Integrating Human Biomimetic Liver Microphysiology System with Quantitative Systems Toxicology Modeling to Predict DILI

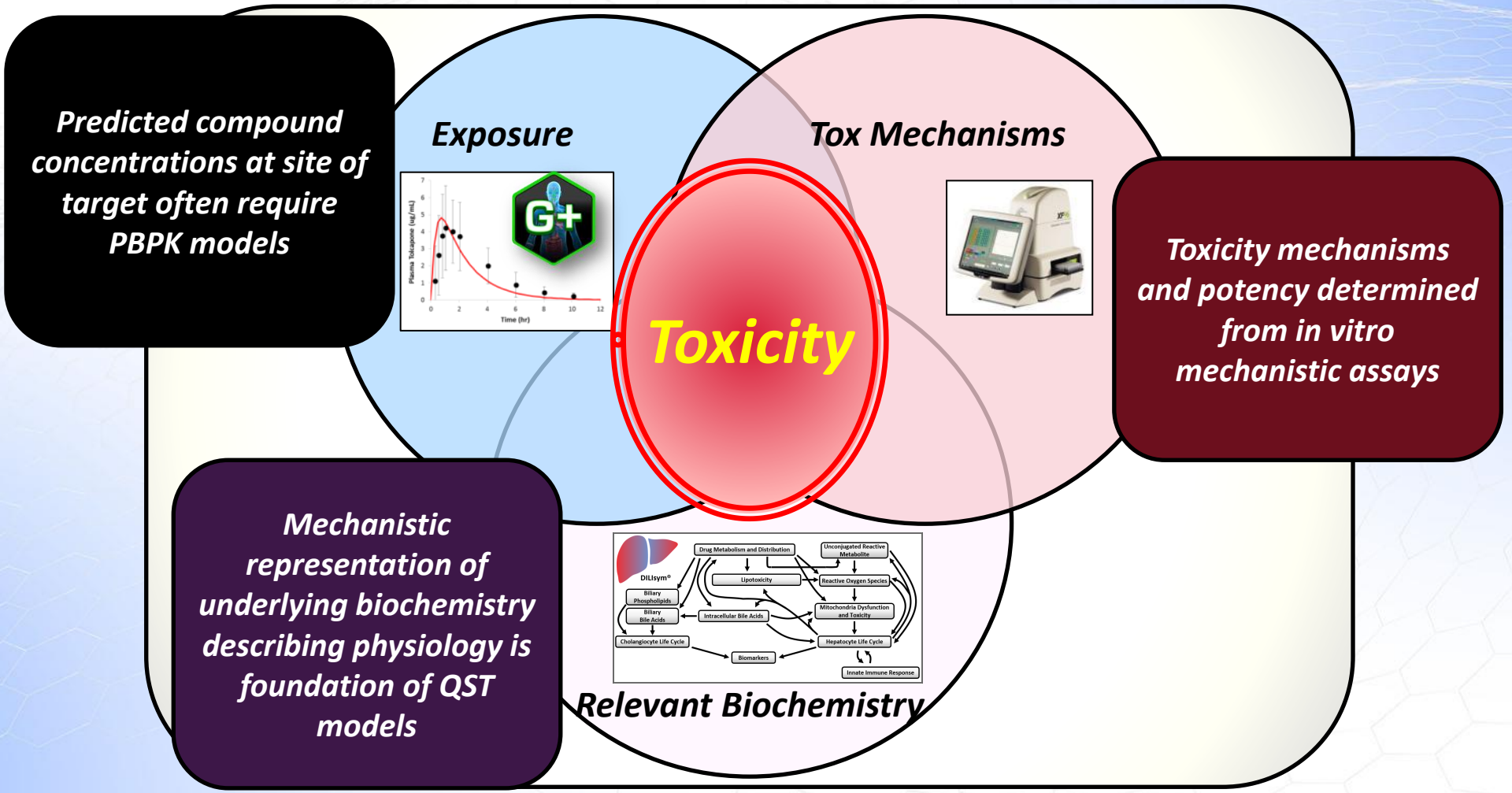
Kyunghee Yang

2024 Drug-Induced Liver Injury Conference

Agenda

- Quantitative systems toxicology (QST) modeling of DILI
  - Liver safety assessment of small molecules using DILIsym
- Application of QST modeling and liver microphysiology system in the liver safety assessment of biologics
  - Tocilizumab
  - Immune checkpoint inhibitors
- Conclusions and perspectives

QST Models Predict Toxicity via the Intersection Between Underlying Biochemistry, Compound Exposure, and Toxicity Mechanisms



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

Excellent Scientific Advisory Boards

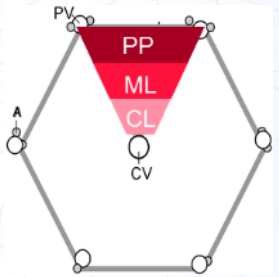
Current DILI-sim / RENAsym Members

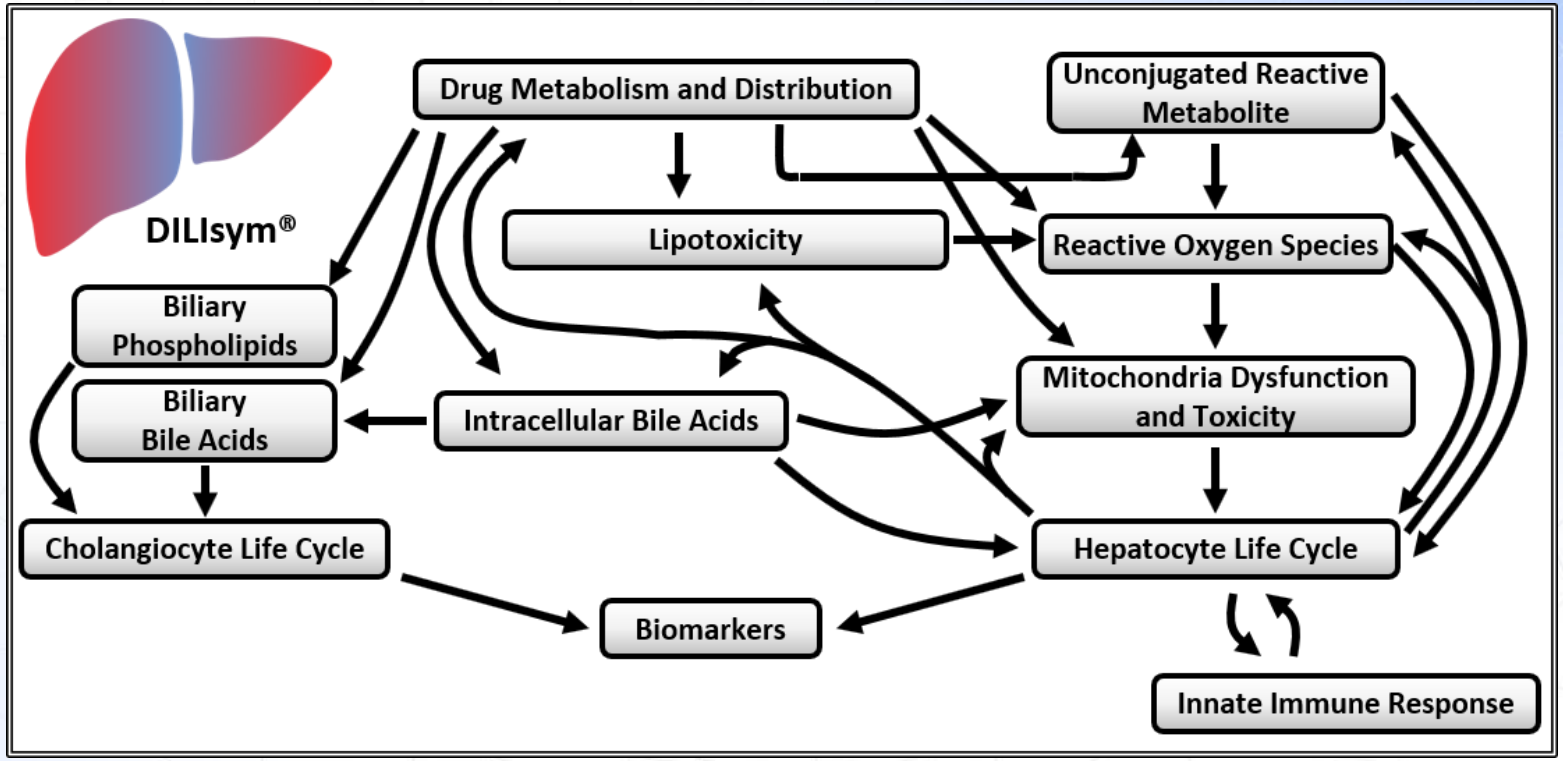
For a comprehensive review of progress, see *Watkins 2020, Current Opinion in Toxicology (23-24:67-73)*

