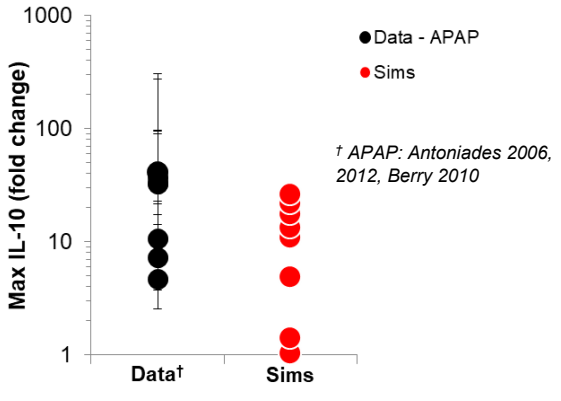
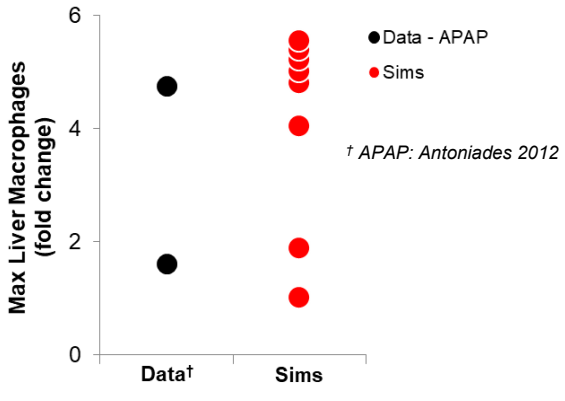
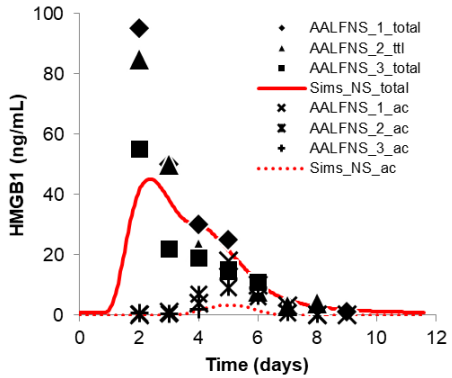
Human Simulations Consistent with Preponderance of Data

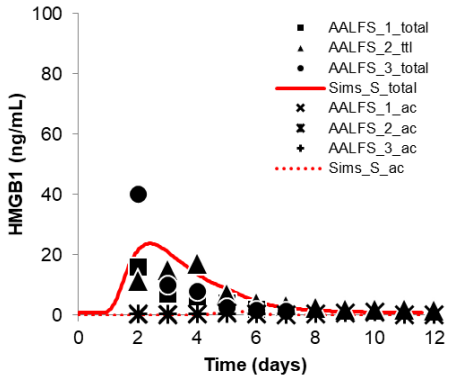
- Use of APAP overdose data (minimal data for dose, *i.e.*, model input)
- Focus on ability to approximate data range using alternate APAP doses











APAP: Antoine 2012

Clinical Data & Simulation Results

DILIsym Services

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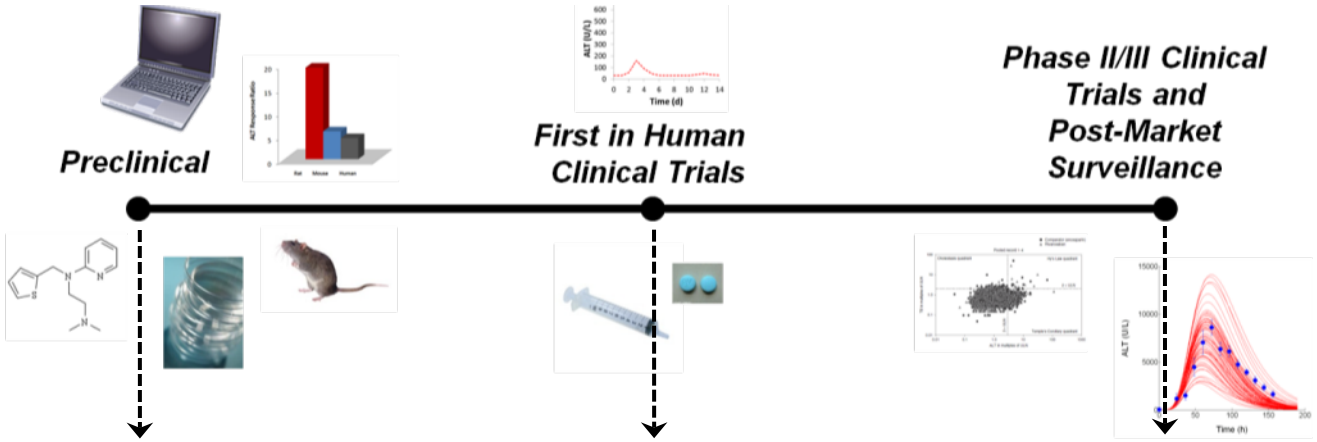
Applications of DILIsym Along the Drug Development Pipeline

