DILIsym Services

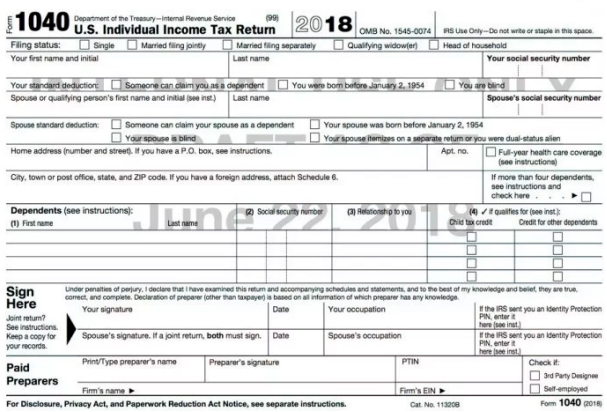
A SIMULATIONS PLUS COMPANY

Game Changing: The Latest Developments in the Machine Learning / PBPK / QST Modeling Space

March 11, 2019

Society of Toxicology Annual Meeting

Several certainties in life…



Version H, Cycle 1

Form **1040** Department of the Treasury—Internal Revenue Service (99) **U.S. Individual Income Tax Return** **2018** OMB No. 1545-0074 IRS Use Only—Do not write or staple in this space.

Filing status: ☐ Single ☐ Married filing jointly ☐ Married filing separately ☐ Qualifying widow(er) ☐ Head of household

Your first name and initial | Last name | **Your social security number**

Your standard deduction: ☐ Someone can claim you as a dependent ☐ You were born before January 2, 1954 ☐ You are blind

Spouse or qualifying person's first name and initial (see inst.) | Last name | **Spouse's social security number**

Spouse standard deduction: ☐ Someone can claim your spouse as a dependent ☐ Your spouse was born before January 2, 1954 ☐ Your spouse is blind ☐ Your spouse itemizes on a separate return or you were dual-status alien

Home address (number and street). If you have a P.O. box, see instructions. | Apt. no. | ☐ Full-year health care coverage (see instructions)

City, town or post office, state, and ZIP code. If you have a foreign address, attach Schedule 6. | If more than four dependents, see instructions and check here . . . ▶ ☐

Dependents (see instructions): | (1) First name Last name | (2) Social security number | (3) Relationship to you | (4) ✓ if qualifies for (see inst.): Child tax credit | Credit for other dependents

Sign Here Joint return? See instructions. Keep a copy for your records. Under penalties of perjury, I declare that I have examined this return and accompanying schedules and statements, and to the best of my knowledge and belief, they are true, correct, and complete. Declaration of preparer (other than taxpayer) is based on all information of which preparer has any knowledge.

Your signature | Date | Your occupation | If the IRS sent you an Identity Protection PIN, enter it here (see inst.)

Spouse's signature. If a joint return, **both** must sign. | Date | Spouse's occupation | If the IRS sent you an Identity Protection PIN, enter it here (see inst.)

Paid Preparers Print/Type preparer's name | Preparer's signature | PTIN | Check if: ☐ 3rd Party Designee ☐ Self-employed

Firm's name ▶ | Firm's EIN ▶

For Disclosure, Privacy Act, and Paperwork Reduction Act Notice, see separate instructions. Cat. No. 11320B Form **1040** (2018)

Model "driven" R&D: How do I change my R&D process to reflect the availability of *in silico* tools and support regulatory interactions?

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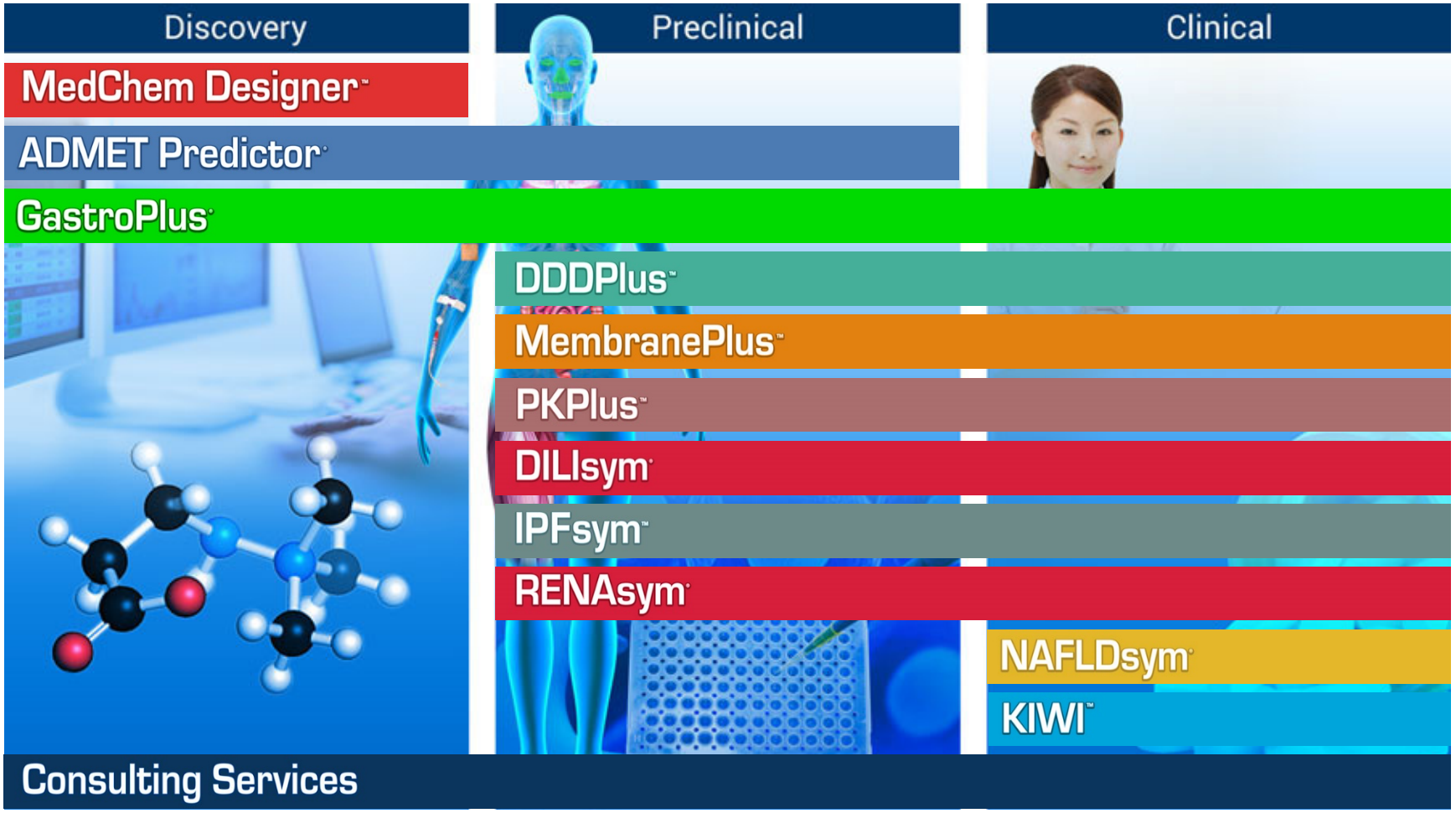
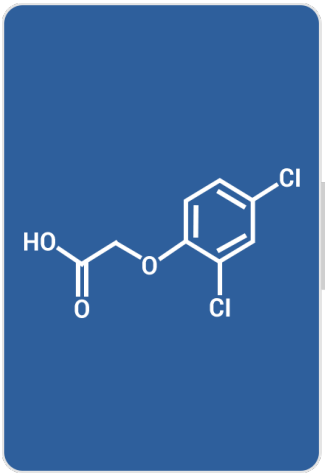
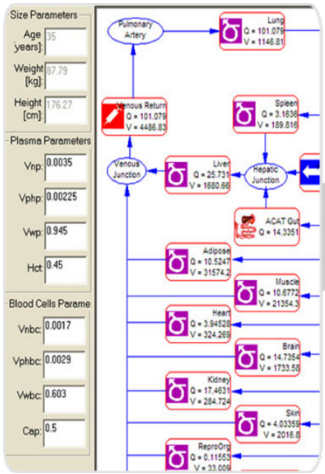
Simulations Plus (NASDAQ: SLP): Your end-to-end M&S solutions provider



Saying “I do” to the Machine Learning / PBPK / QST marriage…



Permeability, solubility vs. pH, pKa(s), logD vs. pH, Fup, blood:plasma ratio, tissue Kps, CLint, CLfilt



Local & systemic exposure, drug distribution, parent and metabolite levels, patient variability

Quantitative Structure Activity Relationships (QSAR)

ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™

Quantitative Systems Pharmacology/Toxicology (QSP/QST)

RENAsym™

DILIsym®

IPFsym™

NAFLDsym™

DILIsym Services

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Simulations Plus: 2018-19 Headlines!

- **US FDA purchases licenses to the DILIsym® platform**
  - Workshops being scheduled to train more FDA reviewers using the tool
- **Cosmetics Europe consortium funds GastroPlus® dermal model improvements**
  - Improvements to support alternative approaches to animal testing
- **DILIsym Services awarded SBIR grant to fund development of drug-induced kidney injury model**
  - RENAsym® will be applied to investigate and screen for possible drug-induced kidney damage
- **FDA extends funded collaboration for GastroPlus® ocular absorption modeling**
  - Incorporate new functionality to aid innovator/generic companies designing new eye treatments
- **Simulations Plus receives new grant award from FDA**
  - Integrate formulation characteristics into the GastroPlus™ mechanistic dermal PBPK model
- **Simulations Plus & DILIsym Services partner with large pharmaceutical company on pulmonary PBPK/QSP modeling**
  - Funding provided to create new IPFsym™ platform for idiopathic pulmonary fibrosis

Common theme:

Simulations Plus is advancing science in new areas and reinforcing partnerships with global organizations…

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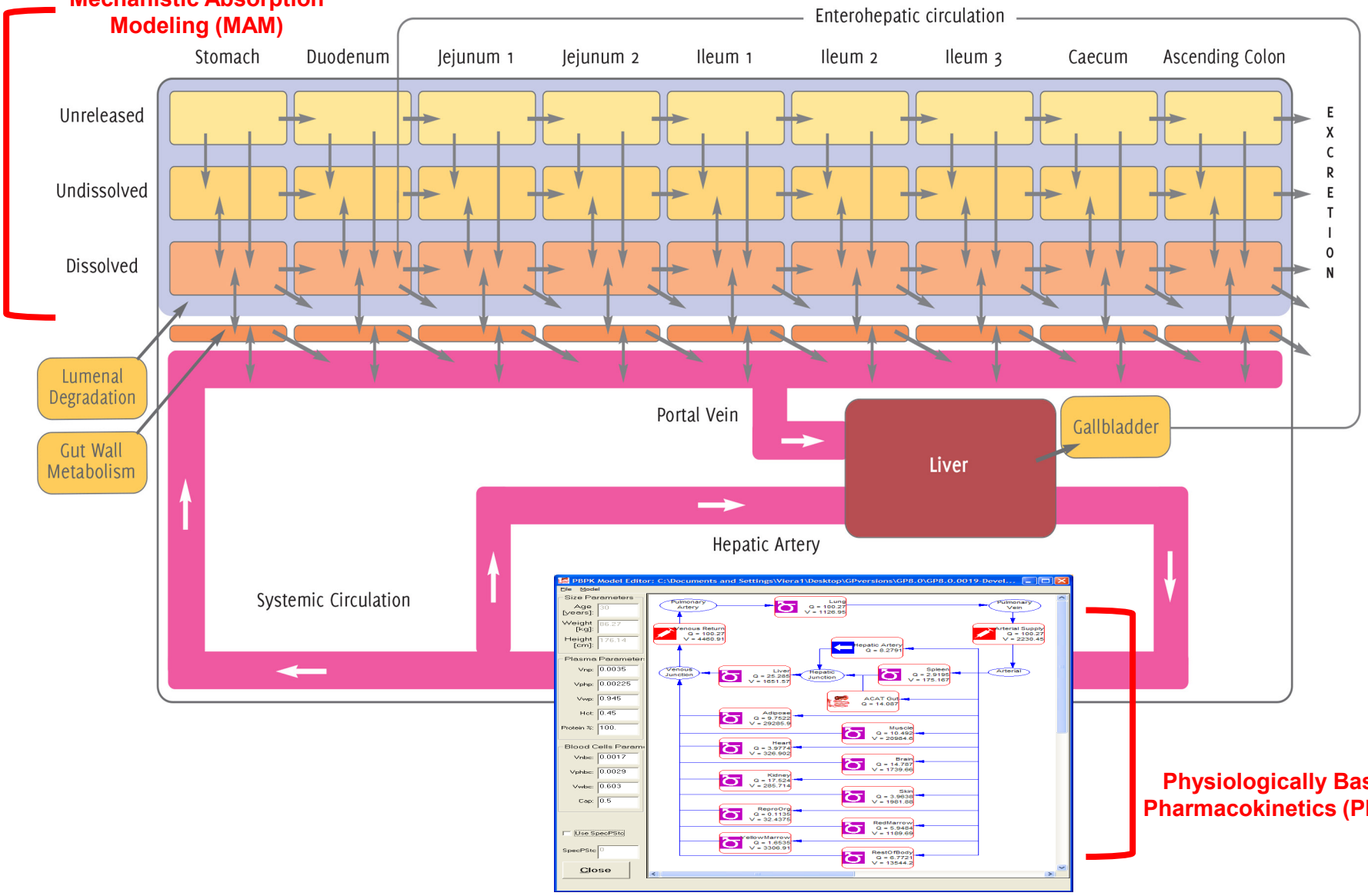
Advanced Compartmental Absorption and Transit Model (ACAT™)