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



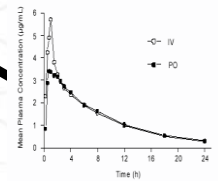


SimulationsPlus

DILIsym Utilizes Various Data Types to Inform Decisions

Exposure (PBPK modeling)

Pharmacokinetics



Mechanisms

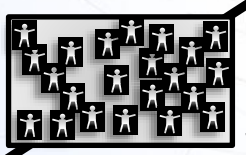
Bile Acid Transporter Inhibition

Mitochondrial Respiration

ROS Generation

Interpatient Variability

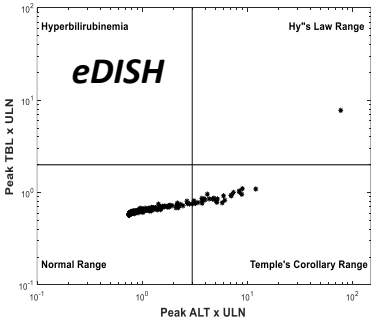
Unique Parameter Combinations

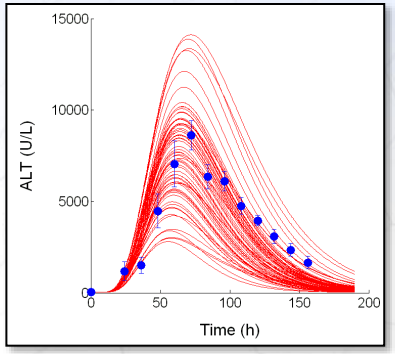


SimPops™

DILIsym®

Simulated Frequency & Severity of Liver Injury





SimulationsPlus

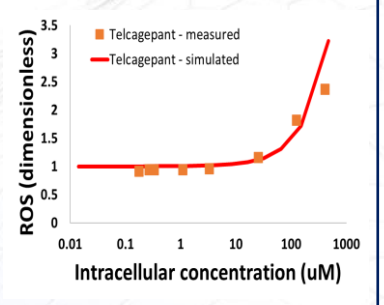
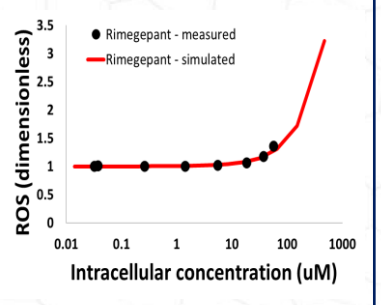
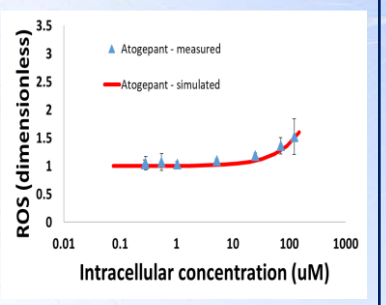
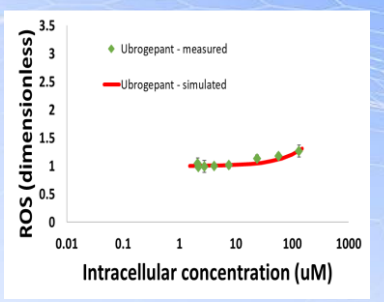
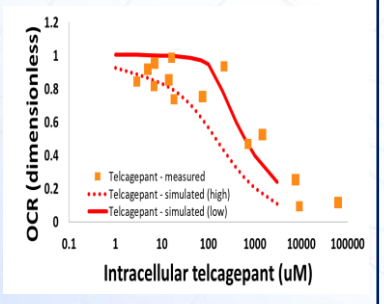
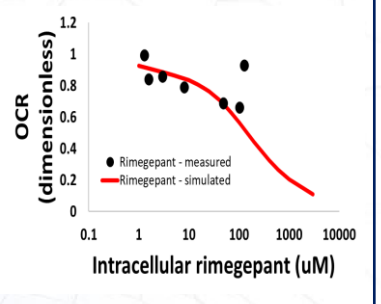
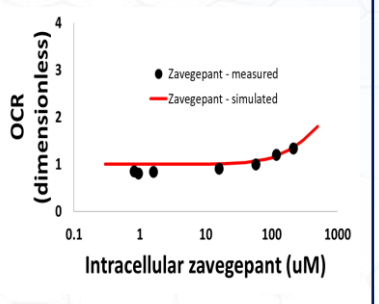
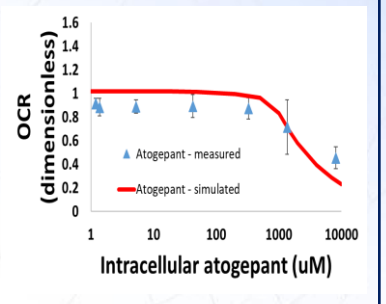
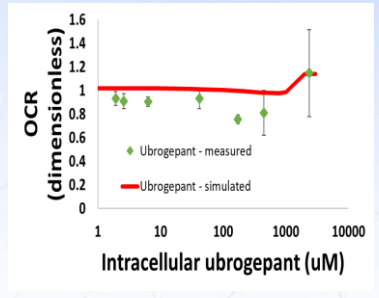
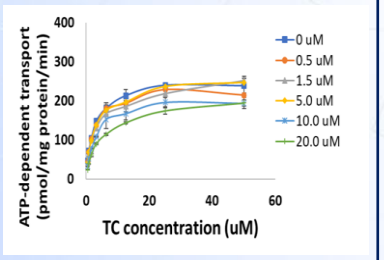
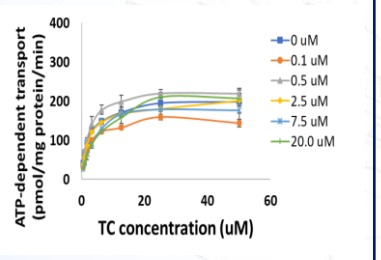
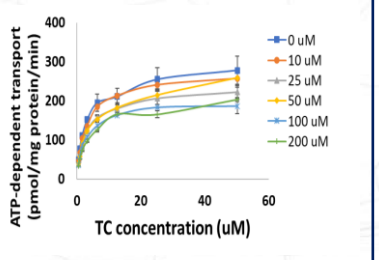
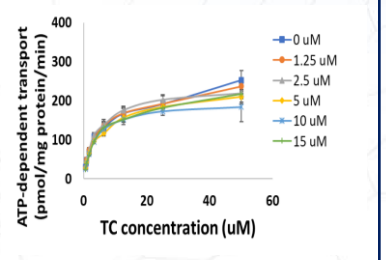
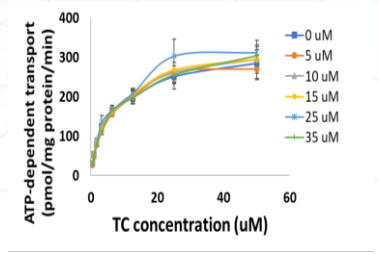
QST Modeling of CGRP Receptor Antagonists to Assess Liver Safety

- DILIsym simulations performed with telcagepant using clinical trial dosing protocols
  - Goal is to **recapitulate clinically observed toxicity**

- DILIsym simulations performed with rimegepant, zavegepant, atogepant, and ubrogepant
  - Goal is to **predict likelihood of toxicity**

Woodhead et al., Tox Sci (2022)

In Vitro Mechanistic Toxicity Signals Observed for Telcagepant, Rimegepant, Zavegepant, Atogepant, and Ubrogepant

Mechanism	In Vitro Assay	Telcagepant	Rimegepant	Zavegepant	Atogepant	Ubrogepant
Oxidative Stress	HepG2 cells; High content imaging			***No ROS Signal***		
Mitochondrial Dysfunction	HepG2 cells; Seahorse XF analyzer					
Bile Acid Transporter Inhibition	Membrane vesicles & transfected cells; Transport of taurocholate					

Woodhead et al., Tox Sci (2022)

SimulationsPlus

CGRP Receptor Antagonists Modeling Results

- DILIsym modeling **retrospectively** predicted liver toxicity for telcagepant consistent with clinical experiences
  - The mechanisms involved in the predicted liver injury for *telcagepan*t were mainly *inhibition of bile salt transport* and *mitochondrial ECT inhibition*

- DILIsym **prospectively** predicted liver safety for rimegepant, zavegepant, atogepant, and ubrogepant at clinically relevant doses
  - Liver safety confirmed by clinical trials, validating model prediction

Woodhead et al., Tox Sci (2022)

Liver Safety of Ubrogepant Confirmed in Clinical Trials

Original Article

Cephalalgia
An International Journal of Headache

International Headache Society

Cephalalgia
2019, Vol. 39(14) 1753–1761

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Safety and tolerability of ubrogepant following intermittent, high-frequency dosing: Randomized, placebo-controlled trial in healthy adults

Peter J Goadsby[1], Stewart J Tepper[2], Paul B Watkins[3], Girma Ayele[4], Rosa Miceli[4], Matthew Butler[4], Lawrence Severt[4], Michelle Finnegan[4], Armin Szegedi[4], Joel M Trugman[4] and Abhijeet Jakate[4]

No significant liver signals shown at one of the simulated dosing protocols: 100 mg QD, 2 days on, 2 days off, for 56 days (28 total doses)

Table 3. Hepatic laboratory parameters.