Predictions of hepatotoxicity for humans and preclinical animal models



• Mechanism exploration • Rank candidates for DILI potential • Extrapolation from animal and in vitro findings to humans	• **Dose optimization (risk versus presumed benefit)** • Infer magnitude of injury based on measured biomarkers • Extrapolation from healthy volunteers to patient groups • Guide incorporation of emerging biomarker measurements in clinical trials • **Analysis of mechanisms underlying observed liver signals**	• Inform choice and timing of biomarker measurement • Aid identification of risk factors leading to personalized medicine approaches • Analysis of mechanisms underlying observed liver signals

DILIsym Services

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Project Example Executive Summary

DILIsym Validation Using Clinical Data for Compound X

- ALT elevations were predicted in DILIsym simulations of previous Compound X clinical protocols where liver injury occurred clinically
  - Predicted delayed ALT elevations due to accumulation of a Compound X metabolite over time within DILIsym
  - Compound X metabolite-mediated mitochondrial electron transport chain (ETC) inhibition and oxidative stress (ROS) were responsible for predicted ALT signals

Prospective Compound X Development using DILIsym

- Optimal, prospective (much lower) dosing protocols were identified to achieve maximum drug efficacy using the DILIsym software and a custom SimPops with Compound X PK variability included
- ALT elevations were not predicted to occur in DILIsym simulations of Compound X dosing at the optimal, prospective clinical dose levels identified from the exposure simulations

Final DILIsym Input Parameters For Compound X and Compound X Metabolite

Compound	Mechanism	Parameter	Unit	Value*
Compound X	Mitochondrial Dysfunction	Coefficient for ETC Inhibition 1	µM	3.5×10^{6}
Compound X Metabolite	Oxidative Stress	RNS/ROS production rate constant 1	mL/mol/hr	3×10^{-5}
	Mitochondrial Dysfunction	Coefficient for ETC Inhibition 2	µM	2000
		Coefficient for ETC Inhibition 3	µM	50
		Max inhibitory effect for ETC inhibition 3	Dimensionless	0.4

* Values shown in the table for DILIsym input parameters should not be interpreted in isolation with respect to clinical implications, but rather, should be combined with exposure in DILIsym to produce simulations that have predictive and insightful value

Compound X Clinical Protocols for DILIsym Hepatotoxicity and Exposure Simulations

Past Clinical Studies

- 0.3X mg Compound X, 16 weeks
- 0.5X mg Compound X, 16 weeks
- 1X mg Compound X, 16 weeks

No clinical stop protocol

DILIsym Services

A SIMULATIONS PLUS COMPANY

Hepatotoxicity Correctly Predicted for Retrospective Compound X Protocols

- Compound X effects simulated in SimPops (n=285) that represent variability in toxicity mechanisms and PK
- DILIsym predicted delayed hepatotoxicity with varying grades for previous clinical protocols

	Comp X Protocol	Grade 1 (ALT 1-2.5X ULN*)		Grade 2 and above (ALT > 2.5X ULN)		
		Observed	Simulated†	Observed	Simulated†	
Prospective	0.07X load / 0.03X steady, 32 weeks‡					Prospective
Prospective	0.13X load / 0. 07X steady, 32 weeks‡					
Previous	0.3X, 16 weeks	25% (13/52)	0.35% (1/285)	3.8% (2/52)	0.35% (1/285)	Previous
Previous	0.5X, 16 weeks	14% (1/7)	8.4% (24/285)	0% (0/7)	22.5% (64/285)	
Previous	1X, 16 weeks	20% (1/5)	4.9% (14/285)	0% (0/5)	37.5% (107/285)	

*upper limit of normal (ULN) in DILIsym is 40 U/L.

†SimPops™ Human_ROS_apop_mito_BA_v4A_1 (n=285) combined with Compound X PK variability used.

‡PROSPECTIVE clinical protocols

Simulation Results and Clinical Data

No clinical stop protocol

DILIsym Services

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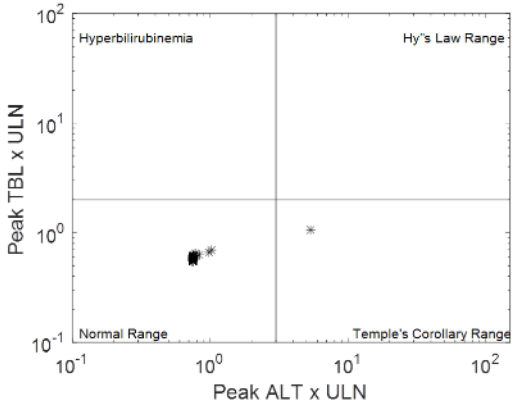
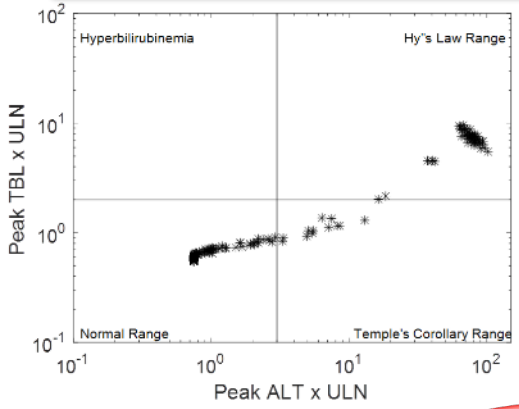
Hepatotoxicity Correctly Predicted for Retrospective Compound X Protocols