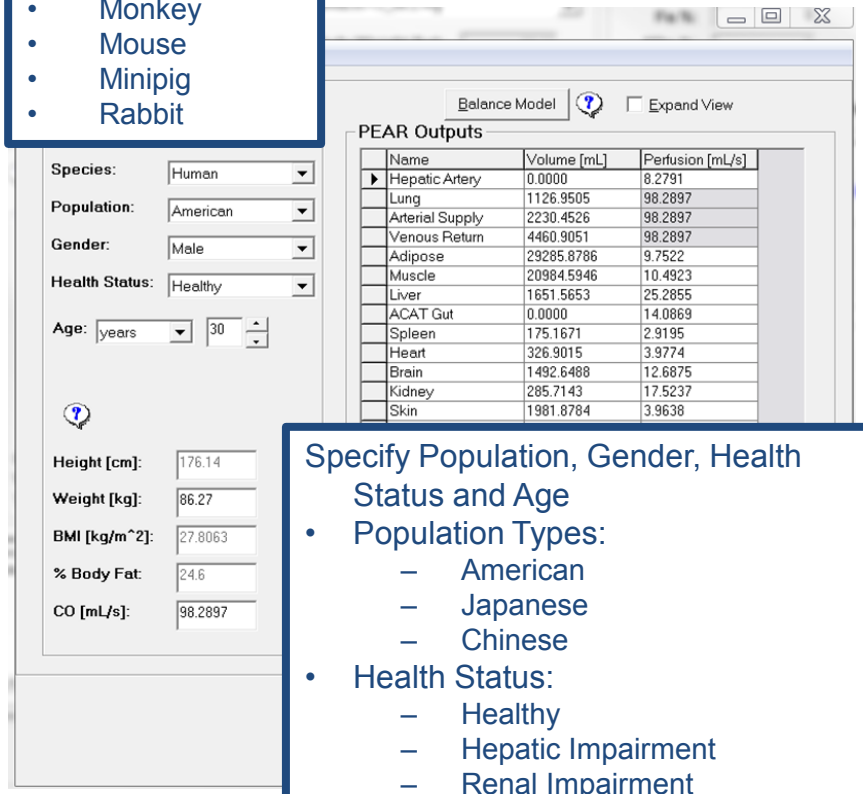
Mechanistic Absorption Modeling (MAM)



Physiologically Based Pharmacokinetics (PBPK)

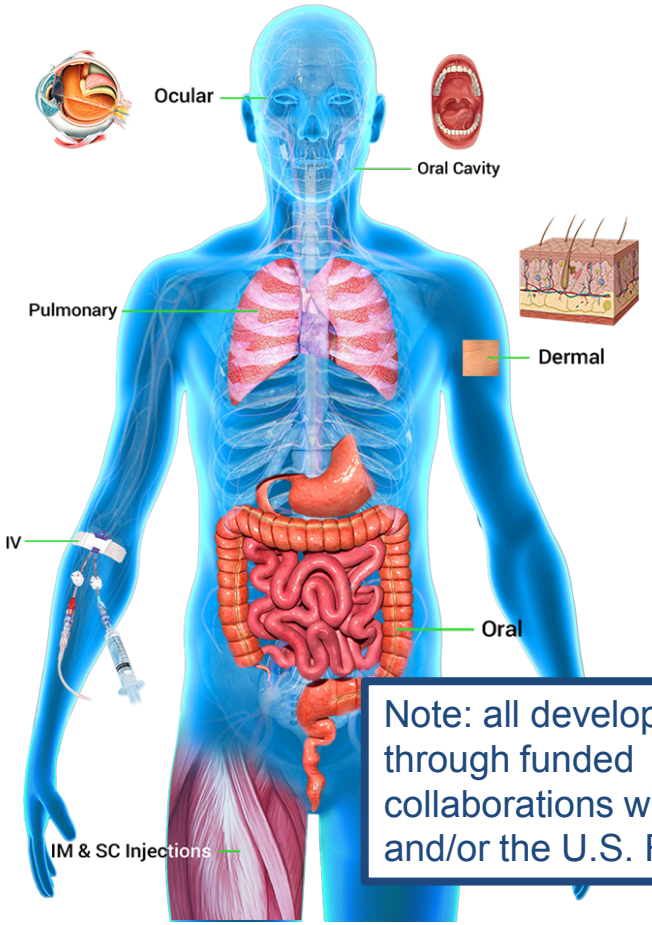
Validated System Models in GastroPlus®

Select Species:
- Human
- Rat
- Dog
- Monkey
- Mouse
- Minipig
- Rabbit



Name	Volume [mL]	Perfusion [mL/s]
Hepatic Artery	0.0000	8.2791
Lung	1126.9505	98.2897
Arterial Supply	2230.4526	98.2897
Venous Return	4460.9051	98.2897
Adipose	29285.8786	9.7522
Muscle	20984.5946	10.4923
Liver	1651.5653	25.2855
ACAT Gut	0.0000	14.0869
Spleen	175.1671	2.9195
Heart	326.9015	3.9774
Brain	1492.6488	12.6875
Kidney	285.7143	17.5237
Skin	1981.8784	3.9638

Specify Population, Gender, Health Status and Age
- Population Types:
  - American
  - Japanese
  - Chinese
- Health Status:
  - Healthy
  - Hepatic Impairment
  - Renal Impairment
  - Obesity
  - NEW! Pregnancy
- Age:
  - Day 1 of birth (16 weeks premature) -> 85 years old



Note: all developed through funded collaborations with industry and/or the U.S. FDA

Services
PLUS COMPANY

Notable Publications: 2018-19
New Strategies for FIH Predictions with PBPK Modeling

- The GastroPlus® User Group published several articles in 2018-19
- New strategies for FIH predictions using PBPK modeling include:
  - Flowcharts describing steps going from structure -> preclinical model building -> FIH predictions
  - Case studies showing comparisons between measured vs. predicted outcomes across different compound classes
- **Open access – free copies available in our booth #3736**

Miller et al. Clin Pharmacokinet. 2019

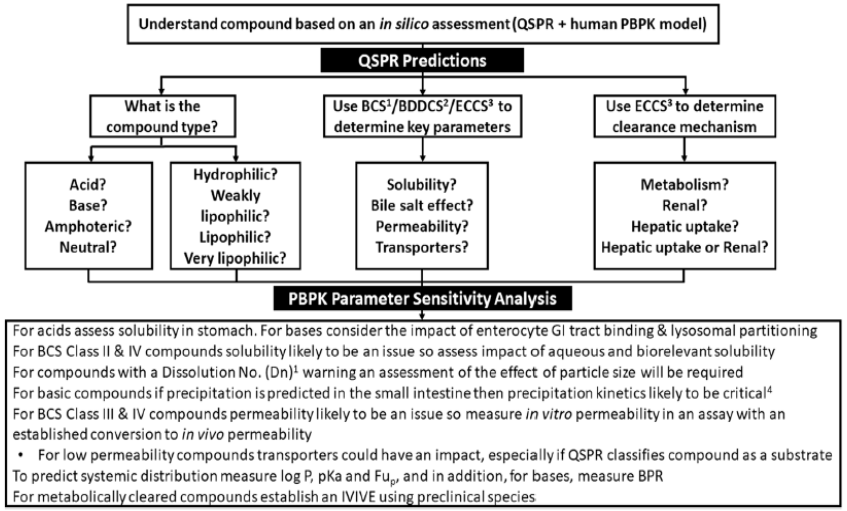
Clinical Pharmacokinetics
https://doi.org/10.1007/s40262-019-00741-9

REVIEW ARTICLE

Check for updates

Physiologically Based Pharmacokinetic Modelling for First-In-Human Predictions: An Updated Model Building Strategy Illustrated with Challenging Industry Case Studies

Neil A. Miller[1] · Micaela B. Reddy[2] · Aki T. Heikkinen[3] · Viera Lukacova[4] · Neil Parrott[5]



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Notable Publications: 2018-19
PBPK modeling to predict toxicokinetics of chemicals

- Dow Chemical performed an evaluation of the machine learning-PBPK marriage for several dosing routes:
  - Oral exposure
    - 88% predicted within 10-fold
  - Dermal exposure
    - 83% predicted within 10-fold
  - Inhaled exposure
    - 63% predicted within 10-fold
- Additional validation performed on key physicochemical inputs:
  - pKa(s)
  - logP
  - Henry's Law Constant
  - Intrinsic clearance
  - Plasma protein binding

SAR AND QSAR IN ENVIRONMENTAL RESEARCH
https://doi.org/10.1080/1062936X.2018.1518928
Taylor & Francis
Taylor & Francis Group
Check for updates

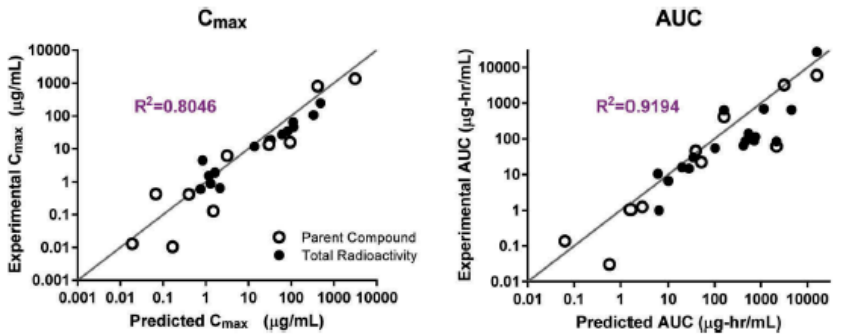
Performance evaluation of the GastroPlus™ software tool for prediction of the toxicokinetic parameters of chemicals

F. Zhang[a], M. Bartels[b], A. Clark[a], T. Erskine[a], T. Auernhammer[a], B. Bhhatarai[c], D. Wilson[a] and S. Marty[a]

[a]The Dow Chemical Company, Midland, MI, USA; [b]ToxMetrics.com LLC, Midland, MI, USA; [c]Novartis Institute for Biomedical Research, Cambridge, MA, USA

ABSTRACT
The accurate prediction of toxicokinetic parameters arising from oral, dermal and inhalation routes of chemical exposure is a key

ARTICLE HISTORY
Received 2 July 2018
Accepted 30 August 2018



Total radioactivity: Total C_{max} or AUC of all radioactivity after dosing
Parent compound: Total C_{max} or AUC of parent compound after dosing

Figure 4. Correlation of predicted C_{max} and AUC versus empirical data following oral exposure.

Zhang et al. SAR QSAR Environ Res 2018

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Notable Publications: 2018-19
Structural/functional PK analogs for PBPK evaluation

- P&G evaluated the application of a validated PBPK model for a target chemical to structural and functional PK analogs to predict oral exposure
- Baseline models were developed for 51 chemicals using available *in silico / in vitro / in vivo* data
- Functional and structural analogs were generated from the target chemical; baseline model applied with analog-specific inputs substituted
  - Functional PK analog pairs
    - 87% predicted within 3-fold
  - Structural PK analog pairs
    - 67% predicted within 3-fold

Ellison CA. Regul Toxicol Pharmacol. 2018

Regulatory Toxicology and Pharmacology 99 (2018) 61–77

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Structural and functional pharmacokinetic analogs for physiologically based pharmacokinetic (PBPK) model evaluation

Corie A. Ellison

The Procter & Gamble Company, 8700 Mason Montgomery Road, Cincinnati, OH, 45040, USA

ARTICLE INFO

Keywords:
Pharmacokinetics
Physiologically based pharmacokinetics

ABSTRACT

Physiologically based pharmacokinetic (PBPK) models enable simulations of absorption, distribution, metabolism, and elimination of chemicals from the body. Model evaluation is a key step in the PBPK model development processes whereby model predictions are compared to pharmacokinetic (PK) data. A prerequisite for PBPK

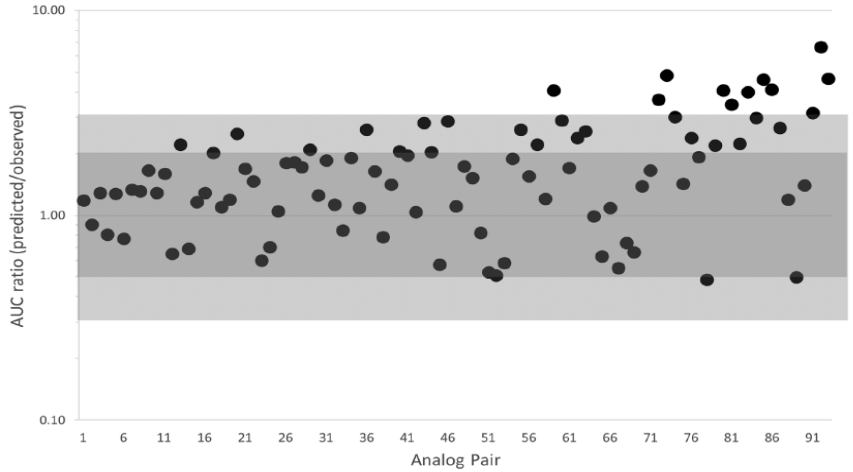

Fig. 5. Comparison of AUC predicted by the target chemical PBPK model and PK data from the source chemical for the ninety-three functional PK analog pairs. Symbols within dark gray shading are within a factor of 2 when comparing predicted and observed AUCs, while symbols in light gray shading are within a factor of 3. Analog pairs are in order of most similar (analog pair 1, difference score = 3.1) to least similar (analog pair 93, difference score = 5.6).

Common Theme of Recent Work…

- Simulations Plus continues to lead in the areas of PBPK modeling to support regulatory submissions and alternatives to animal testing

Physiologically Based Pharmacokinetic Analyses — Format and Content

Guidance for Industry

IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WHO

IPCS Harmonization Project

Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment

DILIsym Services Inc., a SLP Company

"Our vision is safer, effective, more affordable medicines for patients through modeling and simulation."