	Placebo (n = 260)	Ubrogepant 100 mg (n = 256)
ALT, U/L	n = 258	n = 256
Baseline, mean (SD)	20.5 (7.2)	21.1 (9.1)
End of trial, mean (SD)	21.7 (7.7)	21.3 (8.7)
Change from baseline, mean (SD)	1.2 (7.4)	0.1 (8.4)
Post baseline ≥ 3 × ULN, n (%)	3 (1.2)	2 (0.8)

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DILIsym Supported Dose Optimization of Fezolinetant

FDA Approves Fezolinetant (VEOZAH), a First-of-Its-Kind Nonhormonal Drug for Hot Flashes [1]

May 15, 2023

FDA NDA review [2]:

"*Prior to Phase 3 development*, Astellas conducted Quantitative systems Toxicology (QST) modeling (DILIsym) which predicted the adverse hepatic findings observed phase 2 dosing. Based on these reported findings, phase 3 fezolinetant development was limited to 30 and 45 mg fezolinetant dosage strengths".

1).https://www.everydayhealth.com/menopause/fda-approves-fezolinetant-first-of-its-kind-non-hormonal-drug-for-hot-flashes/

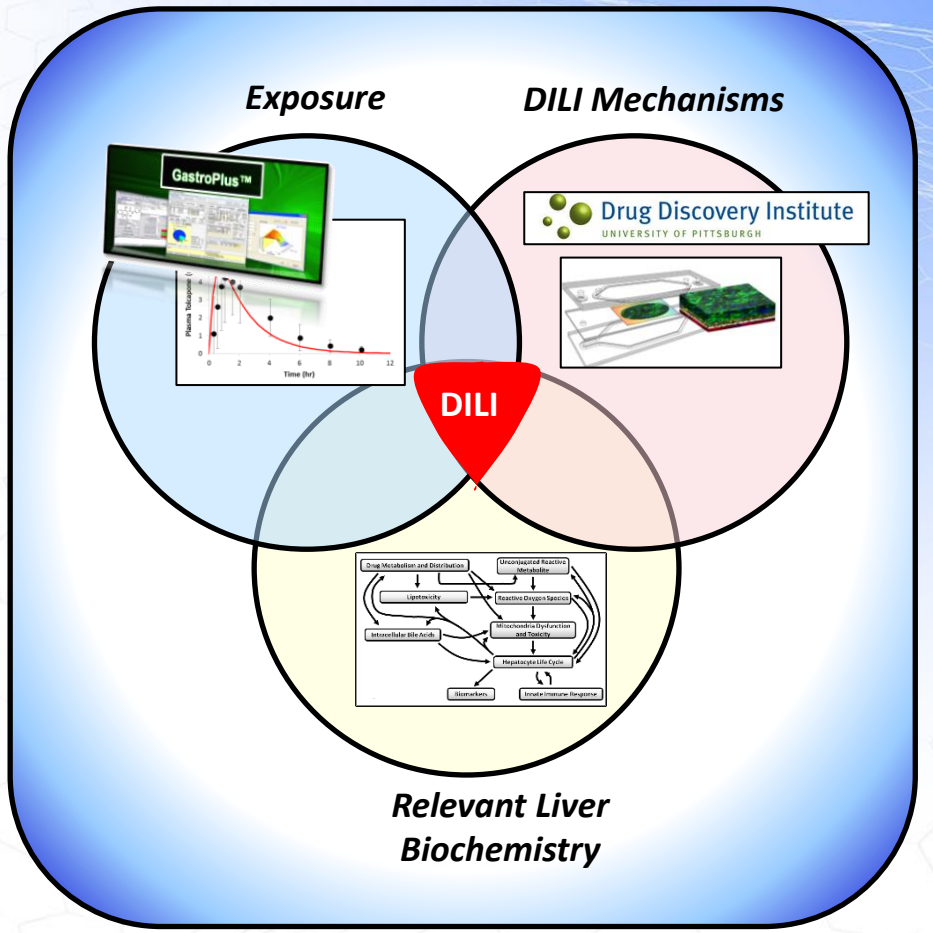
2).https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=BasicSearch.process

Agenda

- Quantitative systems toxicology (QST) modeling of DILI
  - Liver safety assessment of small molecules using DILIsym
- Application of QST modeling and liver microphysiology system in the liver safety assessment of biologics
  - Tocilizumab
  - Immune checkpoint inhibitors
- Conclusions and perspectives

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

- BIOLOGXsym is a mechanistic, mathematical model which is being developed to identify biologics-induced liver injury liabilities in new biologic drug candidates and predict clinical liver injury outcomes
  - Represents mechanistic pathways specific to biologics such as receptor-mediated indirect responses and target-mediated effects
  - *Collaborative efforts between DILIsym Services and University of Pittsburgh Drug Discovery Institute (UPDDI) were made to leverage data from mechanistic experiments in a human liver biomimetic (LAMPS)*
- Phase 1 development supported by NIH Small Business Innovation Research (SBIR) grant was completed successfully
  - A prototype BIOLOGXsym model was developed
  - Two exemplar compounds, GGF2 and tocilizumab, were represented in BIOLOGXsym to show proof-of-concept predictions of BILI response
- Phase 2 SBIR grant for continued development of BIOLOGXsym has been awarded
  - Twelve exemplar compounds including immune checkpoint inhibitors are being tested



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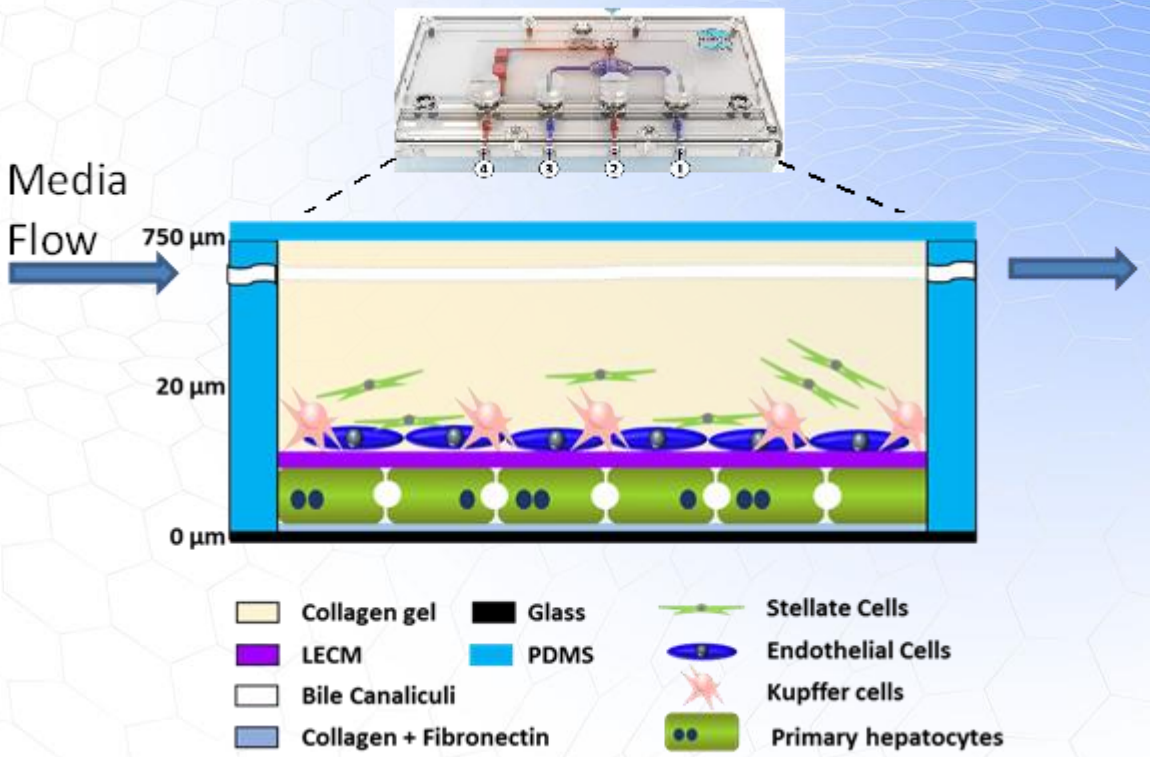
The Liver Acinus Microphysiology System (LAMPS) Provides Mechanistic Inputs for BIOLOGXsym