- Dose dependent DILI frequency and severity predicted for Compound X
- Severity of response not appropriate to consider
  - Clinical stop protocol not included in simulations
  - DILIsym does not yet represent some likely key adaptation mechanisms like mitochondria biogenesis

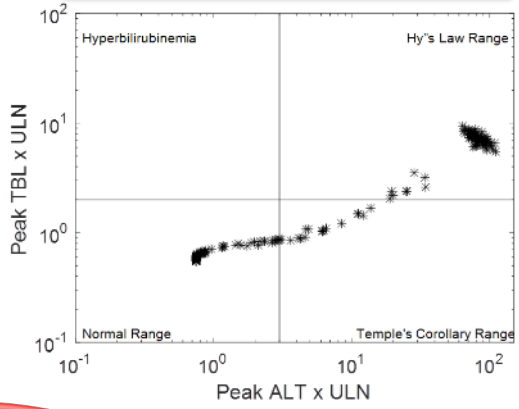
0.3X Compound X Dosing



0.5X Compound X Dosing



1X Compound X Dosing



No clinical stop protocol

Simulation Results

DILIsym Service

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ETC Inhibition and Oxidative Stress Contribute to Simulated Compound X Hepatotoxicity

- 1X Compound X dosing simulated in the sensitive SimCohorts (n=16) for 16 weeks to investigate underlying mechanisms of toxicity
  - One or two mechanisms eliminated sequentially
- Compound X Metabolite-mediated ETC inhibition and oxidative stress are main drivers of predicted toxicity
  - No toxicity predicted in simulations without Compound X metabolite effects (case VI)
- Parent Compound X has a negligible impact on predicted hepatotoxicity

Case	DILI Mechanism			Simulated Grade 1 ALT and Above
	Compound X ETCi	Compound X Metabolite ETCi	Compound X Metabolite ROS	
I	On	On	On	15/16
II	**Off**	On	On	15/16
III	On	**Off**	On	14/16
IV	On	On	**Off**	15/16
V	**Off**	**Off**	On	14/16
VI	On	**Off**	**Off**	0/16
VII	**Off**	On	**Off**	14/16

*SimCohorts Human_ROS_apop_mito_BA_v4A_1_Multi16 (n=16) used.
**Upper limit of normal (ULN) in DILIsym is 40 U/L.
ETCi – mitochondrial electron transport chain inhibition.
OS – oxidative stress.

Compound X Clinical Protocols for DILIsym Hepatotoxicity and Exposure Simulations

Past Clinical Studies

- 0.3X mg Compound X, 16 weeks
- 0.5X mg Compound X, 16 weeks
- 1X mg Compound X, 16 weeks

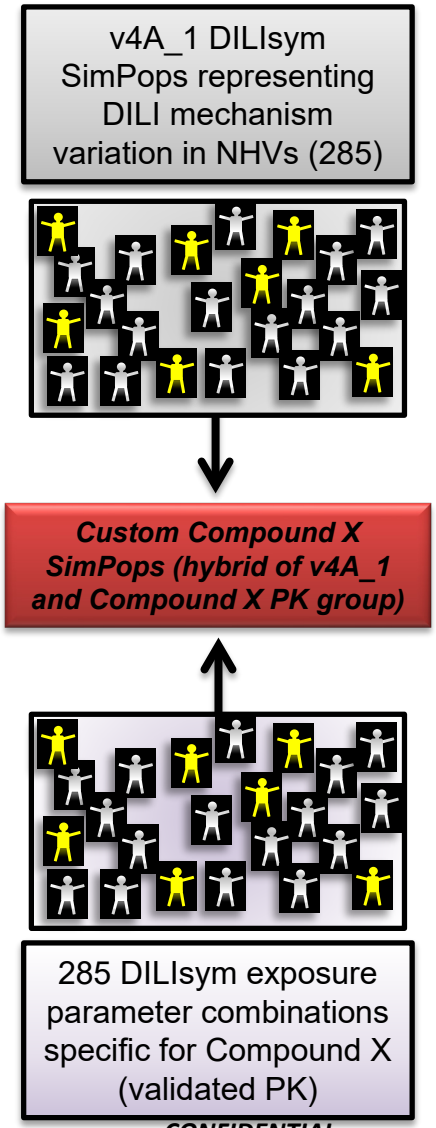
Prospective Studies

- 0.13X Compound X loading dose / 0.07 Compound X steady state dose, 32 weeks total
- 0.07X Compound X loading dose / 0.03 Compound X steady state dose, 32 weeks total