- DILIsym Services, Inc. offers comprehensive program services:
  - **DILIsym** software licensing, training, development (DILI-sim Initiative)
  - **NAFLDsym** software licensing, training, development
  - **DILIsym** and **NAFLDsym** simulation consulting projects
  - Consulting and data interpretation; *in vitro* assay experimental design and management
  - **RENAsym** and **IPFsym** software in development

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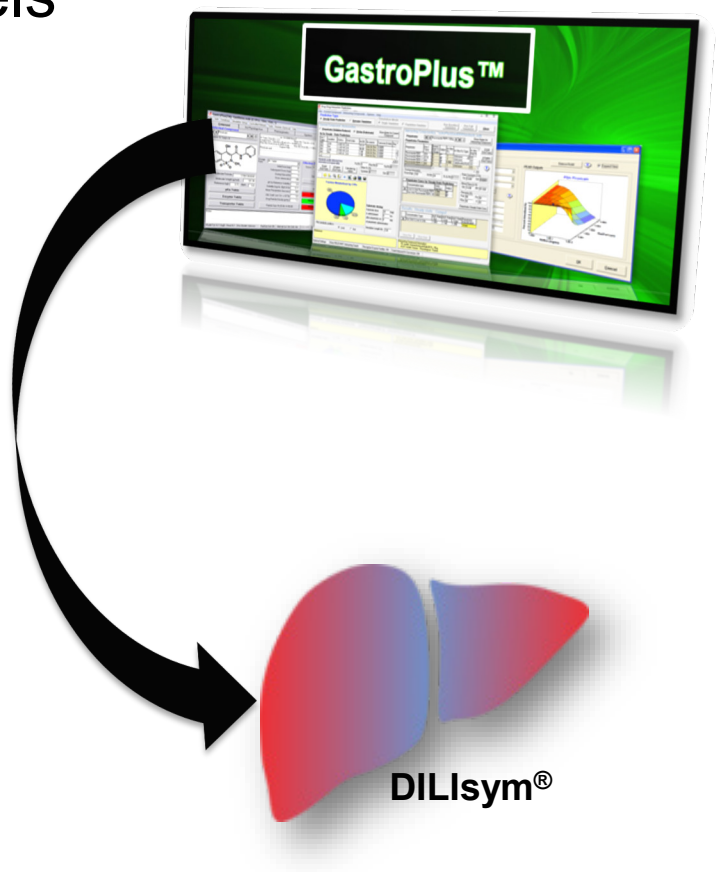
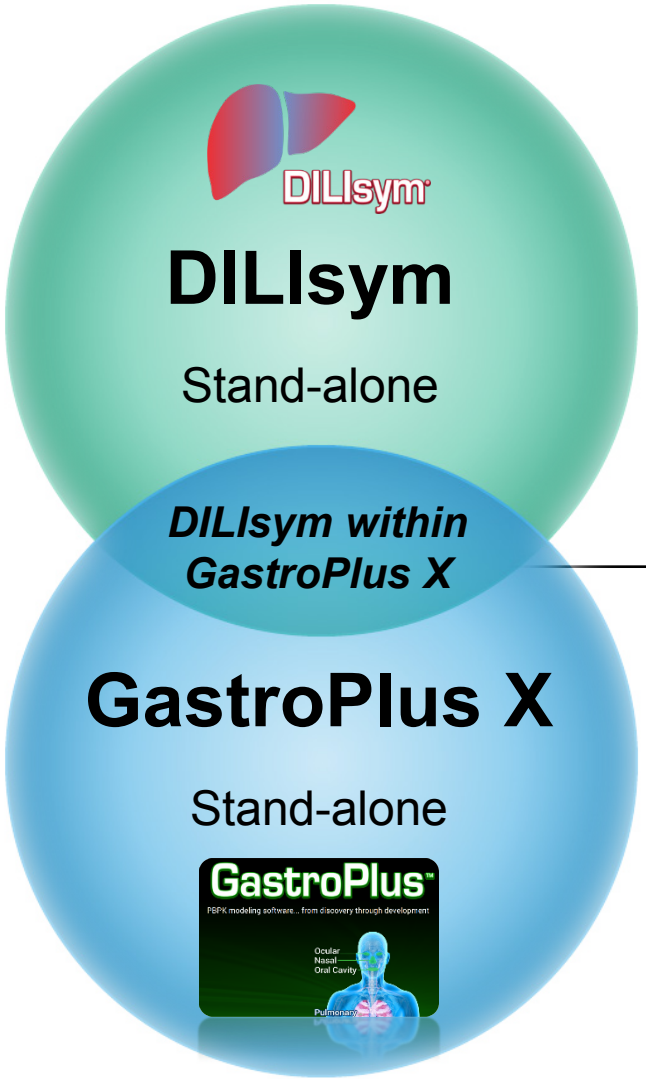
GastroPlus Allows for Efficient Use of GastroPlus PBPK Models in Combination with DILIsym SimPops

- GastroPlus users build PBPK models within GastroPlus
- The “DILIsym” simulation mode in v9.7 allows users to select a mapping of GastroPlus outputs to DILIsym PK inputs
- **Easy, simple-to-use template automatically generated for GastroPlus users to import into DILIsym and drive simulations!**



Refactored DILIsym and GastroPlus Will Be Integrated for More Efficient and Powerful Predictions

Both DILIsym and GastroPlus X will operate independently of each other



- Integration will occur via an interoperability plugin
- During integration, DILIsym will utilize GastroPlus X's ODE system for running simulations.

DILIsym Services QST and QSP Models

DILIsym®

- Predicts drug-induced liver disease
- v8A released Q1 2019
- Includes mechanistic representation of normal hepatic biochemistry
- Evaluated >60 compounds with 25 companies

RENAsym™

NAFLDsym®

So how can DILIsym help my organization?

- Predict DILI liabilities beforehand and save **$$$**
- Choose the lead candidate ***most likely to succeed*** from a DILI standpoint
- Communicate with regulators on safety issues with information they have requested from others numerous times and from a platform they license (FDA)
- ***Keep patients safer….***

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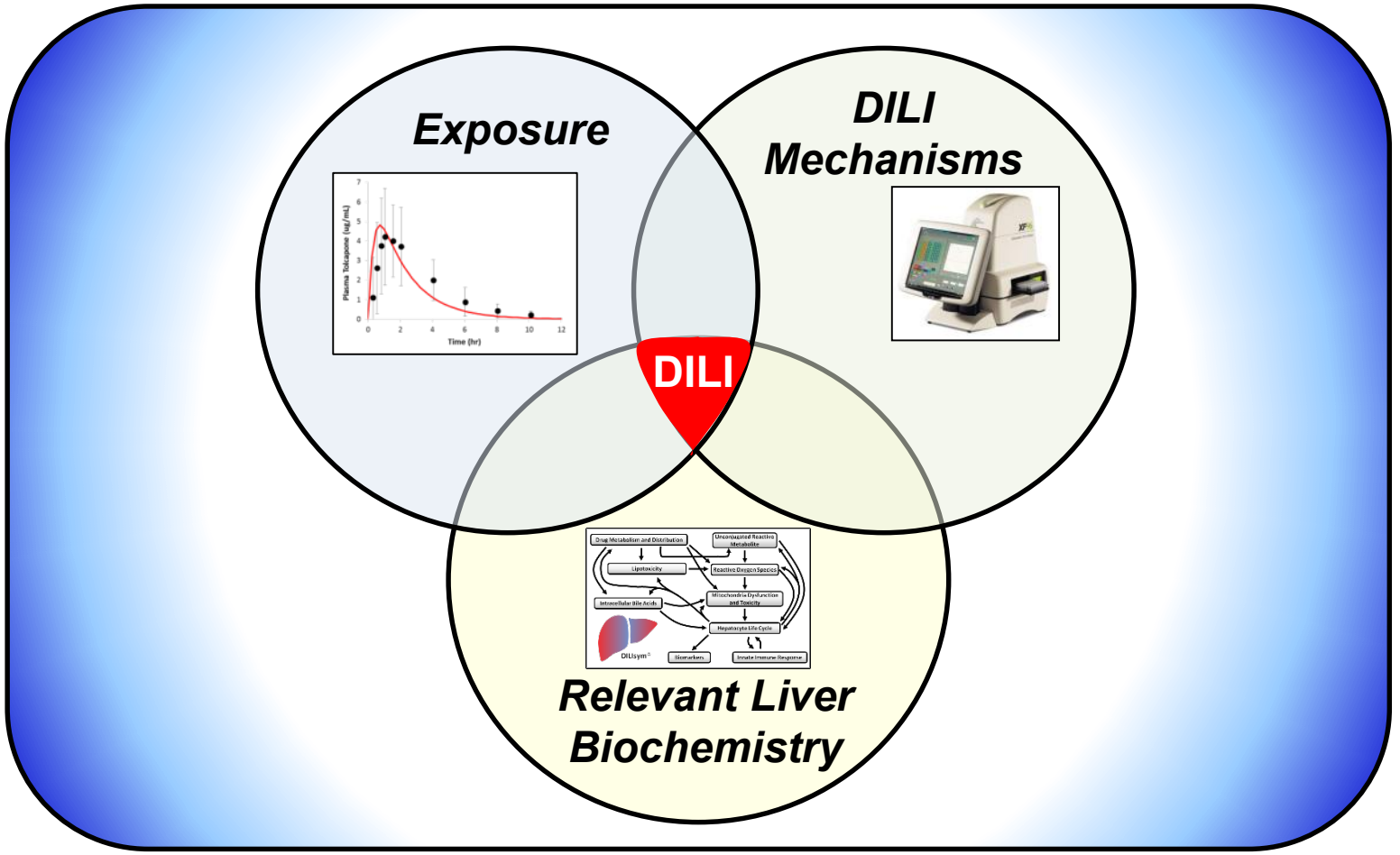
DILIsym Predicts DILI via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



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

Select Sample of Current Companies Licensing DILIsym

- *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 $9 million total invested in project*

FDA

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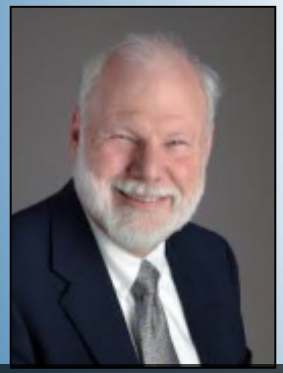
DILI-sim Scientific Advisory Board

Dr. Neil Kaplowitz
Professor of Medicine
USC Thomas H. Brem Chair in Medicine
Chief, Division of Gastroenterology and Liver Diseases

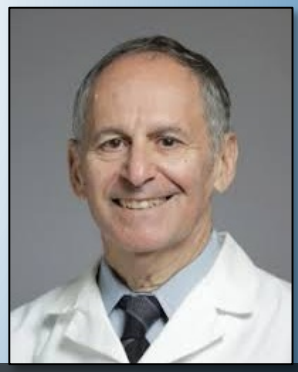
Dr. Paul B. Watkins
DIRECTOR, INSTITUTE FOR DRUG SAFETY SCIENCES
HOWARD Q. FERGUSON DISTINGUISHED PROFESSOR OF MEDICINE
UNC Eshelman School of Pharmacy

Dr. Kevin Park
Head of Institute of Translational Medicine / Director, MRC Centre for Drug Safety Science, University of Liverpool



Dr. Jack Uetrecht
Professor, Canada Research Chair in Adverse Drug Reactions
University of Toronto

Dr. Robert Roth
Distinguished Professor of Pharmacology & Toxicology
Director, Graduate Training Program in Environmental and Integrative Toxicological Sciences, Center for Integrative Toxicology
Michigan State University



David Pisetsky
Professor of Medicine
Professor of Immunology
Member of the Duke Cancer Institute
Member of the Duke Human Vaccine Institute

Support of the DILI-sim Initiative Has Led to Significant Research Achievements

- Eight versions of DILIsym released, including DILIsym v8A in Jan 2019
- At least **18** applications of DILIsym directly related to regulatory submissions for drug development (that we are aware of)
- More than **35** pharmaceutical companies have utilized DILIsym via consulting contracts for projects related to regulatory issues or applications, internal validation, or DILIsym use help internally
  - Insights go directly back into software for members
- **80%** of the simulation scenarios evaluated within DILIsym have generally been predicted well (of the 66 cases and 59 compounds simulated)
- **27** accepted manuscripts and **5+** more in final preparation focused on DILIsym content
  - Many of these are co-publications between DILIsym Services and a member or non-member pharma company
- DILIsym related publications have been cited **444** times as of September 2018
- Academic and government licenses issued for teaching and research, including to FDA across multiple divisions

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DILI-sim Initiative Membership Benefits

Access to DILIsym software, equations, and support

- DILI-sim members receive access to the DILIsym software during their active membership term
- DILI-sim members receive a PDF copy of all equations included in each version of the DILIsym software released during their active membership term
- DILI-sim members have exclusive access to DILIsym training materials and support, including 10 hours of one-on-one support, free training once per year at annual meeting, and reduced rates on off-site workshops
- DILI-sim members have access to online DILIsym documentation (i.e., software use, modeling rationale)
- Tier 1 (3 year) members receive a 31% discount on consulting; Tier 2 (annual) members receive a 17% discount (compared to non-member pricing)
- DILI-sim members have exclusive access to the DILIsym Discovery Support Program (DDSP); not available to non-members or academics

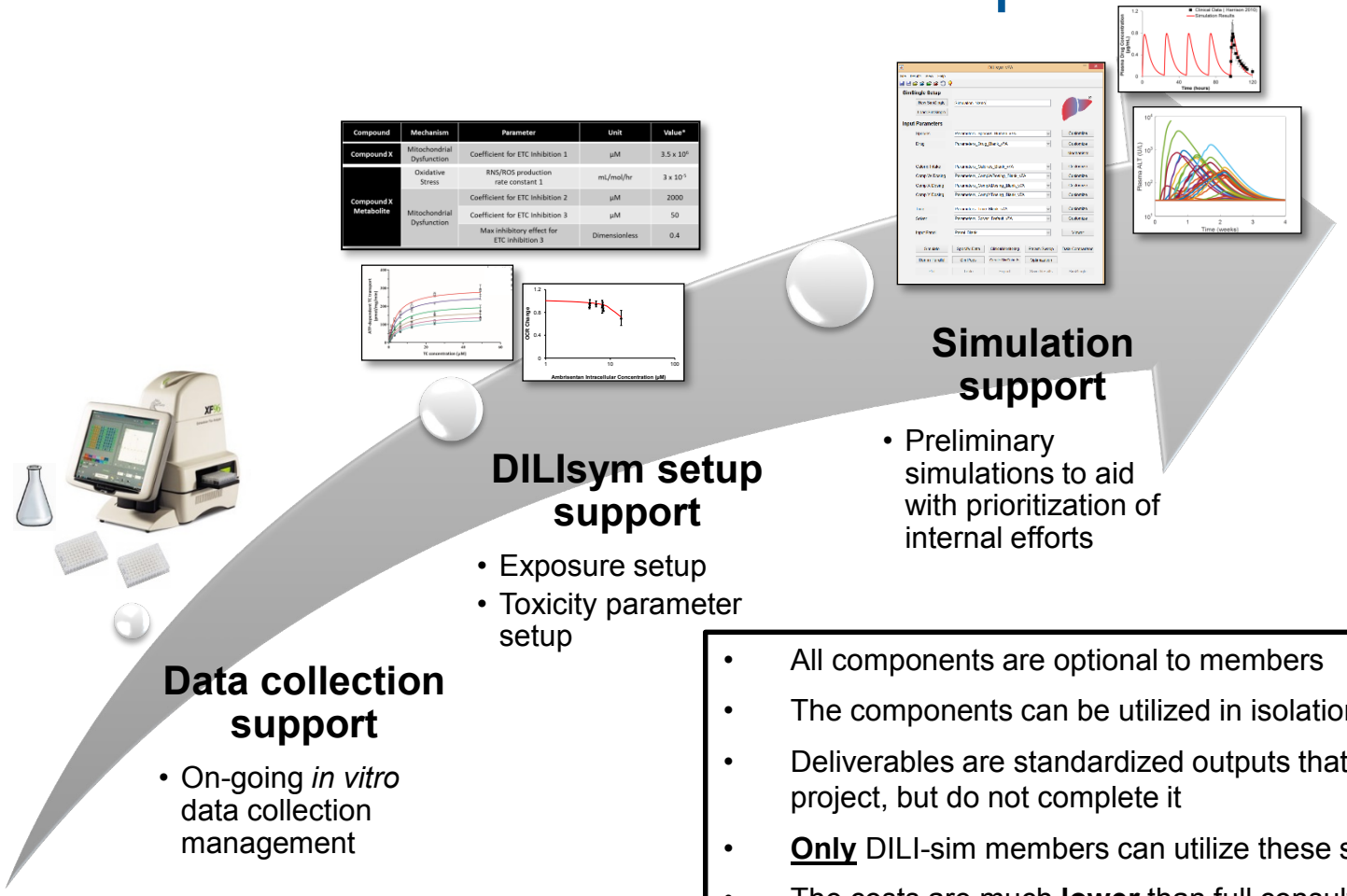
Influence over DILIsym development

- Member companies guide DILIsym development through voting
- DILI-sim members have option to donate data from current or failed compounds to serve as exemplars for DILIsym