- Organ-on-a-chip microphysiological systems have emerged as a powerful platform to mimic a particular human tissue, organ, and multiple organs for drug discovery and drug development
- LAMPS is a human biomimetic liver model that includes four key liver cells
  - Hepatocytes, stellate cells, liver sinusoidal endothelial cells, Kupffer cells
  - Structurally organized as a liver sinusoidal unit; 10-14 day functionality
  - Recapitulates periportal to perivenous oxygen and metabolic zonation



Frevert (2005) PLoS Biol

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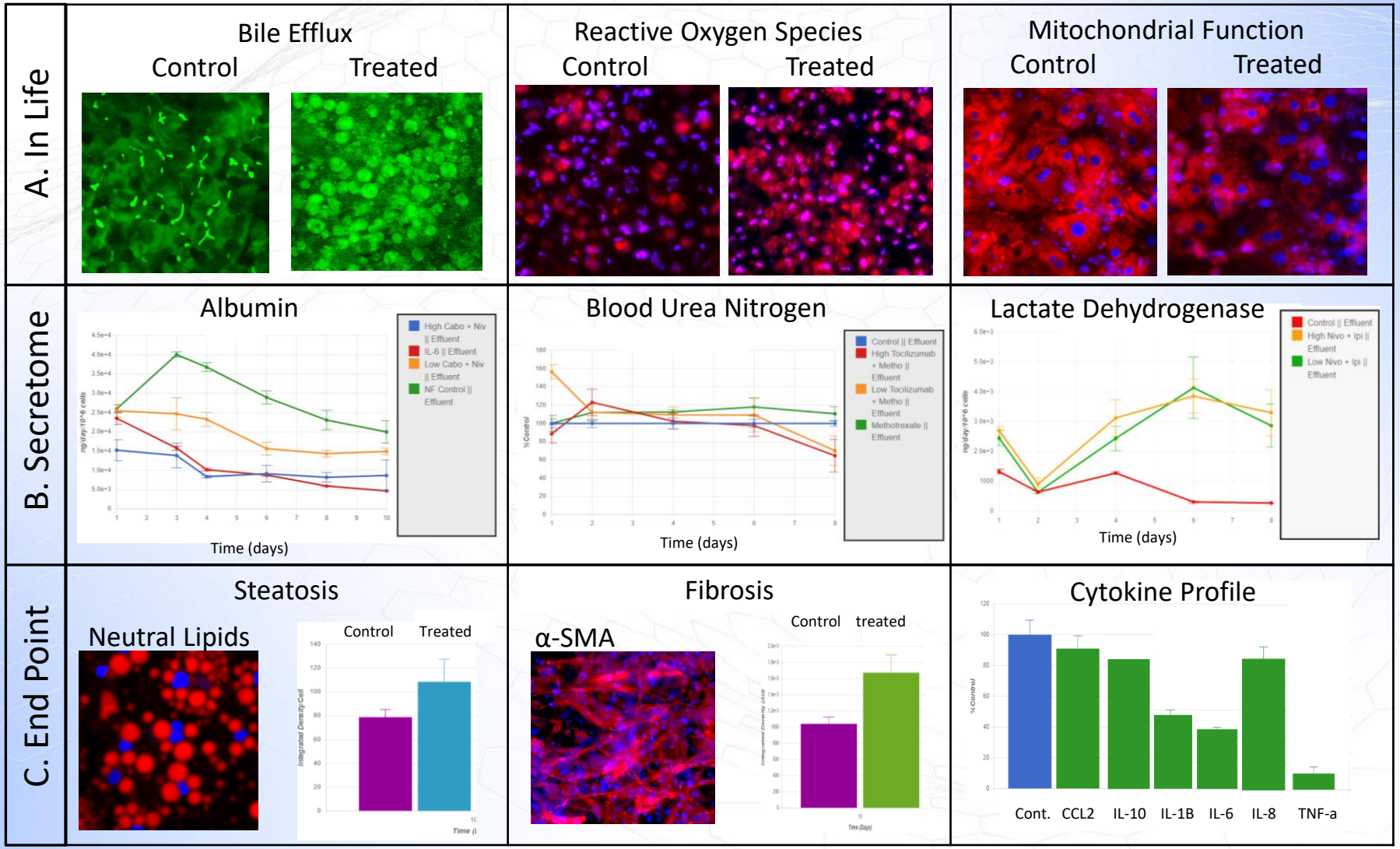

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Frequent Mild Liver Injury Associated with Tocilizumab

- Tocilizumab (TCZ), a humanized mAb to IL-6R, approved for various autoimmune or inflammatory diseases, is associated with modest ALT elevations
- Meta-analysis including five phase 3 studies demonstrates relatively frequent ALT elevations >1x ULN but less frequent >3x or >5x ULN
  - For patients undergoing dose reduction, most continued therapy
- Relatively rare case studies identified for severe liver injury, sometimes after months to years of TCZ treatment

Table 6 Changes in ALT/AST values from normal at baseline to highest value in the all-control and in the all-exposed population

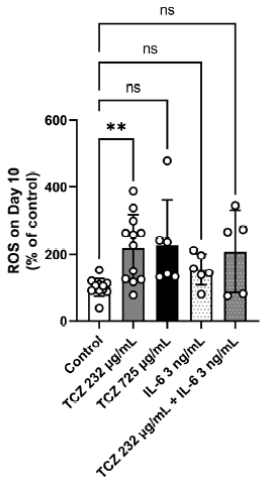
	Controlled, double-blind study population					All-exposed population
	Tocilizumab 8 mg/kg monotherapy, % (*n*) *n* = 288	Methotrexate (control), % (*n*) *n* = 284	Tocilizumab 4 mg/kg + DMARDs, % (*n*) *n* = 774	Tocilizumab 8 mg/kg + DMARDs, % (*n*) *n* = 1,582	DMARD monotherapy, % (*n*) *n* = 1,170	Tocilizumab, % (n/n) *n* = 4,009[c]
ALT,[a] *n* = normal at baseline	*n* = 269	*n* = 269	*n* = 706	*n* = 1,465	*n* = 1,080	
> 1-3× ULN	33.8 (91)	32.0 (86)	42.8 (302)	45.9 (672)	19.1 (206)	57.3 (2,112/3,696)
> 3-5× ULN	1.1 (3)	2.6 (7)	4.0 (28)	4.3 (63)	0.8 (9)	7.2 (267/3,696)
> 5× ULN	0.7 (2)	1.1 (3)	1.0 (7)	1.4 (20)	0.3 (3)	2.2 (83/3,696)
AST,[a] *n* = normal at baseline	*n* = 283	*n* = 269	*n* = 743	*n* = 1,502	*n* = 1,123	
> 1-3× ULN	20.8 (59)	24.9 (67)	32.4 (241)	38.8 (583)	14.5 (163)	51.4 (1,961/3,818)
> 3-5× ULN	0.4 (1)	1.1 (3)	0.9 (7)	1.5 (23)	0.3 (3)	2.6 (98/3,818)
> 5× ULN	0.7 (2)	0.4 (1)	–	0.2 (3)	0.1 (1)	0.6 (22/3,818)
Dose held[b]	8.0 (23)	9.9 (28)	2.5 (19)	2.5 (39)	0.7 (8)	10.3 (413/4,009)
Discontinued	0.3 (1)[b]	1.4 (4)[b]	1.3 (10)[b]	1.3 (21)[b]	0.2 (2)[b]	2.3 (91/4,002)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMARD, disease-modifying antirheumatic drug; ULN, upper limit of normal. [a]Percentages are based on number of patients with normal ALT (or AST) at baseline. [b]Percentages are based on total treatment-group sample size. [c]Excluding patients with missing values.