No clinical stop protocol

Customized SimPops Constructed to Recapitulate Compound X PK Variability

- Variability in parameters specific to Compound X and Compound X metabolite exposure superimposed on the existing human normal healthy volunteer SimPops (v4A_1 SimPops)
  - Existing SimPops: Human_ROS_apop_mito_BA_v4A_1 (N = 285)
  - Existing SimPops includes variability in oxidative stress, mitochondrial function, and bile acid transport
  - 285 Compound X ADME parameter combinations assigned randomly to existing individuals in v4A_1 SimPops within DILIsym
- Clinical PK data used to optimize and validate the Compound X PK SimPops
  - Clinical PK data employed for optimization
- Log-normal distribution used to select parameter values
  - 15 parameters varied
  - Metabolism Vmax distribution taken from distribution reported in literature
  - Standard deviation of 80% and 53% mean parameter value used for Compound X- and Compound X metabolite-related parameters, respectively, to represent observed PK variability



DILIsym Services

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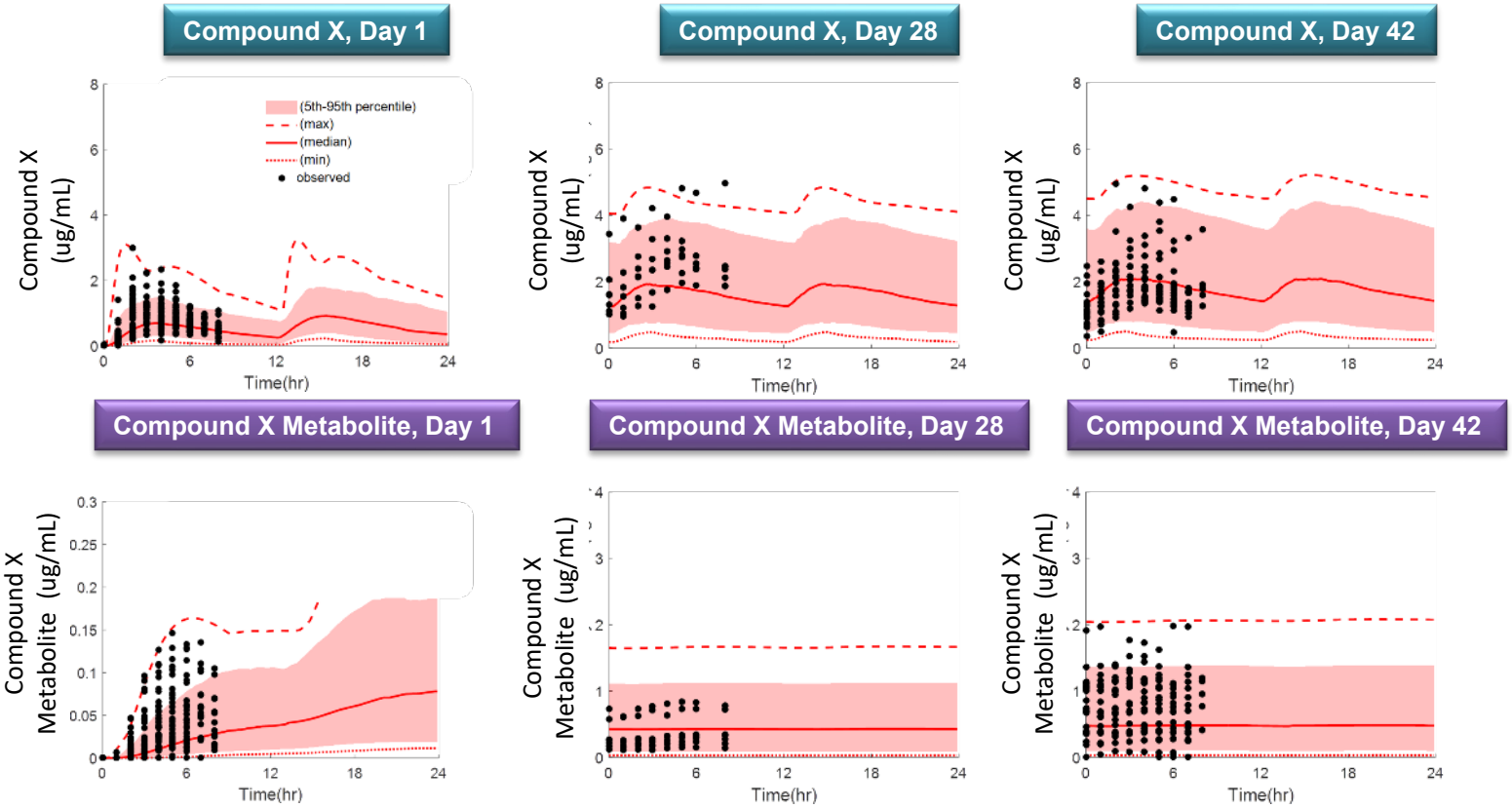
Custom Compound X PK SimPops Covers Observed Plasma Concentration Ranges