Participation in regular meetings with colleagues

- Representatives from member companies attend quarterly DILI-sim update meetings to monitor progress and provide feedback, along with model design review sessions
- Members gather in person once per year for a more comprehensive overview during the annual DILI-sim Face to Face Meeting
- Attendance, voting, and data generation are optional benefits of membership

The DILIsym Discovery Support Program (DDSP) Enables Internal Use by DILI-sim Member Companies



Data collection support

- On-going *in vitro* data collection management

DILIsym setup support

- Exposure setup
- Toxicity parameter setup

Simulation support

- Preliminary simulations to aid with prioritization of internal efforts

- All components are optional to members
- The components can be utilized in isolation or together
- Deliverables are standardized outputs that facilitate the project, but do not complete it
- **Only** DILI-sim members can utilize these services
- The costs are much **lower** than full consulting projects conducted by DILIsym Services

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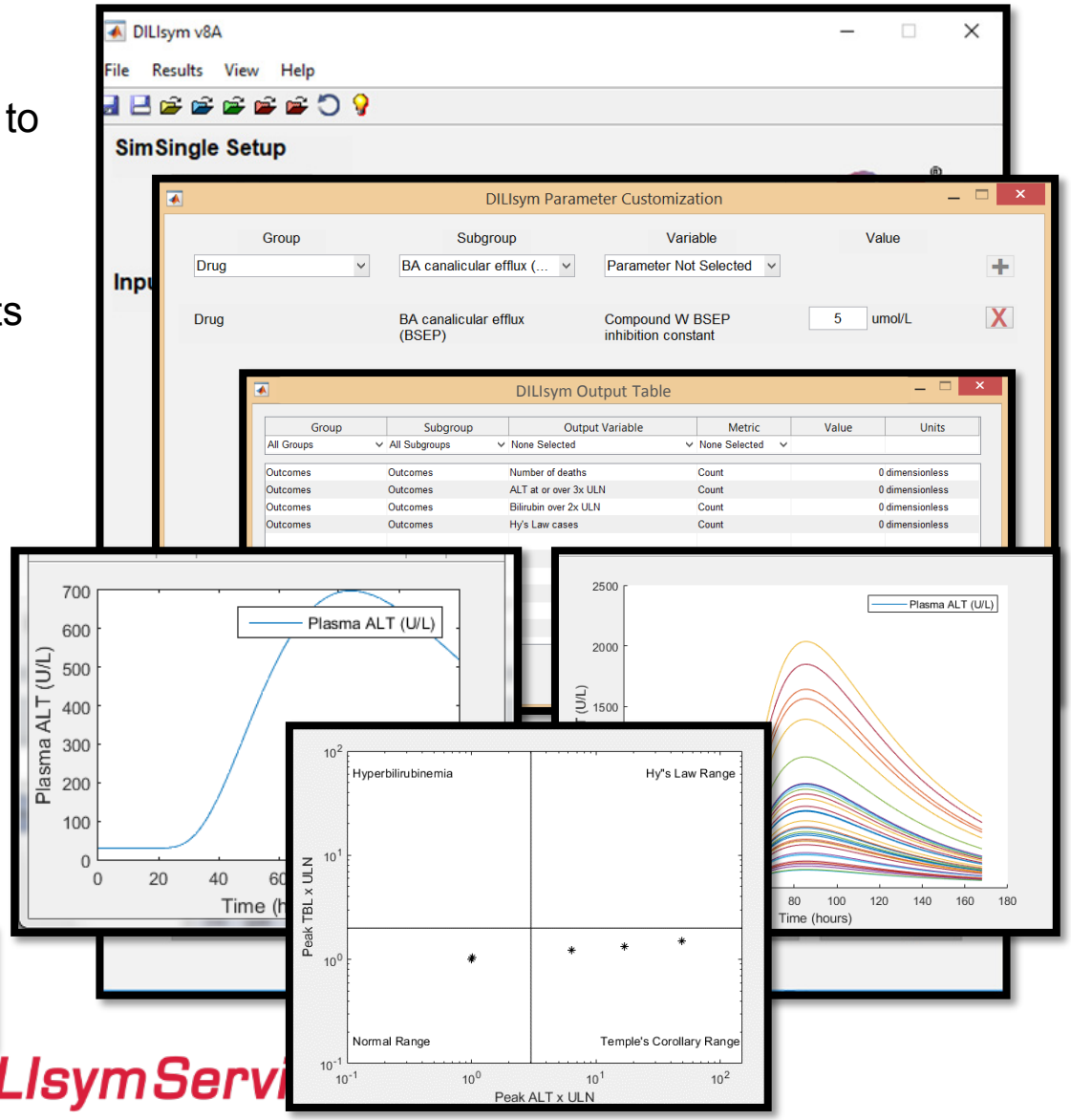
DILIsym Software Licenses Are Available to Industry and Academia

- DILI-sim membership not required to license the DILIsym modeling software
- Training provided in various formats
  - Training courses and workshops
  - Web-based videos
  - User manual
  - Documentation
- Academic and regulatory licenses also available
- Access to the MITOsym modeling software is also provided



MITOsym®: A Mechanistic, Mathematical Model of Hepatocellular Respiration and Bioenergetics

Y. Yang • S. Nadanaciva • Y. Will • J. L. Woodhead • B. A. Howell • P. B. Watkins • S. Q. Siler

DILIsymServi

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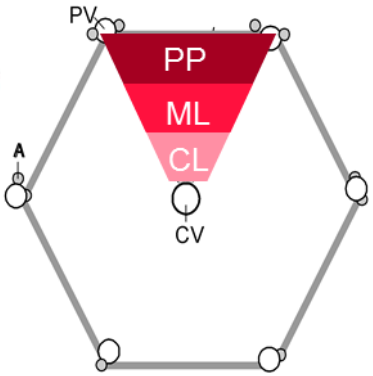
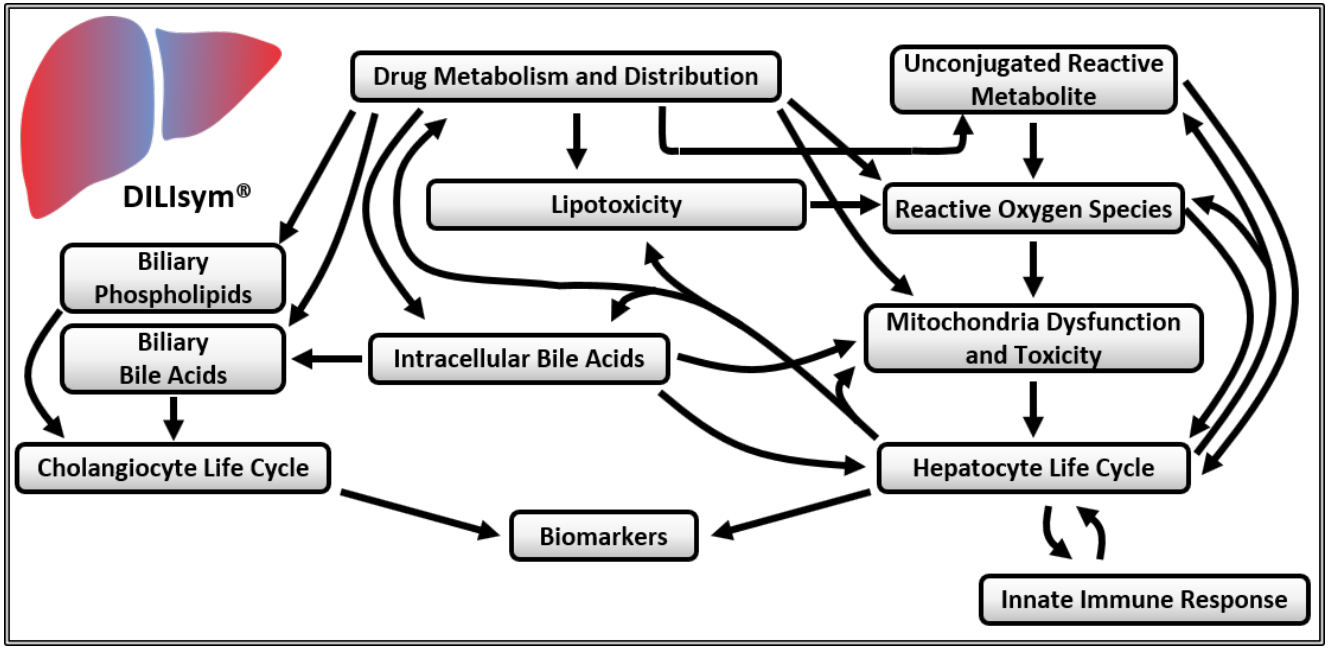
DILIsym Overview

- **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
  - Cholangiocyte apoptosis and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers



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



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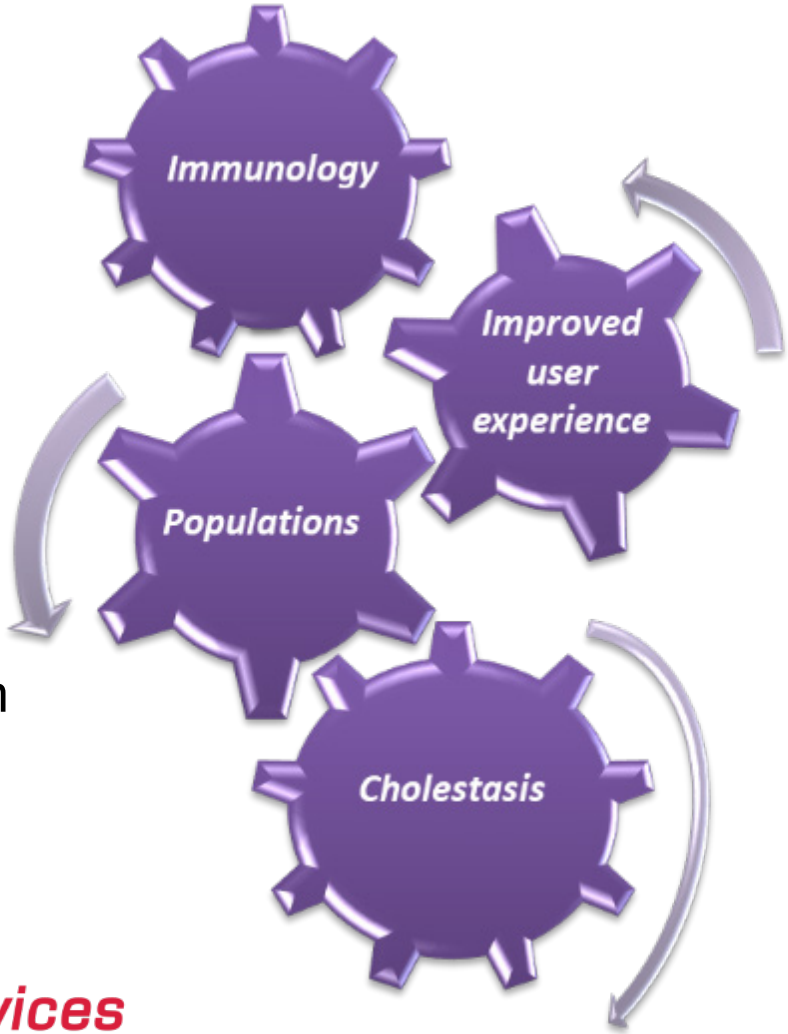
Immunology, Cholestasis, Population Variability and Enhanced Software Highlight Stage 3 Areas of Focus

- Immunology is a critical area for latent DILI and many oncology related DILI events
- Cholestasis has become increasingly important from a regulatory standpoint
  - Phospholipids and MDR3 an important consideration
- Population variability must include all relevant factors such as disease, environment and genetics
- Software enhancements stemming from acquisition by Simulations Plus will propel user friendliness and speed
- Improved *in vitro* data to feed the software



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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

DILIsym®

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 / phospholipid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP, (MDR3)*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

cyprotex AN EVOTEC COMPANY

SOLVO® BIOTECHNOLOGY THE TRANSPORTER COMPANY

Clinical Data / Information

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

DILIsym Services

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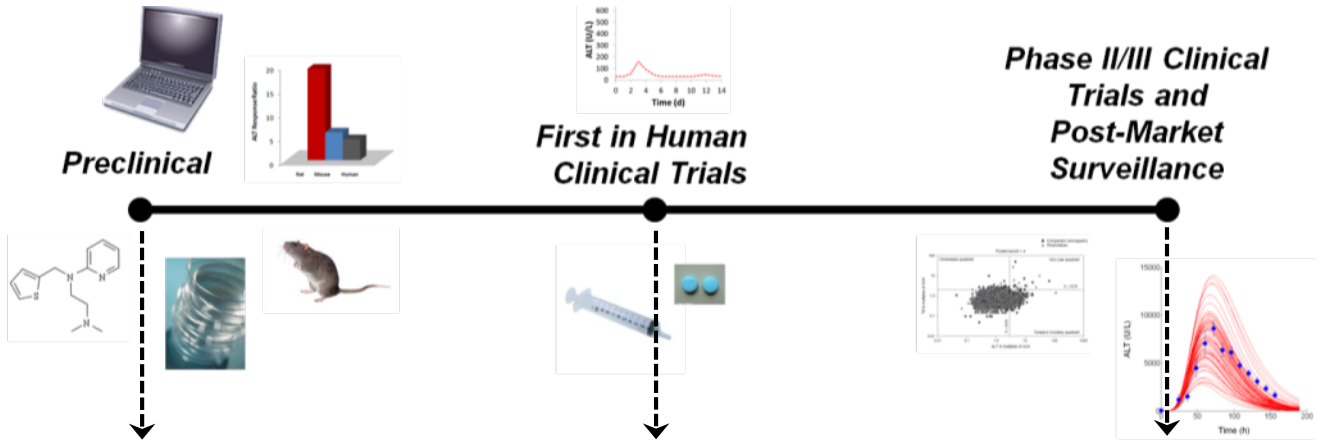
Applications of DILIsym Along the Drug Development Pipeline