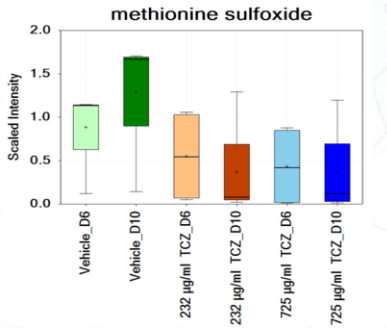
Schiff 2011

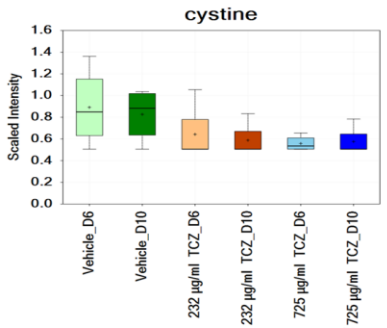
LAMPS Data Indicated Tocilizumab-Induced Oxidative Stress

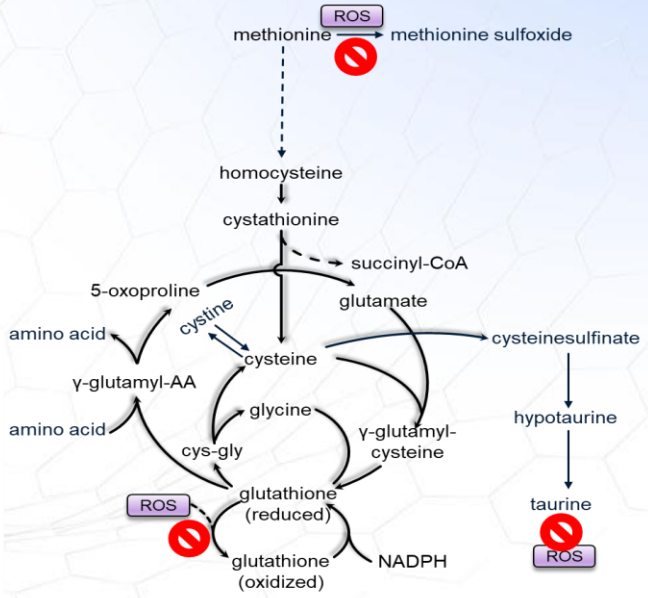
- Tocilizumab was tested at 1.6 µM and 5 µM in the LAMPS models under continuous media flow for 10 days
  - 1.6 µM is the human Cmax at the IV dose of 8 mg/kg
- Tocilizumab significantly increased production of RNS/ROS
  - Not reversed by co-incubation with IL-6
- Metabolomics profiling of the LAMPS secretome showed persistent and significant alterations in several metabolic markers of oxidative stress
- Tocilizumab did not significantly change bile acid handling and mitochondrial function in LAMPS assays



Sub Pathway	Biochemical Name	232 µg/ml TCZ D6 Vehicle_D6	232 µg/ml TCZ D10 Vehicle_D10	725 µg/ml TCZ D6 Vehicle_D6	725 µg/ml TCZ D10 Vehicle_D10
Methionine, Cysteine, and Taurine Metabolism	methionine	0.58	0.43	0.45	0.41
	methionine sulfone	0.54	0.29	0.44	0.29
	methionine sulfoxide	0.48	0.22	0.38	0.22
	cystathionine	0.53	0.21	0.35	0.12
	cysteine	1.11	1.25	0.44	0.41
	cystine	0.63	0.63	0.55	0.62
	taurine	4.06	3.51	1.02	2.46
Glutathione Metabolism	cysteine-glutathione disulfide	0.34	0.38	0.43	0.45
	5-oxoproline	0.74	0.70	0.45	0.40
Gamma-glutamyl Amino Acid	gamma-glutamylhistidine	0.86	0.62	0.61	0.60
	gamma-glutamylisoleucine*	0.57	0.24	0.41	0.22
	gamma-glutamylleucine	0.78	0.58	0.59	0.53
Nicotinate and Nicotinamide Metabolism	quinolinate	12.55	16.96	2.06	5.66
	nicotinate	45.24	57.07	1.54	18.58
	nicotinamide adenine dinucleotide (NAD+)	1.00	1.00	1.22	1.00





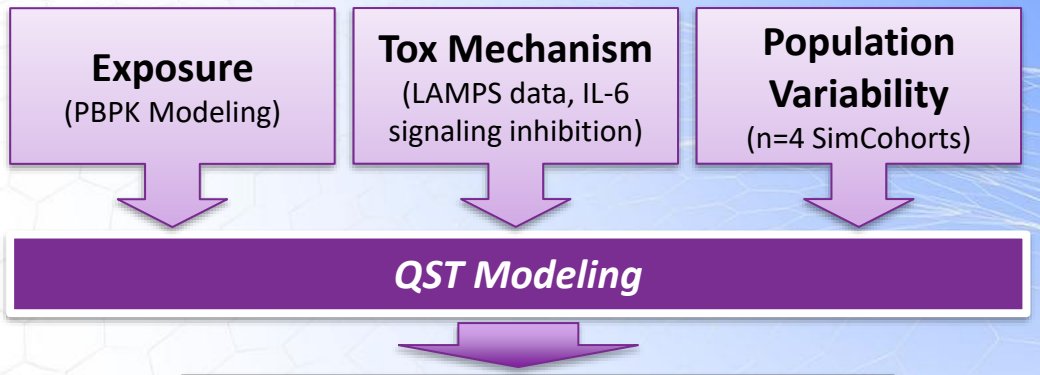


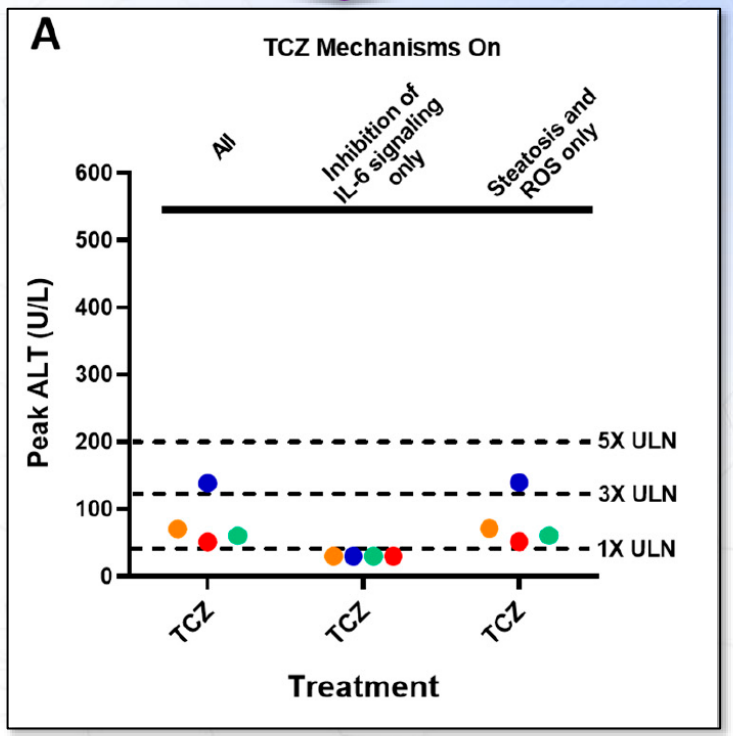
Beaudoin et al., IJMS (2023)

SimulationsPlus

BIOLOGXsym Simulations Recapitulated Clinically Observed Modest ALT Elevations by Tocilizumab

- Tocilizumab-mediated hepatotoxicity was simulated within BIOLOGXsym by integrating:
  - Tocilizumab clinical exposure which as simulated by PBPK modeling using GastroPlus (i.e., IV 8 mg/kg Q2 weeks)
  - Tocilizumab-mediated oxidative stress parameters optimized to the LAMPS data
  - Tocilizumab-mediated inhibition of major downstream effects of IL-6 signaling (i.e., hepatocyte regeneration, macrophage recruitment, CYP suppression)
  - Population variability in a small SimCohorts (N=4)
- Tocilizumab proof-of-concept simulations with clinical dosing protocol predicted modest ALT elevations within ~2 weeks of treatment initiation, consistent with clinical data
  - Attributed to tocilizumab-mediated oxidative stress