- Custom SimPops compared to data from studies several Compound X clinical studies
- Observed concentration ranges for Compound X and Compound X metabolite recapitulated by PK SimPops; some profiles extend beyond max and min values measured (by design)



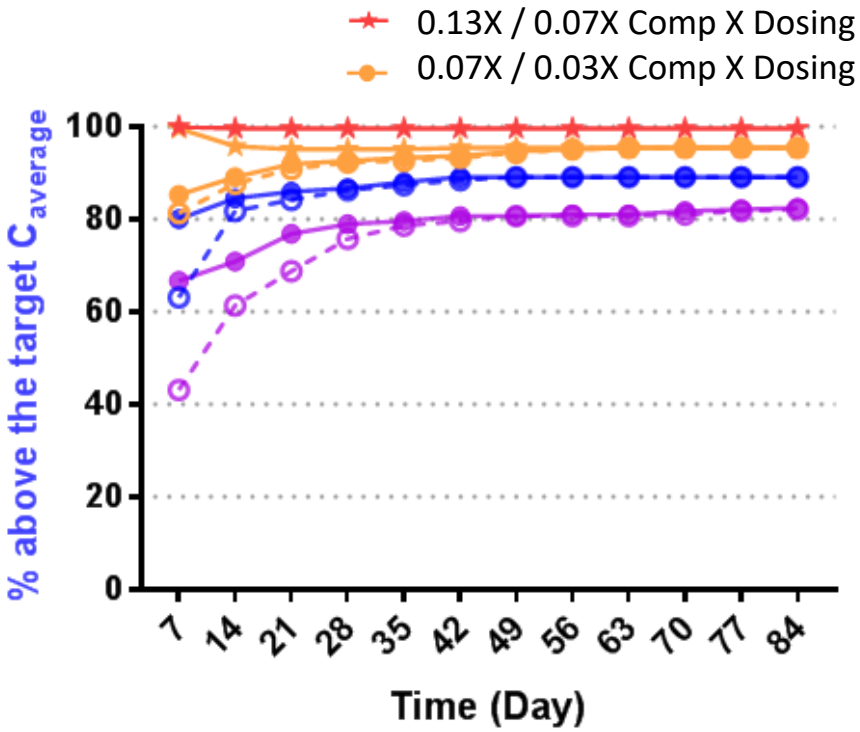
Simulation Results and Clinical Data

DILIsym Services
A SIMULATIONS PLUS COMPANY

Prospective Doses Identified Showing Good Compound X Exposure

- The percentage of the simulated population who achieved the target Compound X $C_{average}$ at the steady-state increases with increasing daily maintenance dose
  - Two protocols taken forward for safety simulations

- The higher the loading dose, the greater percentage of the simulated population achieving the target during the first few weeks



No Hepatotoxicity Predicted for Additional Prospective Clinical Protocols

- Compound X effects simulated in SimPops (n=285) that represent variability in toxicity mechanisms and PK
- DILIsym predicted delayed hepatotoxicity with varying grades for previous clinical protocols
- **No ALT elevations predicted for additional prospective clinical protocols**

	Comp X Protocol	Grade 1 (ALT 1-2.5X ULN*)		Grade 2 and above (ALT > 2.5X ULN)	
		Observed	Simulated†	Observed	Simulated†
Prospective	0.07X load / 0.03X steady, 32 weeks‡	-	0% (0/285)	-	0% (0/285)
Prospective	0.13X load / 0. 07X steady, 32 weeks‡	-	0% (0/285)	-	0% (0/285)
Previous	0.3X, 16 weeks	25% (13/52)	0.35% (1/285)	3.8% (2/52)	0.35% (1/285)
Previous	0.5X, 16 weeks	14% (1/7)	8.4% (24/285)	0% (0/7)	22.5% (64/285)
Previous	1X, 16 weeks	20% (1/5)	4.9% (14/285)	0% (0/5)	37.5% (107/285)

*upper limit of normal (ULN) in DILIsym is 40 U/L.

†SimPops™ Human_ROS_apop_mito_BA_v4A_1 (n=285) combined with Compound X PK variability used.