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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Evaluating DILIsym for Pre-clinical Drug Development

DILIsym Annual Meeting
September 2018

Paul Michalski, Valeriu Damian

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

DILIsym Services GSK Proof of Concept Exemplar Compound Summary

Compound (Clinical Dosing Protocol)	Active Toxicity Mechanism(s)	Predicted Toxicity* at 1x Clinical Dose	Predicted Toxicity** at 10x Clinical Dose	Clinically toxic?
Phenoxybenzamine (40 mg TID, 10 days)	BA, mito, ROS	No (0/285)	No (0/285)	No
Zafirlukast (20 mg BID, 3 months)	BA, mito	No (0/285)	No (0/285)	Yes
Fluconazole (400 mg QD, 4 weeks)	mito	Yes (285/285)	NA	Yes
GSK Compound A (150 mg QD, 2 weeks)	BA, mito	Yes (285/285)	NA	Yes
GSK Compound B (900 mg & 1200 mg QD, 2 months)	BA, mito, ROS	Yes (95/285)***	NA	Yes
GSK Compound C (15 mg BID, 60 days)	BA, mito, ROS	Yes (3/285)	NA	Yes
GSK Compound D (600 mg QD, 1 week)	BA, mito, ROS	No (0/285)	Yes (16/16)	Yes

*ALT >3x ULN in human v4A_1 SimPops unless otherwise noted
**ALT >3x ULN in Multi16 SimCohort
*** Same frequency at 900 mg and 1200 mg

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

DILIsym PoC Conclusions

1) DILIsym performs well for compounds with hepatoxicity mechanism which are
   (a) included in DILIsym
   (b) detectable in the DILIsym in vitro assay panel

2) PoC highlighted the importance of mitotox as a hepatotox mechanism. We are now in the process of including mitotox screens in the GSK hepatotox strategy.

3) Assay negative compounds cannot be modeled in DILIsym (seems obvious, but now forms an important flag in screening strategy).

4) The DILIsym assay panel does a poor job of capturing the effects of reactive metabolites. The assay panel is being re-configured to include spheroids instead of primary hepatocytes.

5) Most importantly, DILIsym does provide added value over our current GSK hepatotox strategy.

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

General Pre-clinical Modeling Considerations

How do we use DILIsym to inform go/no-go decisions?

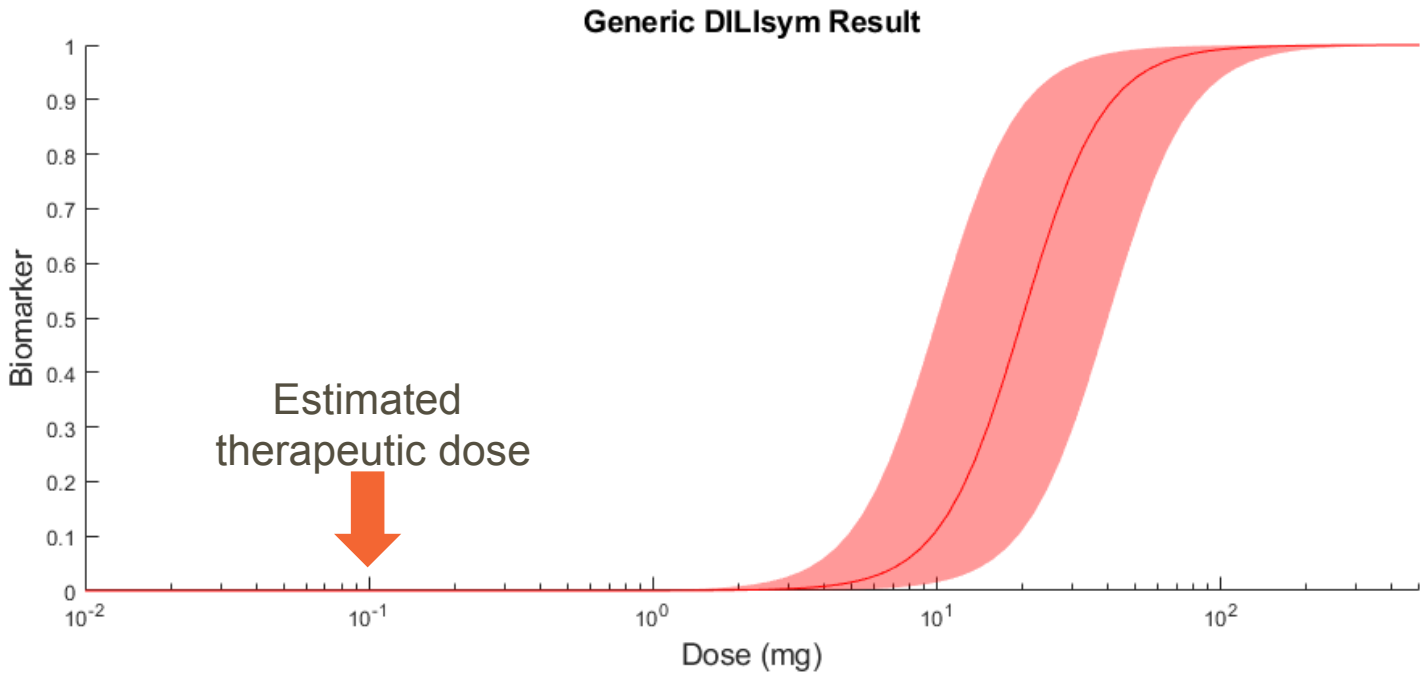
Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

Pre-clinical view of DILIsym

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

In general we will have an *estimated* human therapeutic dose and the DILIsym model results with their associated uncertainty.

Question: How do we use this information to make a go/no-go decision?



Pre-clinical view of DILIsym

DILIsym cannot be used in isolation. Must take into account the category of the drug, pre-clinical tox observations, and the DILIsym predictions for human.

Example: Drug in the “Low dose, assay positive” category
Current GSK strategy says “GO”.

		Exp. Pre-clinical Coverage					
		No data		< 10		> 10	
DS Pred Preclin Cov →		<10	>10	<10	>10	<10	>10
(DS Tox. Dose) / (Est. Eff. Dose)	< 1	**STOP**	**STOP**	**STOP**	**STOP**	GO?	**STOP**
	1 – 10	GO	GO	GO	GO?	GO	GO
	> 10	GO	GO	GO	GO	GO	GO

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

Pre-clinical view of DILIsym

Originally presented at DILI-sim Initiative annual meeting in Research Triangle Park, NC in September of 2018

DILIsym cannot be used in isolation. Must take into account the category of the drug, pre-clinical tox observations, and the DILIsym predictions for human.

GO Low dose, assay neg.

(DS Tox. Dose) / (Est. Eff. Dose)	Exp. Pre-clinical Coverage: No data		< 10		> 10	
DS Pred Preclin Cov →	<10	>10	<10	>10	<10	>10
< 1	**STOP**	**STOP**	**STOP**	**STOP**	GO?	**STOP**
1 – 10	GO	GO	GO	**STOP?**	GO	GO
> 10	GO	GO	GO	GO?	GO	GO

Low dose, assay pos. GO

(DS Tox. Dose) / (Est. Eff. Dose)	Exp. Pre-clinical Coverage: No data		< 10		> 10	
DS Pred Preclin Cov →	<10	>10	<10	>10	<10	>10
< 1	**STOP**	**STOP**	**STOP**	**STOP**	GO?	**STOP**
1 – 10	GO	GO	GO	GO?	GO	GO
> 10	GO	GO	GO	GO	GO	GO

STOP High dose, assay neg.

(DS Tox. Dose) / (Est. Eff. Dose)	Exp. Pre-clinical Coverage: No data		< 10		> 10	
DS Pred Preclin Cov →	<10	>10	<10	>10	<10	>10
< 1	STOP	STOP	STOP	STOP	STOP	STOP
1 – 10	**GO**	**GO**	**GO?**	STOP	**GO?**	**GO**
> 10	**GO**	**GO**	**GO**	**GO?**	**GO**	**GO**

High dose, assay pos. STOP

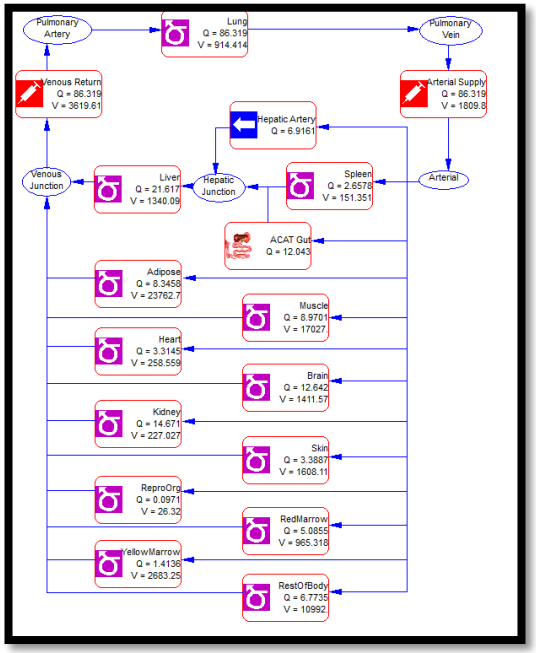
(DS Tox. Dose) / (Est. Eff. Dose)	Exp. Pre-clinical Coverage: No data		< 10		> 10	
DS Pred Preclin Cov →	<10	>10	<10	>10	<10	>10
< 1	STOP	STOP	STOP	STOP	STOP	STOP
1 – 10	STOP	STOP	STOP?	STOP	STOP	STOP
> 10	**Mech?**	**Mech?**	**Mech?**	STOP	**Mech?**	**GO**

Example Project Goal – Assess Compound X and Compound Y

- The primary goal of this simulation work within the DILIsym software was to:
  - quantitatively and mechanistically assess the liver toxicity potential of Compound X and Compound Y combining clinical and mechanistic *in vitro* data with DILIsym and GastroPlus software simulations of previous or prospective clinical dosing paradigms.

GastroPlus PBPK Model Used to Predict Liver Exposure of Compound Y and Compound X

- Data on Compound Y and Compound X pharmacokinetics not available in the literature
  - No plasma time courses available; no *in vitro* or animal studies available either
  - Data on T_{max}, Compound Y $f_{u,plasma}$ available
  - *In vitro* data on liver distribution available from intracellular data collected for this project
- Structure of each compound available online
  - QSAR modeling using ADMET Predictor and GastroPlus provided the best possible estimate of Compound Y and Compound X distribution and pharmacokinetics



- Plasma time course was estimated in GastroPlus and translated into DILIsym using "specified data" option
  - Liver:plasma partition coefficient was calculated from the cell:media ratio in the *in vitro* data and used as input into GastroPlus; the remainder of the parameters were calculated by ADMET Predictor
- Both compounds distribute significantly into the liver
  - Compound Y average cell:media was 18; Compound X average cell:media was 9

Compound Y | Compound X

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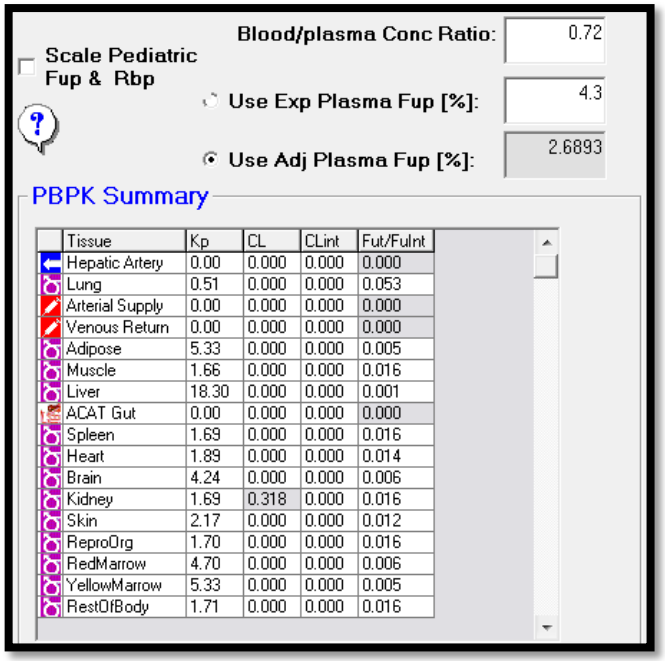
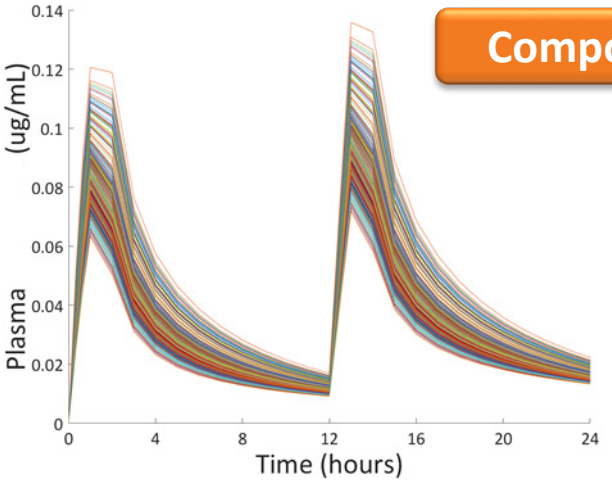
Compound Y PBPK Representation Calculated at Clinical Dose