Beaudoin et al., IJMS (2023)

SimulationsPlus

Frequent Mild Liver Toxicity Signals During Ipilimumab or Nivolumab Administration

- High doses of ipilimumab (anti-CTLA-4 mAb) demonstrate frequent, mild (grade 1-2) and severe (grade 3+) liver adverse events
  - Lower dose ipilimumab has less frequency of hepatic adverse events
  - Some evidence for dose-dependent ipilimumab-induced adverse events (Wolchok 2010)
- Nivolumab (anti-PD-1 mAb) also induces frequent mild liver toxicity signals
  - Some ALT elevations seen at all dose levels, but severe reactions relatively rare (NCT00730639, not shown here)
  - Case studies identified for severe injury ALT profiles (Matsubara 2018, Imoto 2019, Imafuku 2017)

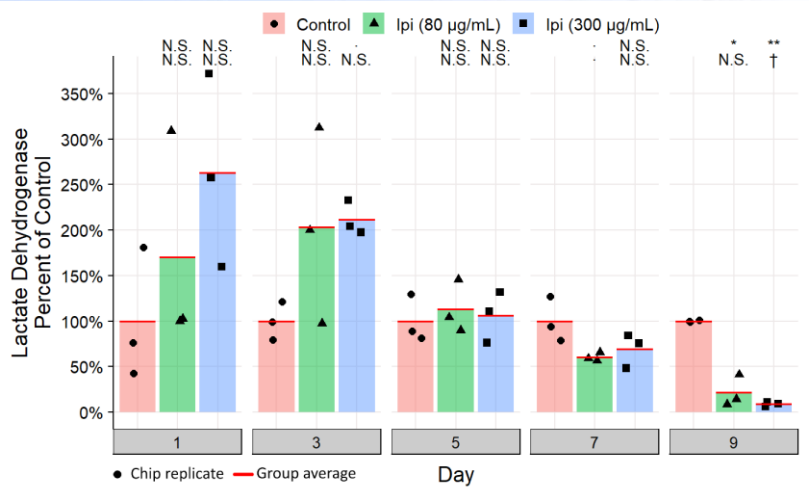
Study details		Any-grade adverse events (grade ≥3 adverse events)							
Study	**Dose (*n*)**	**Diarrhoea**	**Colitis**	**Pulmonary**	**Rash**	**Neurological**	**Endocrinopathy**	**Hepatic**	**Renal**
Ipilimumab									
EORTC 18071 (ref.[17])	10 mg/kg, 3-weekly (471)	41.2% (9.8%)	15.5% (8.2%)	–	34.2% (1.1%)	4.5% (1.9%)	37.8% (7.8%)	24.4% (10.9%)	–
Hodi et al.[166]	3 mg/kg, 3-weekly (131)	27.5% (4.6%)	7.6% (5.3%)	–	19.1% (0.8%)	–	7.6% (3.8%)	3.8% (0%)	–
Nivolumab									
CheckMate 066 (ref.[21])	3 mg/kg, 2-weekly (206)	16% (1%)	1% (0.5%)	1.5% (0%)	15% (0.5%)	–	7.3% (1%)	3.4% (1.5%)	1.9% (0.5%)
CheckMate 057 (ref.[167])	3 mg/kg, 2-weekly (287)	8% (1%)	1% (0.3%)	4.9% (1.4%)	9% (3.5%)	0.3% (0.3%)[a]	10.5% (0%)	10.8% (1.4%)	2% (0%)

Martins 2019

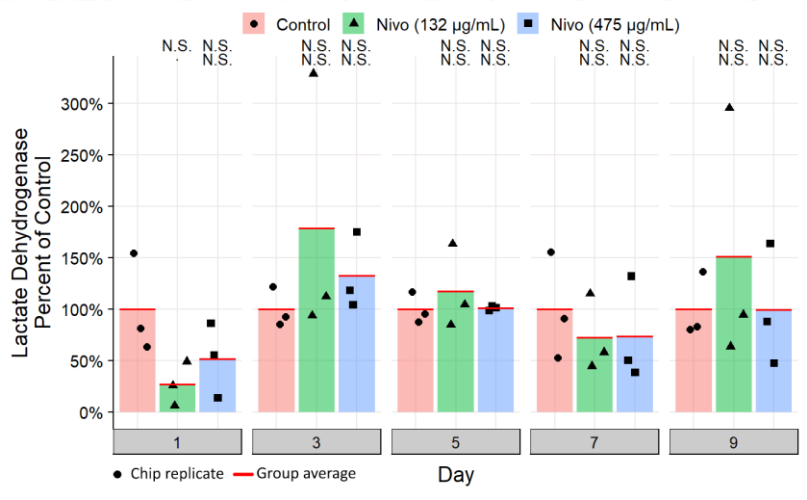
LAMPS Assays Show Synergistic Toxicity Signals for Ipilimumab and Nivolumab

- Ipilimumab (80 and 300 µg/mL) and nivolumab (132 and 475 µg/mL) were tested in the LAMPS models under continuous media flow for 10 days
  - 80 and 300 µg/mL are human Cmax values at the IV dose of 3 and 10 mg/kg ipilimumab, respectively
  - 132 and 475 µg/mL are human Cmax values at the IV dose of 3 and 10 mg/kg nivolumab, respectively
- Synergistic LDH increase was observed with administration of ipilimumab + nivolumab when compared to monotherapy, consistent with clinical findings