‡PROSPECTIVE clinical protocols

Simulation Results and Clinical Data

No clinical stop protocol

DILIsym Services

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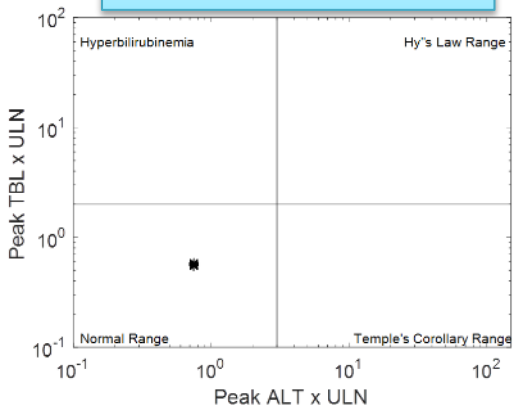
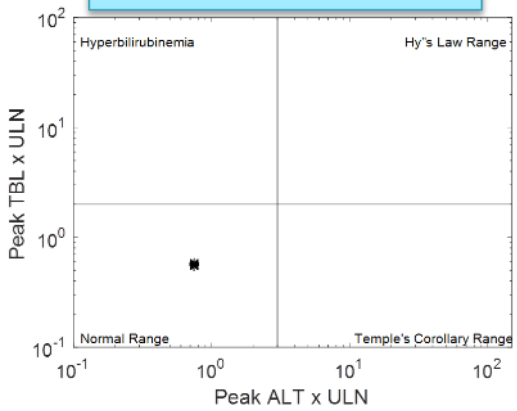
No Hepatotoxicity Predicted for Additional Prospective Clinical Protocols

- Dose dependent DILI frequency and severity predicted for Compound X – prospective dose levels clean
- Severity of response not appropriate to consider
  - Clinical stop protocol not included in simulations
  - DILIsym does not yet represent some likely key adaptation mechanisms like mitochondria biogenesis

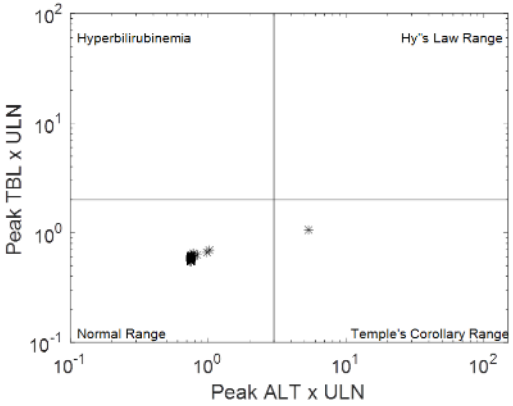
0.07X/0.03X Compound X Dosing



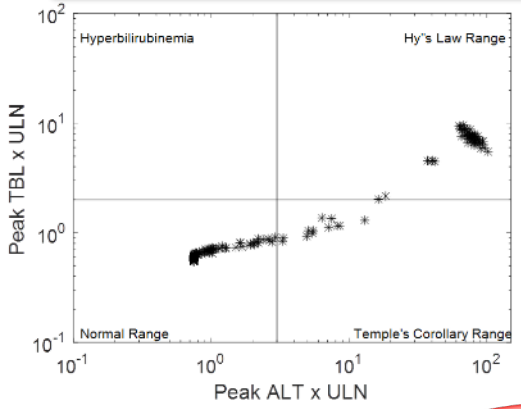
0.13X/0.07 Compound X Dosing



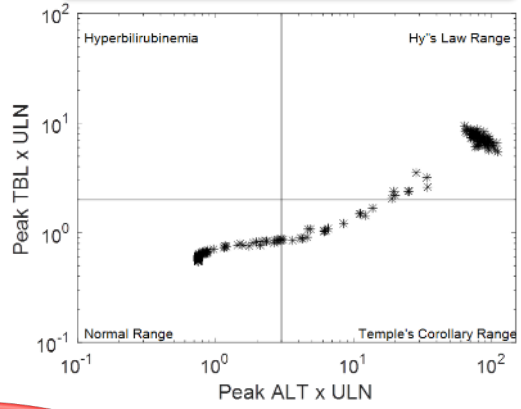
0.3X Compound X Dosing



0.5X Compound X Dosing



1X Compound X Dosing



Simulation Results

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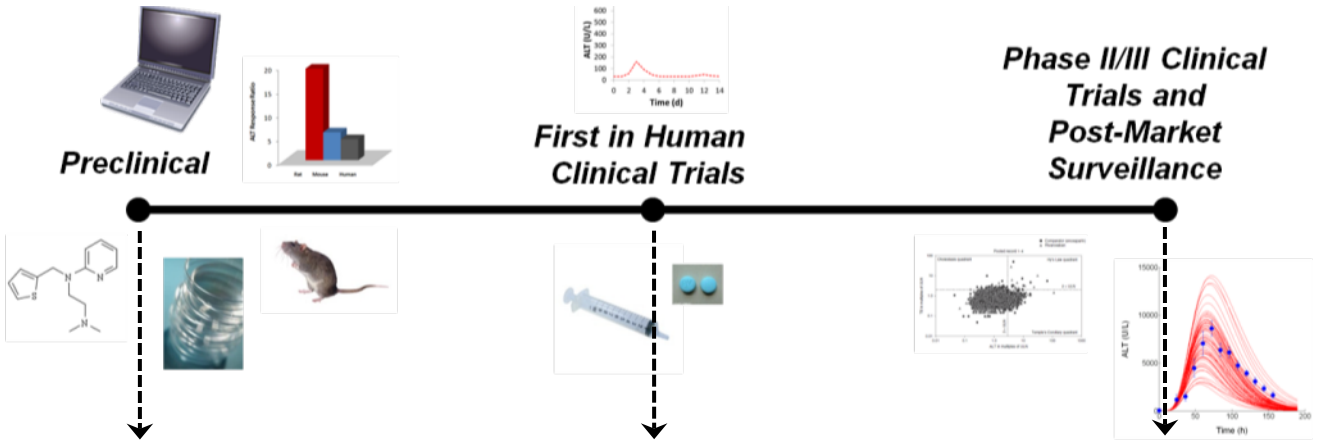
No clinical stop protocol