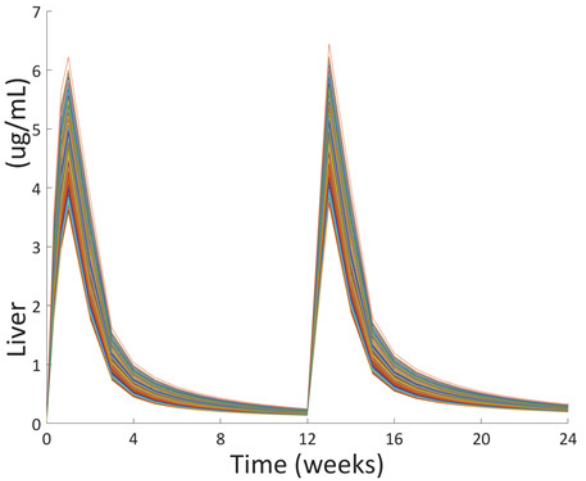
- GastroPlus predictions for liver and plasma at clinical dose shown at right
  - PBPK model specific predictions shown below
  - Dose escalation was simulated



Tissue	Kp	CL	CLint	Fut/FuInt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.51	0.000	0.000	0.053
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	5.33	0.000	0.000	0.005
Muscle	1.66	0.000	0.000	0.016
Liver	18.30	0.000	0.000	0.001
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	1.69	0.000	0.000	0.016
Heart	1.89	0.000	0.000	0.014
Brain	4.24	0.000	0.000	0.006
Kidney	1.69	0.318	0.000	0.016
Skin	2.17	0.000	0.000	0.012
ReproOrg	1.70	0.000	0.000	0.016
RedMarrow	4.70	0.000	0.000	0.006
YellowMarrow	5.33	0.000	0.000	0.005
RestOfBody	1.71	0.000	0.000	0.016

Compound Y





Simulation Results

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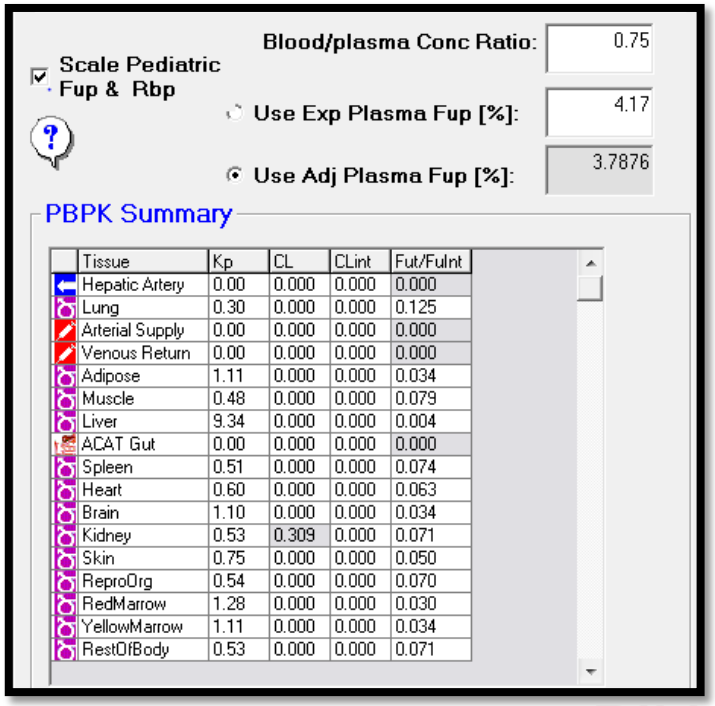
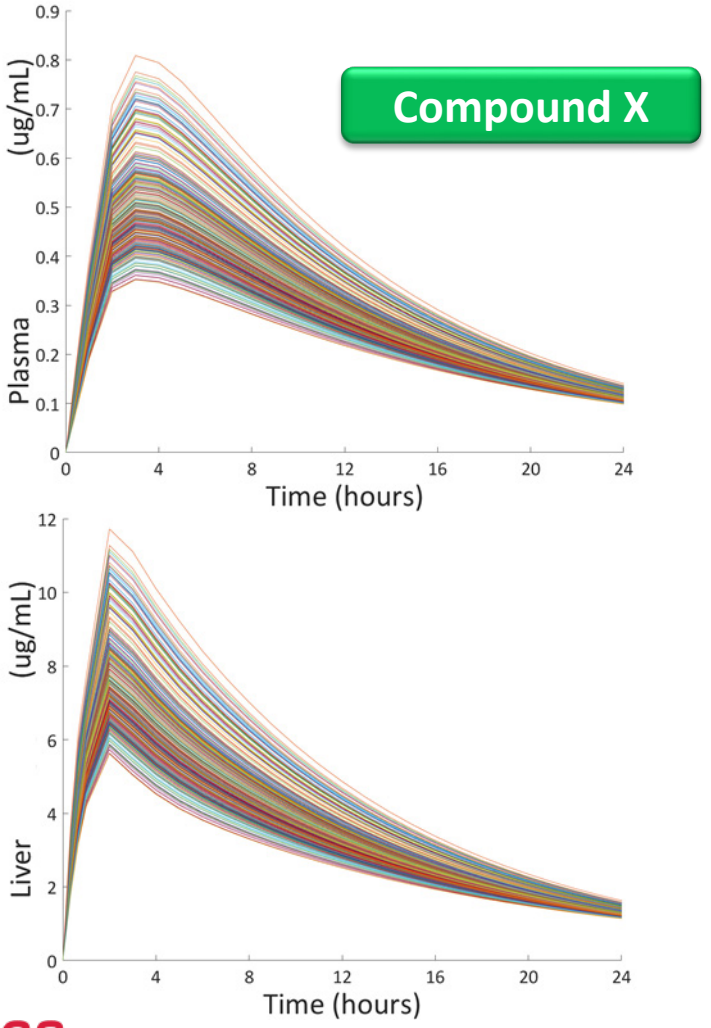
Compound X PBPK Representation Calculated at Clinical Dose

- GastroPlus predictions for liver and plasma at clinical dose for 25 days shown at right
  - PBPK model specific predictions below
  - Dose escalation and alternate protocols were also simulated



Tissue	Kp	CL	CLint	Fut/FuInt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.30	0.000	0.000	0.125
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	1.11	0.000	0.000	0.034
Muscle	0.48	0.000	0.000	0.079
Liver	9.34	0.000	0.000	0.004
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	0.51	0.000	0.000	0.074
Heart	0.60	0.000	0.000	0.063
Brain	1.10	0.000	0.000	0.034
Kidney	0.53	0.309	0.000	0.071
Skin	0.75	0.000	0.000	0.050
ReproOrg	0.54	0.000	0.000	0.070
RedMarrow	1.28	0.000	0.000	0.030
YellowMarrow	1.11	0.000	0.000	0.034
RestOfBody	0.53	0.000	0.000	0.071



Simulation Results

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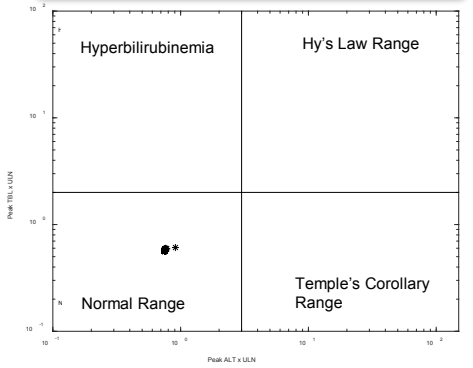
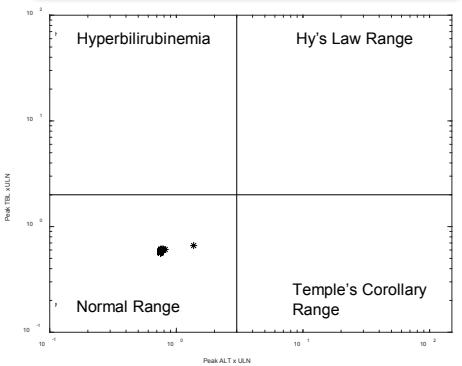
SimPops Results Show Lack of Severe Liver Injury for Both Compound Y and Compound X at Clinical Doses

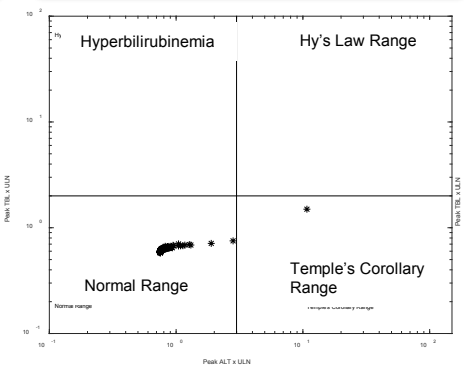
Compound Y; 1X Dose, 12 weeks



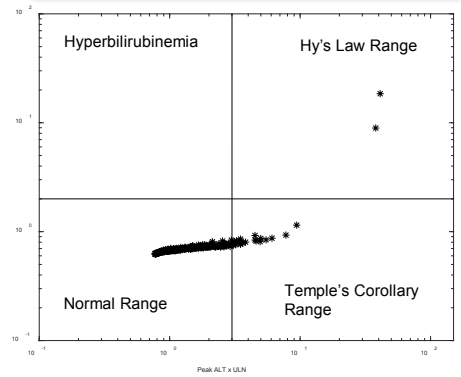
Compound Y; 2X Dose, 12 weeks



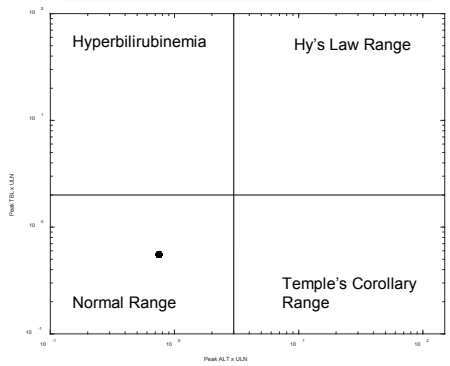
Compound Y; 5X Dose, 12 weeks



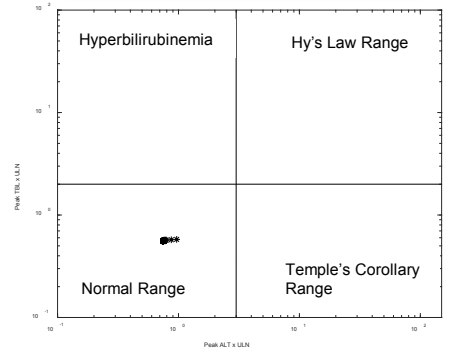
Compound Y; 10X Dose, 12 weeks



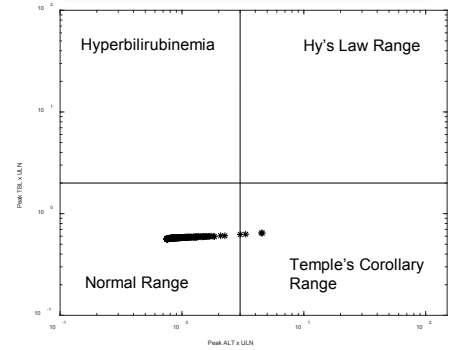
Compound X; 1X Dose



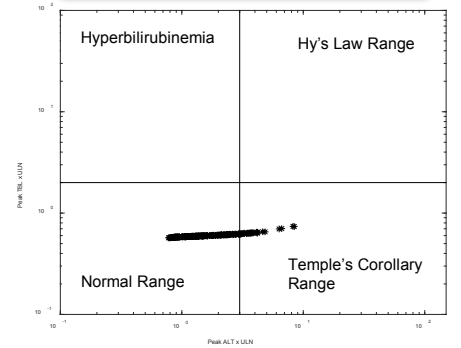
Compound X; 2X Dose



Compound X; 5X Dose



Compound X; 10X Dose



*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used

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

Simulation Results

DILIsym®

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Example Project Summary

- GastroPlus™ software, along with *in vitro* data, was used to construct PBPK representations to predict liver exposures for both compounds
- DILIsym parameters were successfully calculated from *in vitro* data for both compounds
- SimPops results show Compound X and Compound Y to be safe at projected clinical doses
- ALT elevations predicted within DILIsym at higher doses for both compounds
- SimPops results suggest that neither compound is likely to cause severe liver injury
- ***Phase IIb / III clinical trial results have subsequently confirmed the predictions for Compound Y***

ALS Riluzole Project Example Introduction

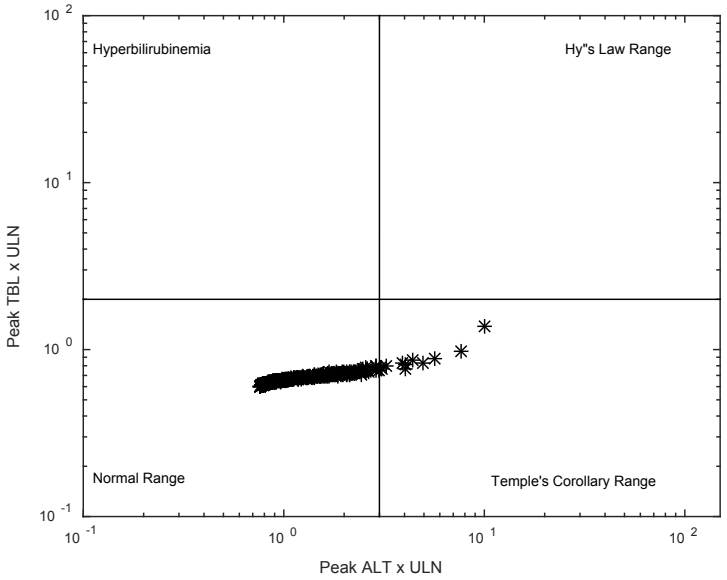
- Riluzole is used to slow the progression of ALS
  - Approved in 1995
  - Largely taken orally, which is difficult for late-stage disease patients
  - Associated with liver enzyme elevations in portion of patients

- Biohaven Pharma is developing an alternative, sublingual formulation (BHV-0223) with the following goals:
  - Improved delivery, compliance, and general ease-of-use for ALS patients, who often have trouble swallowing
  - ***Improved liver safety profile?***