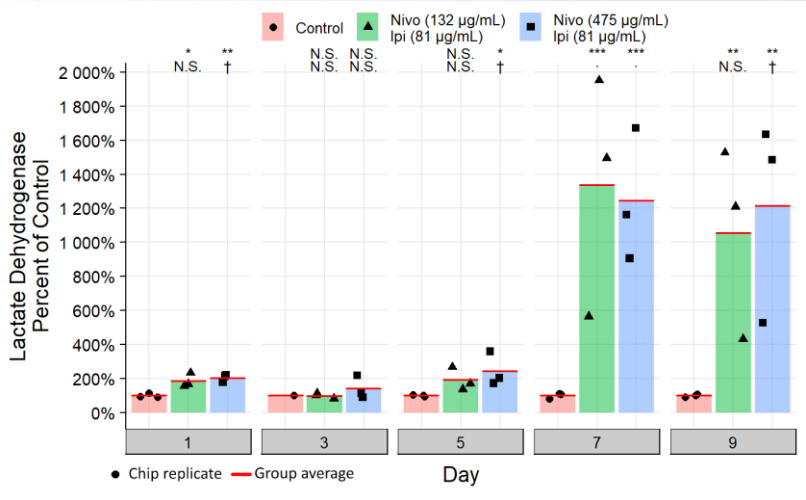
Ipilimumab



Nivolumab



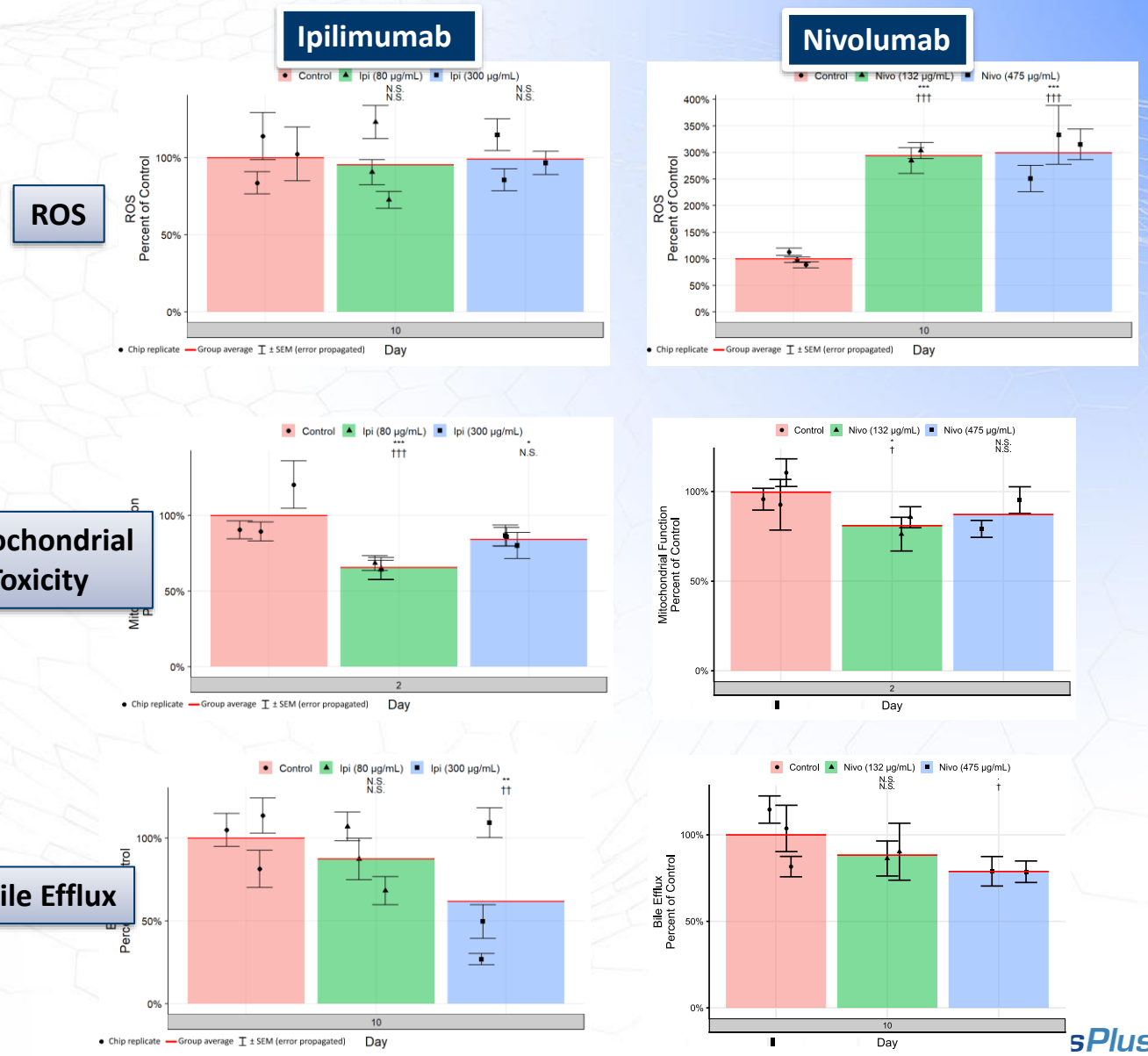
Ipilimumab + Nivolumab



SimulationsPlus

LAMPS Assays Show Hepatocyte Stress Signals for Ipilimumab and Nivolumab

- LAMPS experimental outputs demonstrate early hepatocyte stress signals and mechanisms for ipilimumab and nivolumab
- Ipilimumab significantly decreased mitochondrial function and bile efflux
- Nivolumab significantly increased ROS and decreased mitochondrial function and bile efflux



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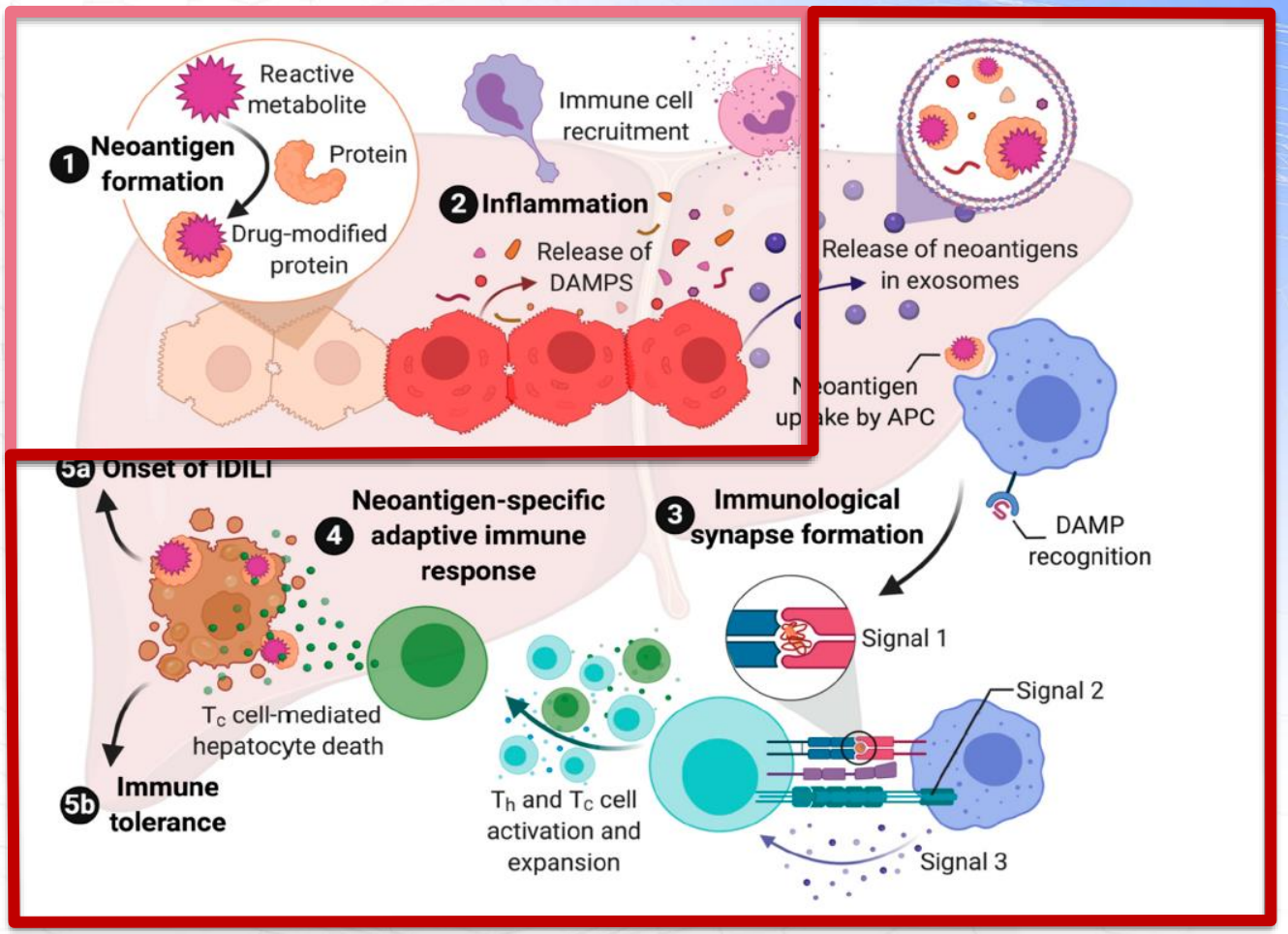
LAMPS Assays Show Hepatocyte Stress Signals for Ipilimumab and Nivolumab