Applications of DILIsym Along the Drug Development Pipeline

DILIsym®

Predictions of hepatotoxicity for humans and preclinical animal models



Preclinical	First in Human Clinical Trials	Phase II/III Clinical Trials and Post-Market Surveillance
• Mechanism exploration • Rank candidates for DILI potential • Extrapolation from animal and in vitro findings to humans	• Dose optimization (risk versus presumed benefit) • Infer magnitude of injury based on measured biomarkers • Extrapolation from healthy volunteers to patient groups • Guide incorporation of emerging biomarker measurements in clinical trials • Analysis of mechanisms underlying observed liver signals	• Inform choice and timing of biomarker measurement • Aid identification of risk factors leading to personalized medicine approaches • Analysis of mechanisms underlying observed liver signals

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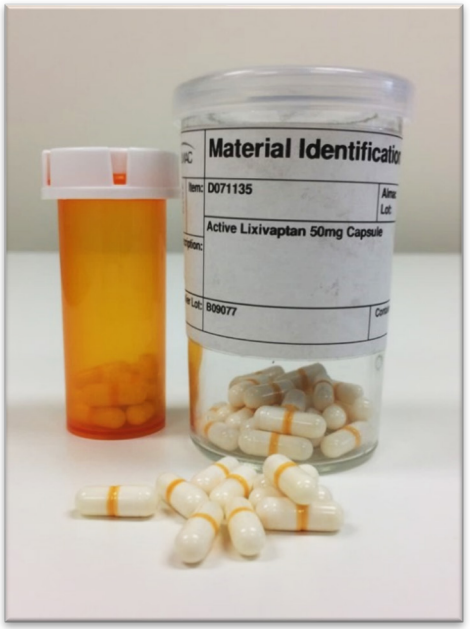
Lixivaptan Background

- Lixivaptan is Palladio Bio's selective, competitive vasopressin V2 receptor antagonist
- Lixivaptan was originally developed by others for the treatment of hyponatremia associated with heart failure and syndrome of inappropriate antidiuretic hormone secretion (SIADH)
- An NDA for lixivaptan was filed in 2011; development was terminated following receipt of a CRL in 2012
- Palladio Biosciences acquired lixivaptan and intends to reposition lixivaptan for the treatment of Autosomal-Dominant Polycystic Kidney Disease (ADPKD)



Lixivaptan DILIsym Project

DILI Background

- An approved compound in the same class had no DILI signals in hyponatremia, but signals were observed in ADPKD patients
- Lixivaptan has had no DILI signals in hyponatremia

Question

- Will lixivaptan experience similar DILI liability as the competitor in ADPKD patients?

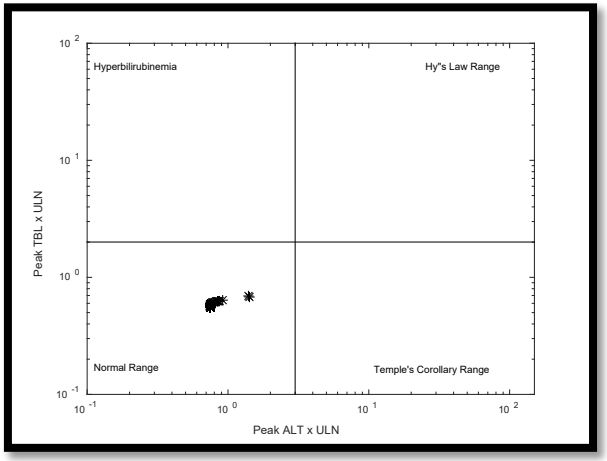
Approach

- Develop a mechanistic representation of lixivaptan in DILIsym, a QST model of drug-induced liver injury (DILI), to assess the potential for liver toxicity with the intended dosing for lixivaptan

Lixivaptan Project Executive Summary

- Simulations of lixivaptan dosing in custom SimPops of 285 simulated individuals with exposure variability show no ALT elevations (0/285 >2X ULN) at 200/100 mg BID dosing
- The DILIsym results suggest that lixivaptan is likely safer than the competitor
  - Competitor had significant ALT elevations at its clinical dose (simulated and clinically observed); lixivaptan simulations predict none

Simulated 200/100 mg dosing over 12 weeks in Custom SimPops of 285 with PK variability



Simulation Results

DILIsym Services

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Lixivaptan Simulations Predict Minimal ALT Elevations at 200/100 mg BID