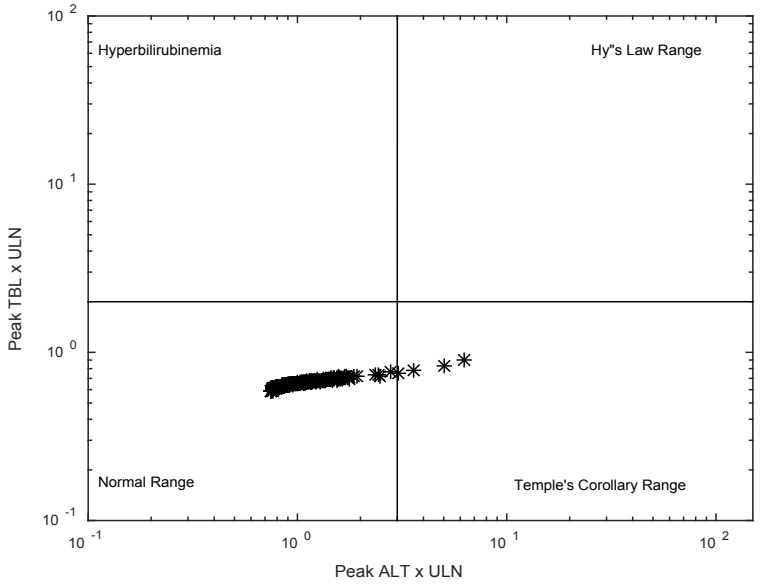
Riluzole Simulations in Normal Healthy Volunteer SimPops Show ALT Elevation Differences Between Oral and Sublingual Dosing with Certain Assumptions

Simulated eDISH Plots

Oral 50 mg BID 12 weeks
High PK, Liver Kb 10
(11/285 > 3x ULN*)



Sublingual 40 mg BID 12 weeks
High PK, Liver Kb 10
(4/285 > 3x ULN*)



Simulation Results

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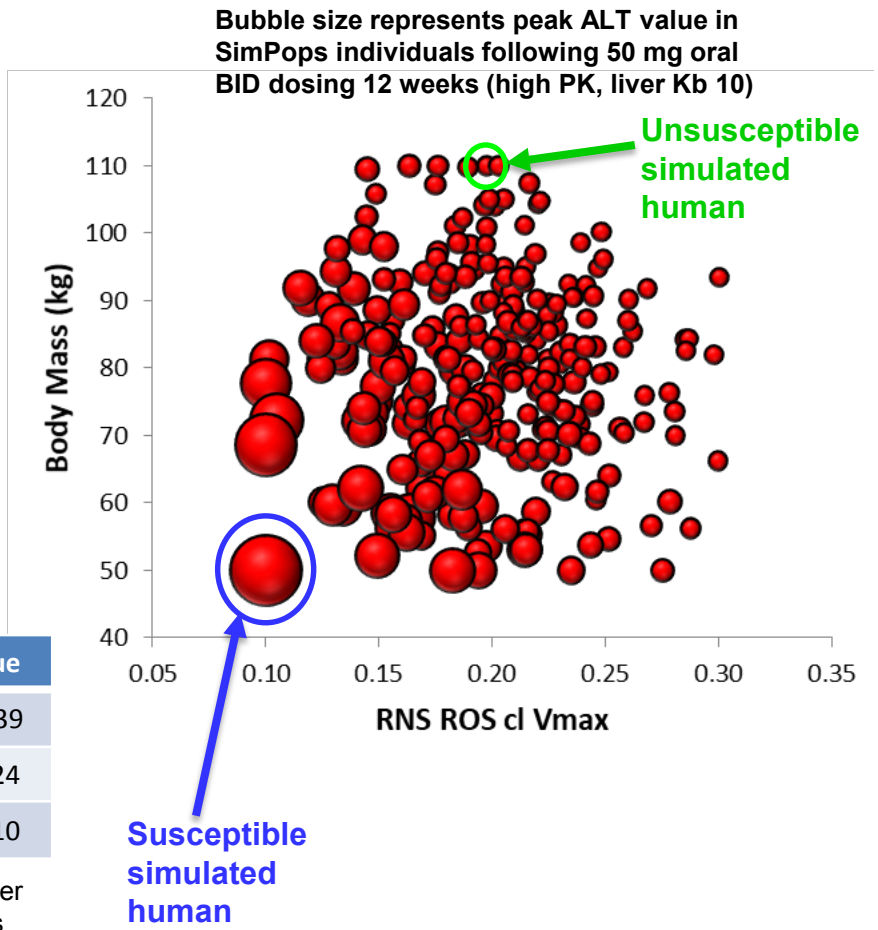
*Upper limit of normal (ULN) in DILIsym is 40 U/L

Multiple Factors Responsible for Differences in the Hepatotoxicity Response to Riluzole Among Simulated Individuals

- Differences in the hepatotoxicity response for the **unsusceptible individual** and the **susceptible individual** due to multiple factors (primarily the combined effect of differences in body mass and RNS/ROS clearance)
- Covariate analysis of the SimPops simulation results revealed 3 SimPops parameters that showed a statistically significant correlation with ALT elevations:

Parameter	Parameter Description	P-value
RNS ROS cl Vmax	Liver RNS/ROS baseline clearance Vmax	7.6 E-39
Body mass	Body mass	8.3E-24
CAS apop scale	Caspase-mediated apoptosis scaling constant	1.4E-10

Regression analysis performed with peak ALT (oral BID dosing, high PK and liver Kb 10 assumptions) as the dependent variable and the 34 SimPops parameters as independent variables



Simulation Results

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ALS Riluzole Project Example: DILIsym Helped Biohaven Assess a Potential Safety Benefit of their Compound

- Riluzole is used to slow the progression of ALS
- Biohaven Pharma is developing an alternative, sublingual formulation (BHV-0223)

Primary Project Outcomes

- DILIsym was used to compare the liver safety profile for both formulations
  - Sublingual formulation is less likely to produce less ALT elevations
  - Benefit largely derived from the reduced dose needed
  - An exposure-response analysis helped to define possible safe exposure cut-offs and identified patient susceptibility factors

- ***The FDA has accepted the NDA filing for Nurtec™ and a final decision on approval is pending***

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Example Compound Comparison Project Scenario and Goal

- A backup drug candidate intended for the treatment of a central nervous system (CNS) disorder is in development
- The lead compound was terminated due to hepatotoxicity
- ***DILIsym was employed to assess the ability to differentiate liver safety between the two compounds***

Example Compound Comparison Project Conclusions

- DILIsym simulations suggested that the backup compound is considerably less likely to pose a DILI risk than the lead compound at the proposed clinical dosing regimens
- SimPops simulations for Lead Compound:
  - Frequency of simulated hepatotoxicity is generally consistent with clinical data from various dosing regimens
  - Provides confidence in this approach for this molecular class and target
- SimPops simulations for Backup Compound:
  - Simulations in DILIsym SimPops up to 1.5x proposed clinical dose show no liver injury
  - Low frequencies of ALT elevations predicted with 2X and 3X proposed clinical dosing

Recently Completed Clinical Trial Indicates that DILIsym Backup Compound Predictions Were Correct

- No liver signals reported for any dosing regimes for the backup compound in clinical trial completed in 2018
- Combination of predicted and measured safety have enabled sponsor to confidently continue clinical development of the backup compound
  - DILIsym also provided a mechanistic understanding of differences between the backup compound and its predecessor

Project Example Introduction

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

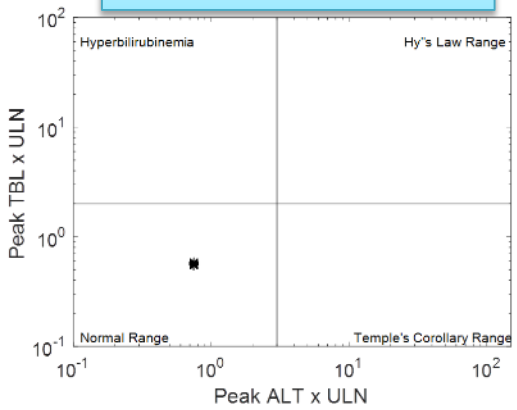
Prospective Compound X Development using DILIsym

- Optimal, prospective (much lower) dosing protocols were simulated within DILIsym to assess efficacy (exposure) and safety

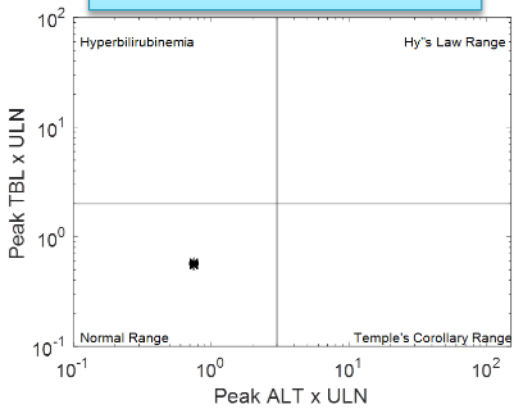
No Hepatotoxicity Predicted for Additional Prospective Clinical Protocols

- Dose dependent DILI frequency and severity correctly predicted for Compound X
- Prospective dose levels predicted to be safe from DILI
- Severity of response not appropriate to consider
  - Clinical stop protocol not included in simulations
  - Simulations may not have included some adaptation mechanisms

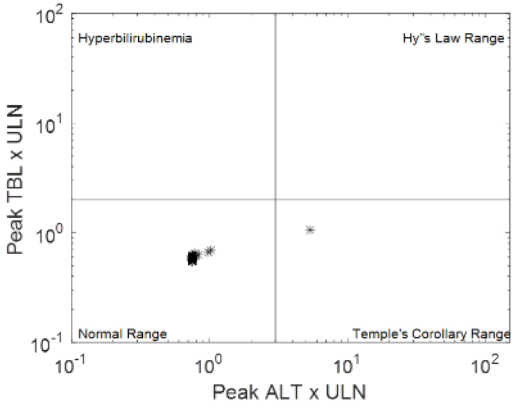
0.07X/0.03X Compound X Dosing



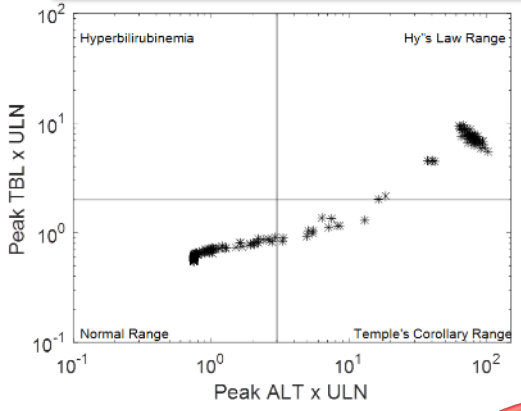
0.13X/0.07 Compound X Dosing



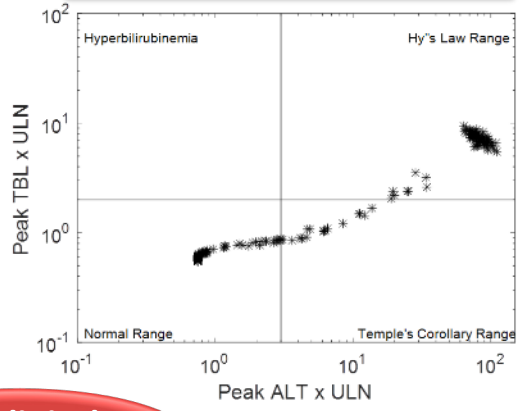
0.3X Compound X Dosing



0.5X Compound X Dosing



1X Compound X Dosing



No clinical stop protocol

Simulation Results

DILIsym Services

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Project Example Executive Summary

DILIsym Validation Using Clinical Data for Compound X

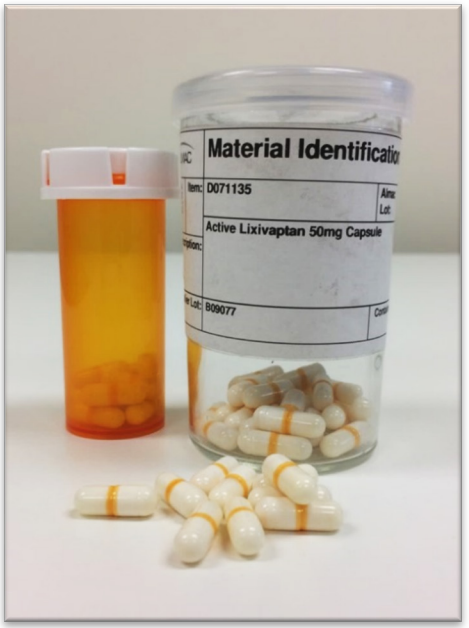
- ALT elevations were predicted in DILIsym simulations of previous Compound X clinical protocols where liver injury occurred clinically
  - Predicted delayed ALT elevations due to accumulation of a Compound X metabolite over time within DILIsym
  - Compound X metabolite-mediated mitochondrial electron 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
- **The Company has received their IND approval from the FDA and is moving forward with clinical studies presently; they will use DILIsym iteratively after each cohort to predict the effects of the next dose selected**

Lixivaptan Background

- Lixivaptan is Palladio Bio's selective, competitive vasopressin V2 receptor antagonist
- **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

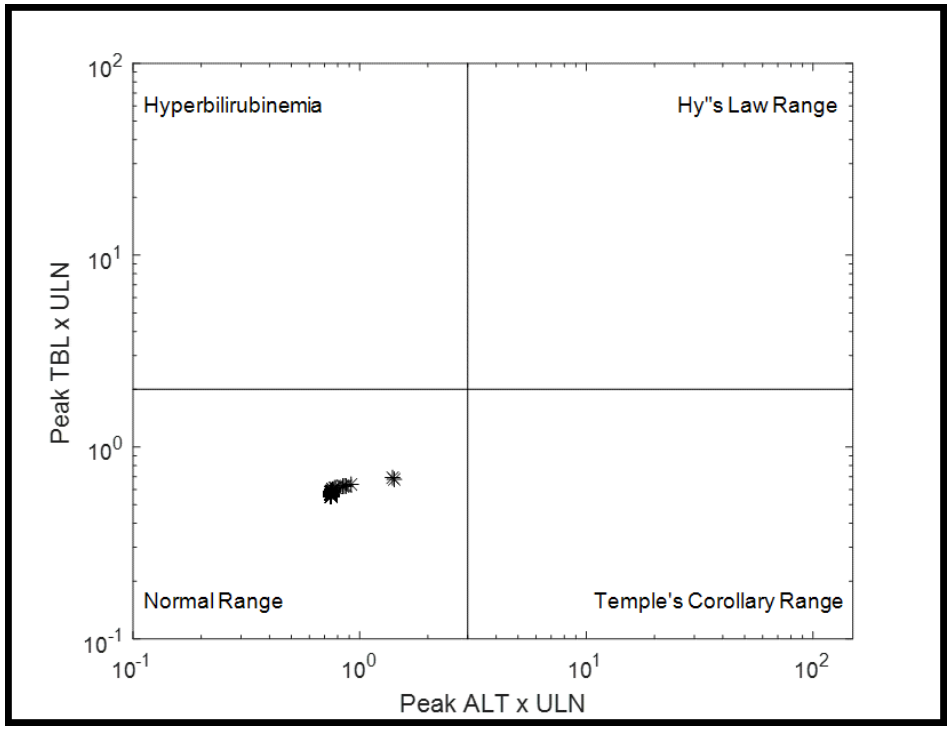
- Will lixivaptan experience similar DILI liability as the competitor in ADPKD patients?