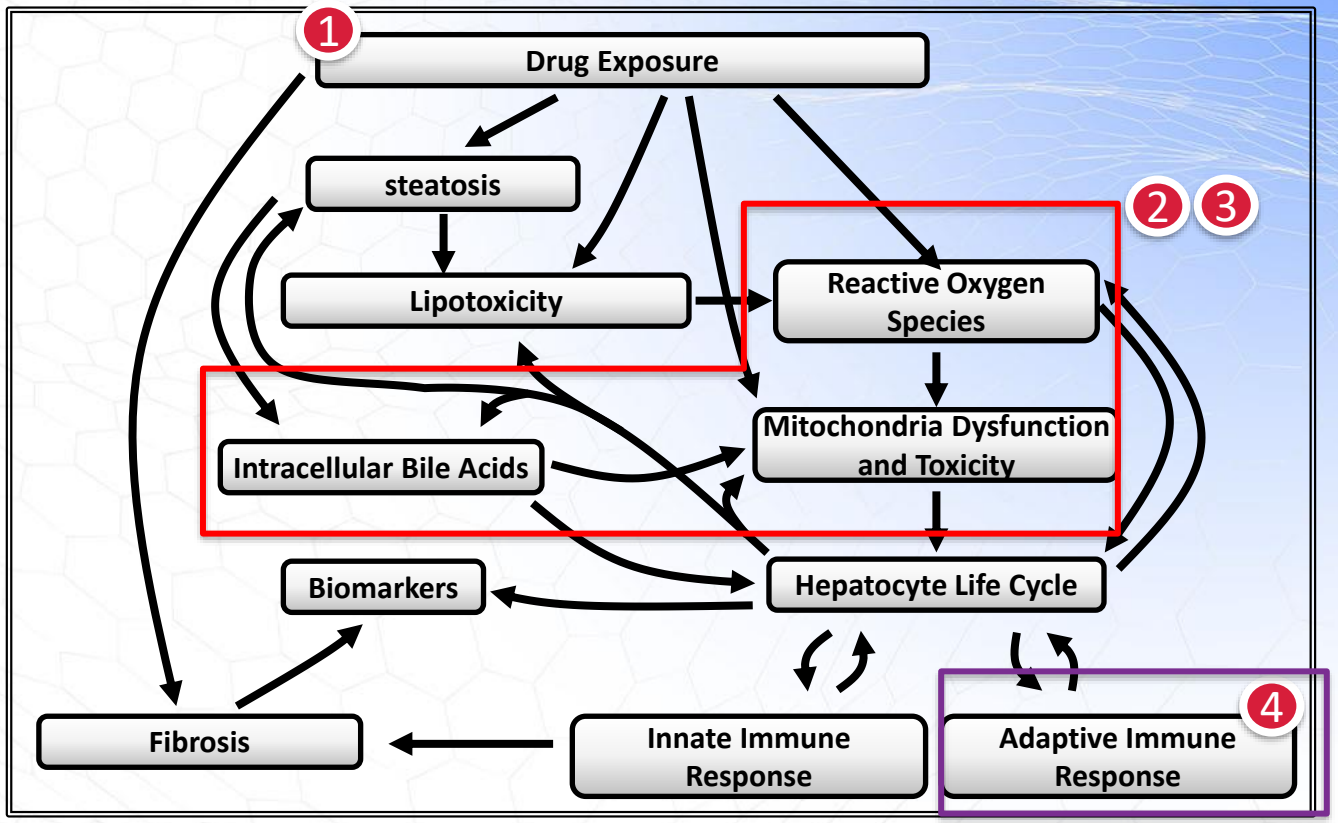
- LAMPS data will be incorporated in BIOLOGXsym to represent hepatocyte stress signals, which set the stage for a potential adaptive immune attack by altering the liver micro-environment to be less tolerogenic and more inflammatory
  - ***Hypothesis: immune checkpoint inhibitors can induce low-level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



Uetrecht et al. (2021) Int J Mol Sci

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

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



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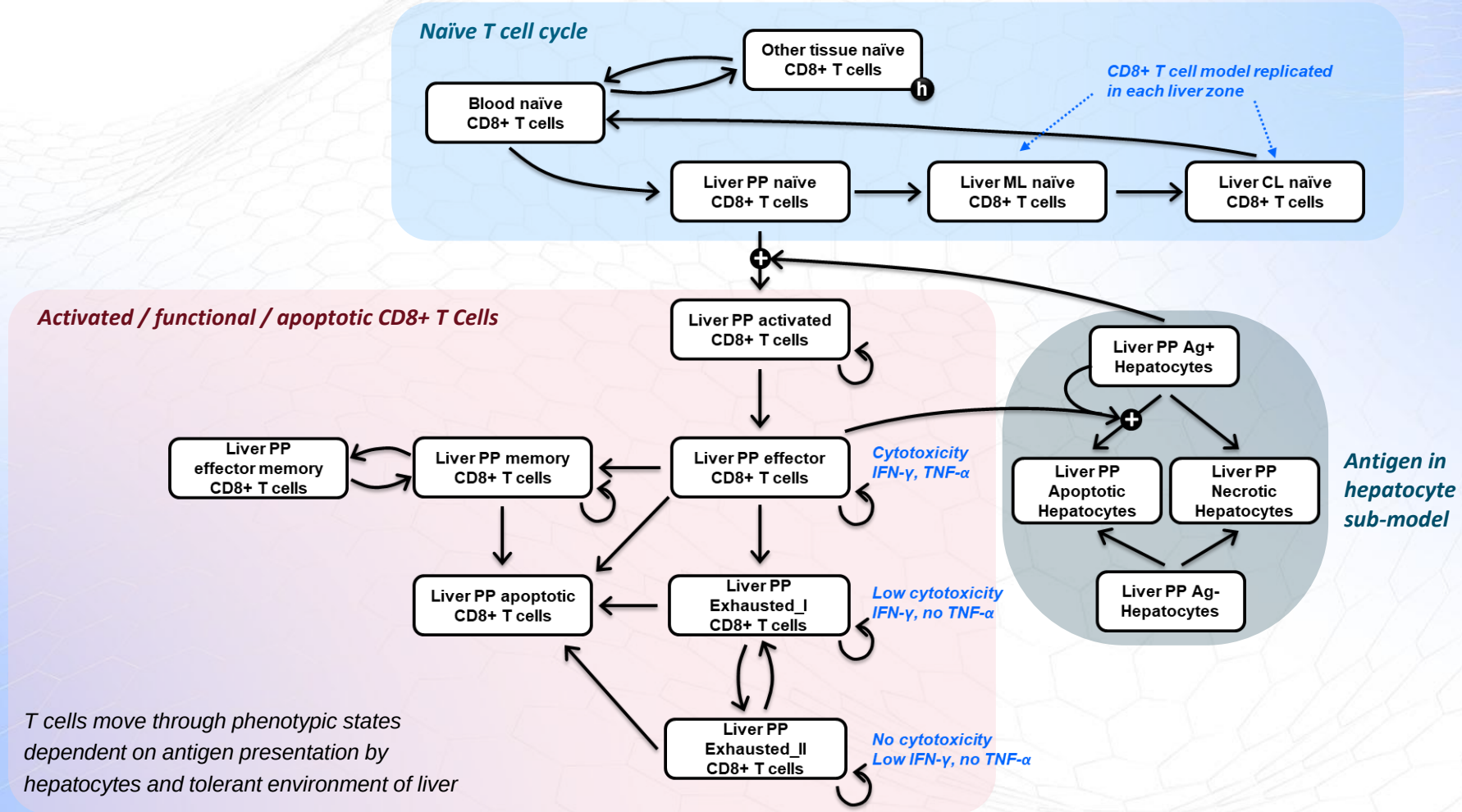
CD8+ T Cell Representation Is Being Developed in BIOLOGXsym to Investigate Requirements for T cell Cytotoxicity to Explain ICI Hepatitis



Not all modeled links shown in diagram, for visual clarity

Agenda

- Quantitative systems toxicology (QST) modeling of DILI
  - Liver safety assessment of small molecules using DILIsym
- Application of QST modeling and liver microphysiology system in the liver safety assessment of biologics
  - Tocilizumab
  - Immune checkpoint inhibitors
- Conclusions and perspectives

Conclusions and Perspectives

- *In vitro* human microphysiology systems can further improve our mechanistic understanding about hepatotoxicity mediated by biologics

- QST modeling that integrates known biochemistry/physiology, *in vitro* mechanistic data, and dynamic exposure can help elucidate DILI mechanisms and evaluate hepatotoxicity of biologics as well as small molecules

Acknowledgements

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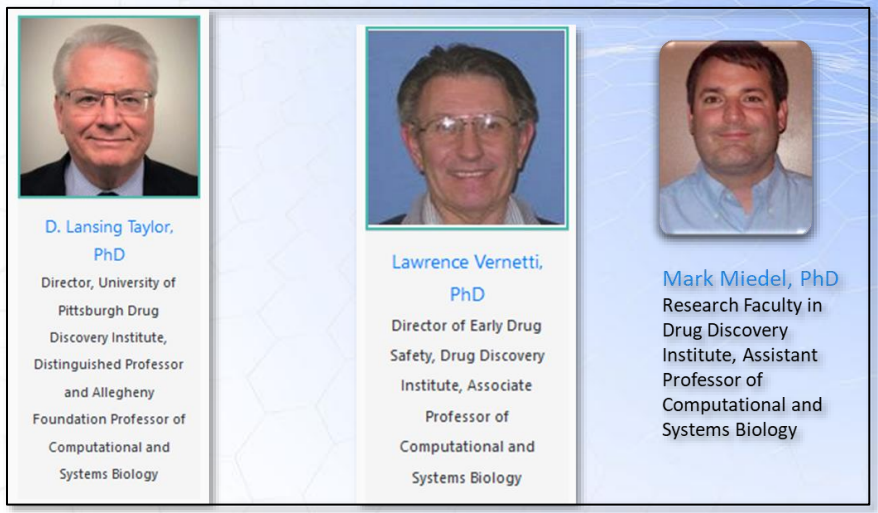

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R43TR003535 & R44 R44TR003535

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