- Lixivaptan simulated in the custom n=285 individual SimPops including PK variability
- No ALT elevations simulated in 100 mg BID 60-day simulation
  - Consistent with observed clinical similarity to placebo
- 7/285 (2.46%) of simulated individuals had ALT elevations with 400 mg BID for 7 days
  - Simulations more conservative than clinical data from a safety standpoint
- No ALT elevations simulated in 200/100 split daily dosing scenario for 12 weeks
  - Maximum intended clinical dosing for ADPKD
  - Highest simulated ALT = 57 U/L
- Dose escalation simulations suggest possible ALT elevations at doses beyond the intended maximum clinical dose (not shown)

Dose and Duration	Parameter Settings	Clinical ALT > 3x ULN	Simulated ALT >3X ULN*
100 mg BID for 60 days	Default measured#	On treatment similar to placebo**	0/285
400 mg BID for 7 days	Default measured#	0/67	7/285
200 / 100 mg for 12 weeks	Default measured#	Study not yet conducted	0/285

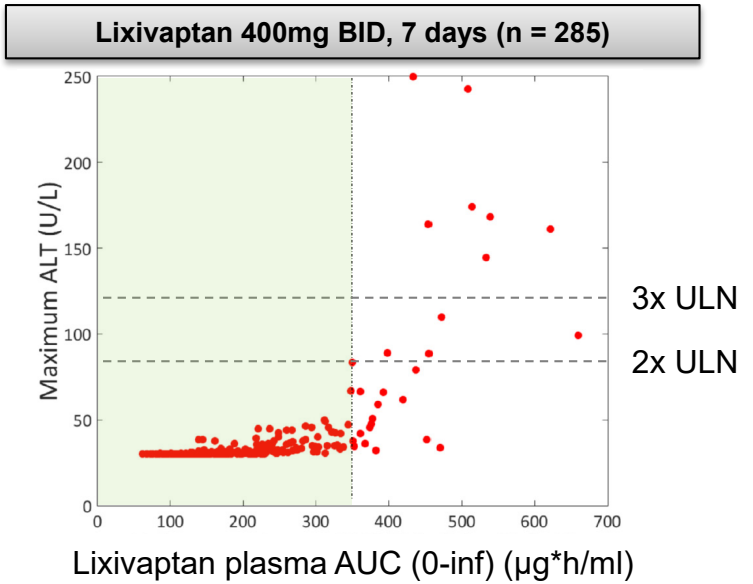
*Upper limit of normal (ULN) in DILIsym is 40 U/L

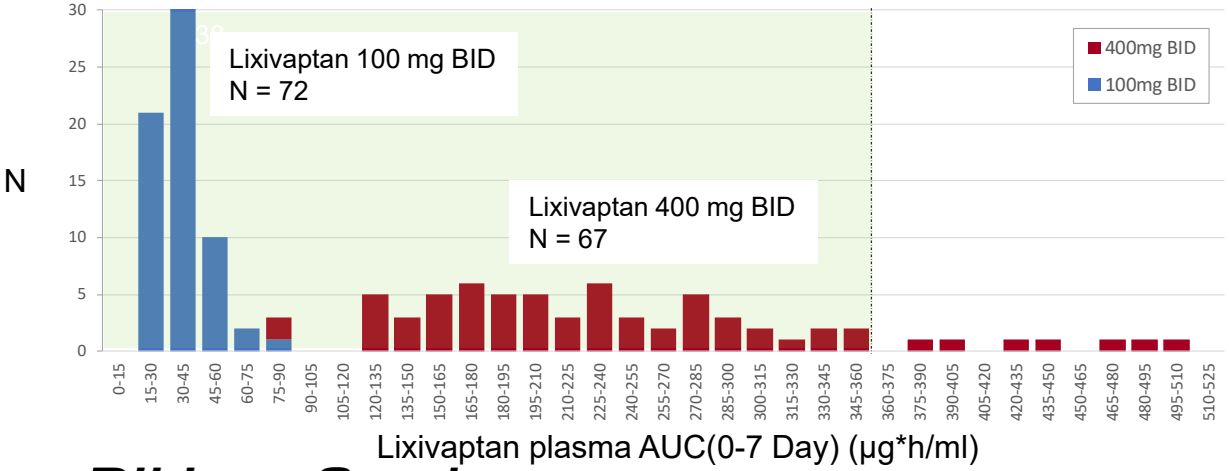
**In study CK-LX3401, 8/315 individuals in the treatment group had ALT > 200 U/L, compared to 6/319 in the placebo group; this was judged to not be a statistically significant increase in AEs due to lixivaptan treatment.

#Default assumption for BA inhibition is mixed inhibition type with $\alpha = 5$ in the absence of K_i studies, based on the experience of the DSS team.

Clinical Application – Dose Selection

- ALT elevations are correlated with total lixivaptan exposure
- Project established exposure threshold below which lixivaptan is safe ($AUC_{0\text{-}7\ days}$< 350 µg*h/ml)
- Existing data indicate lixivaptan exposure rarely exceeds the exposure threshold
- Intended clinical dose not expected to exceed threshold





Clinical Data and Simulation Results

DILIsym Services

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