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

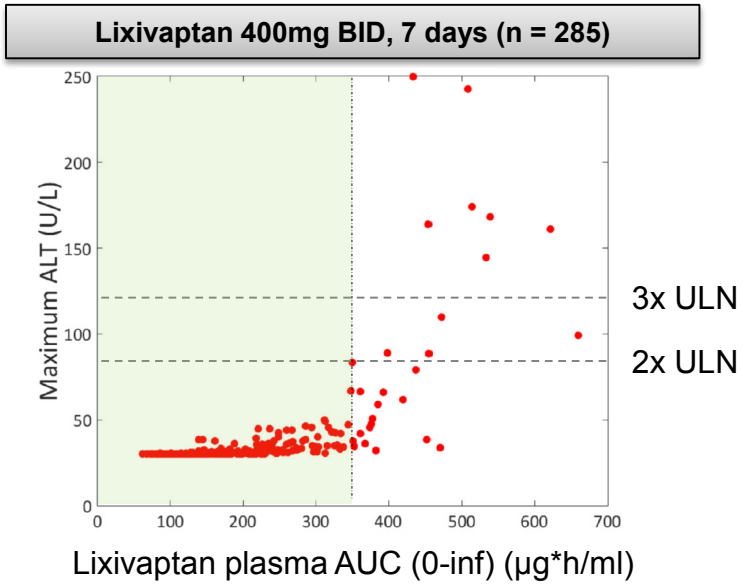
Lixivaptan Simulations Predict Minimal ALT Elevations at 200/100 mg BID

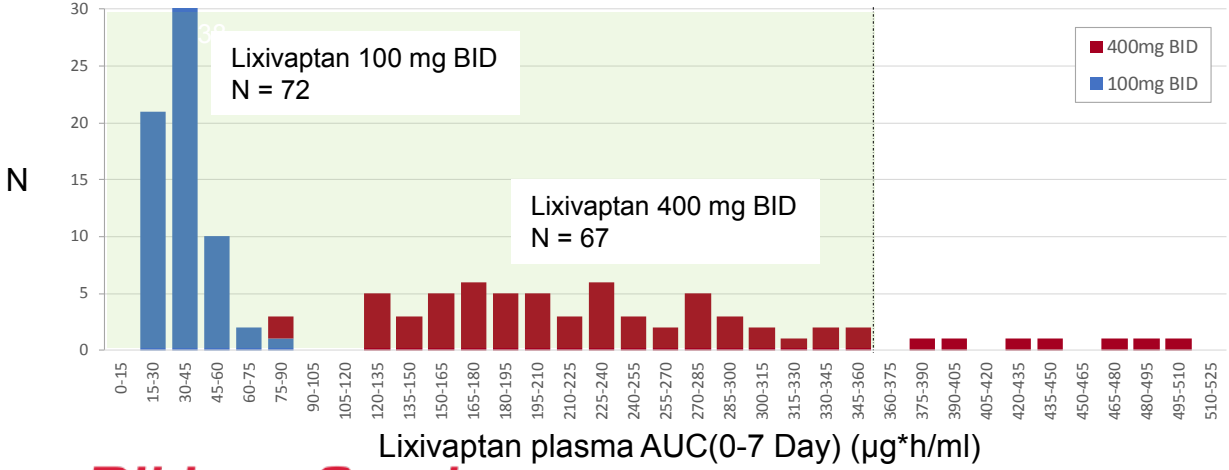
- Lixivaptan simulated in SimPops of N = 285
- No ALT elevations simulated in 100 mg BID 60-day simulation
  - Consistent with observed clinical similarity to placebo (validation)
- **No ALT elevations simulated in 200/100 split daily dosing scenario for 12 weeks**
  - **Maximum intended clinical dosing for ADPKD**



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

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Palladio Biosciences Website

me | Polycystic Kidney Disease | Pipeline | Clinical Trials | Information Resources | About Us | N

Palladio Biosciences Receives FDA IND Clearance to Begin the ELiSA Study, a Phase 2 Clinical Trial with Lixivaptan in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

May 8, 2018

Like 0 | Tweet | G+ | Share

Key Development Milestones

- Results from a state-of-the-art, predictive modeling tool based on in vitro inputs, physiologically based pharmacokinetic modeling and *in silico* simulations suggested that lixivaptan may have a differentiated safety profile compared to tolvaptan with respect to the potential to cause liver injury. Among other key findings, this investigation indicated that lixivaptan may have lower liver exposure than tolvaptan; that lixivaptan does not affect bile acid homeostasis and mitochondrial function, two key mechanisms of liver injury that may contribute to tolvaptan liver toxicity; and that lixivaptan, unlike tolvaptan, may not cause ALT elevations. These findings need to be confirmed in the clinics.
- The U.S. FDA Office of Orphan Drug Products designated lixivaptan as an orphan drug for treating ADPKD. The orphan drug designation is granted to support the development of drugs and biologics intended for the safe and effective treatment, diagnosis or prevention of diseases or disorders that affect fewer than 200,000 people in the U.S., or that affect more than 200,000 people but are not expected to recover the costs of drug development and marketing. Orphan drug designation provides eligibility for certain benefits, including seven years of market exclusivity following receipt of regulatory approval, tax credits for qualified clinical trials, and exemption from FDA application fees. It is an important milestone in the lixivaptan development program.
- In April 2018, the FDA granted IND (investigational new drug) clearance for the ELISA Study (Evaluation of Lixivaptan In Subjects with ADPKD), a Phase 2 clinical trial that will evaluate the safety, pharmacokinetics and pharmacodynamics of multiple doses of lixivaptan in patients with ADPKD with relatively preserved kidney function (chronic kidney disease stages CKD1 and CKD2) and moderately impaired renal function (CKD3). The study is expected to enroll 32 patients beginning the end of June 2018.

PALLADIO BIOSCIENCES

Working to bring new hope to patients with orphan diseases of the kidney

LIFE SCIENCES PA CONNECT - ADVOCATE - ADVANCE

Bio Member

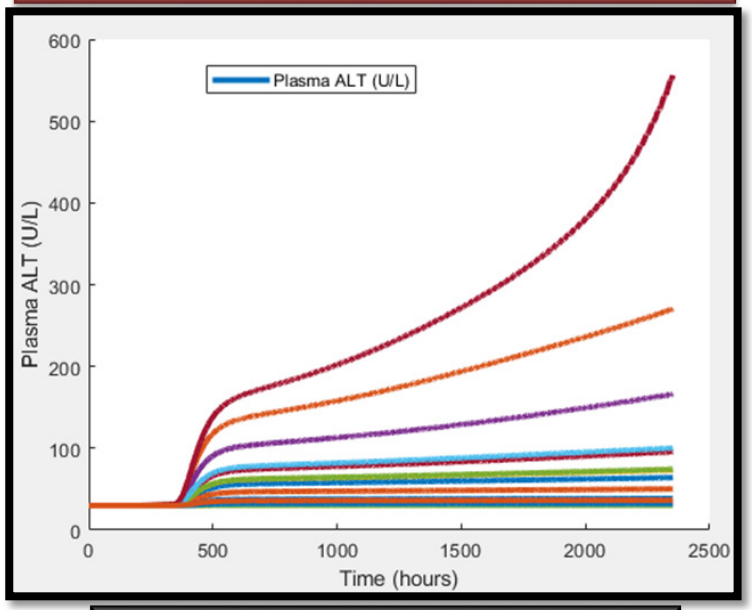
Please note that lixivaptan is for investigational use only.

Palladio Biosciences
12 Penns Trail
Unit A

Plasma ALT from DILIsym Valproate Simulations With Mitochondrial Biogenesis Adaptation Shows ALT Profiles Similar to Clinic with Adaptation

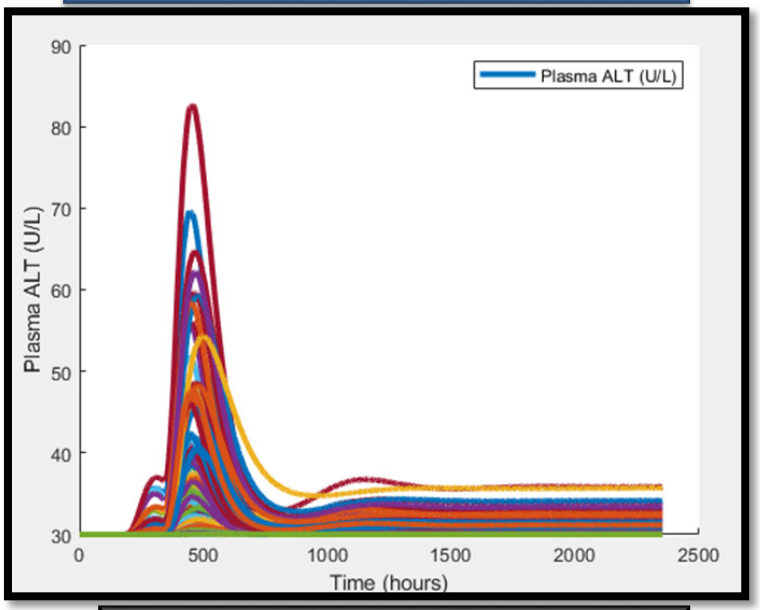
- Valproate titrated from 5 mg/kg BID to 15 mg/kg BID over 3 weeks; dosed at 15 mg/kg BID thereafter
- Without biogenesis, ALT does not resolve; does not look like clinical presentation
- With biogenesis, ALT resolves, looks similar to clinical presentation (5-10% of patients have ALT abnormalities that often resolve upon continued treatment, according to LiverTox NIH website)

WITHOUT BIOGENESIS (Adaptation)



VPA Titrated to 15 mg/kg BID

WITH BIOGENESIS (Adaptation)



VPA Titrated to 15 mg/kg BID

Simulation Results

HUMAN

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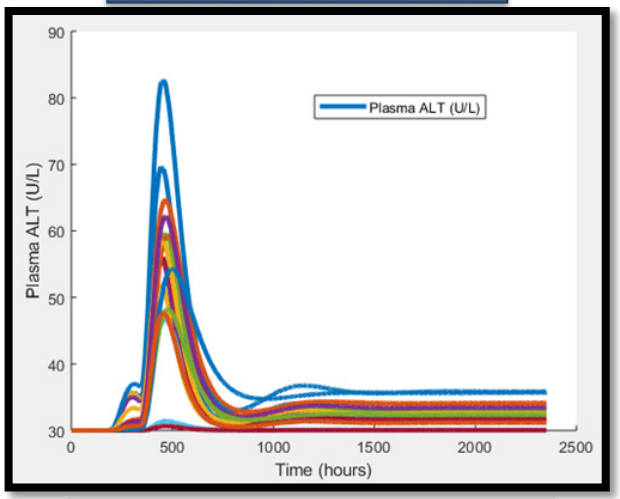
Plasma ALT from Compound ZZZ + Valproate Simulations Show Synergy and Adaptation

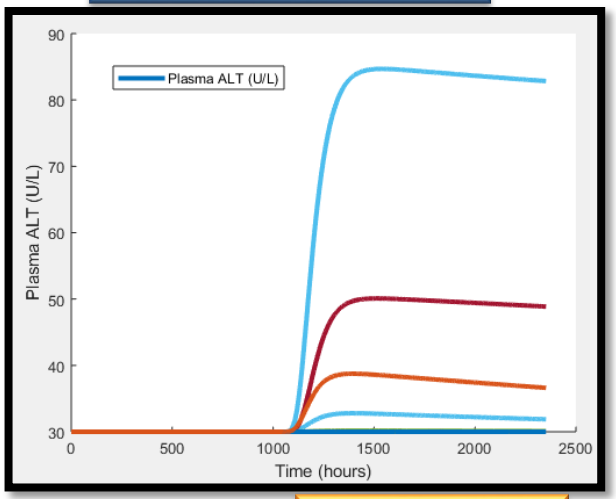
- DILIsym was used to generally test the hypothesis that two mild ETC inhibitors can combine to produce ALT elevations and also that mitogenesis can mitigate the risk
- In DILIsym simulations, Compound ZZZ alone caused minimal change in ALT in most individuals, but the combination of valproate and Compound ZZZ led to enhanced ALT elevations which resolved with continuing dosing
- Simulation results suggest that two mild ETC inhibitors can lead to ALT elevations and that mitochondrial biogenesis may lead to resolution of the issue

WITH BIOGENESIS



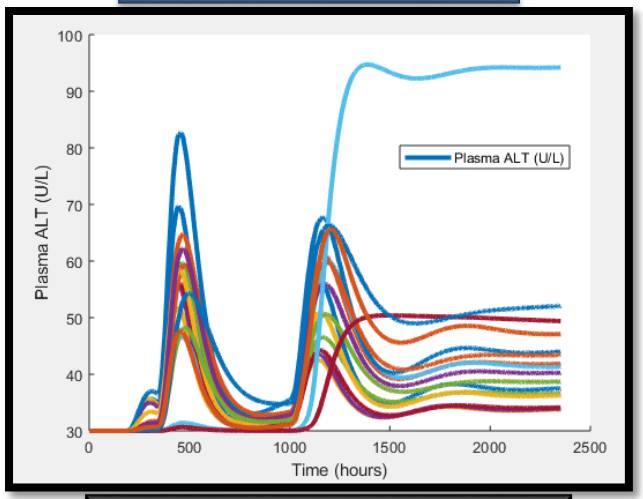
VPA Titrated to 15 mg/kg BID

WITH BIOGENESIS



Compound ZZZ

WITH BIOGENESIS



VPA Titrated to 15 mg/kg BID

Compound ZZZ

Simulation Results

HUMAN

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DILIsym Services QST and QSP Models

DILIsym®

- Predicts drug-induced liver disease
- v8A released Q1 2019
- Includes mechanistic representation of normal hepatic biochemistry
- Evaluated >60 compounds with 25 companies

RENAsym™

- Predicts acute drug-induced kidney injury
- v1A to be released Q3 2020
- Includes mechanistic representation of normal kidney biochemistry
- Not in use as of yet

NAFLDsym

- Predicts improvements in non-alcoholic fatty liver disease with treatment
- v2A to be released Q4 2018
- Includes mechanistic representation of pathophysiology of NAFLD and NASH
- Evaluated 13 compounds with 4 companies

DILIsym Services

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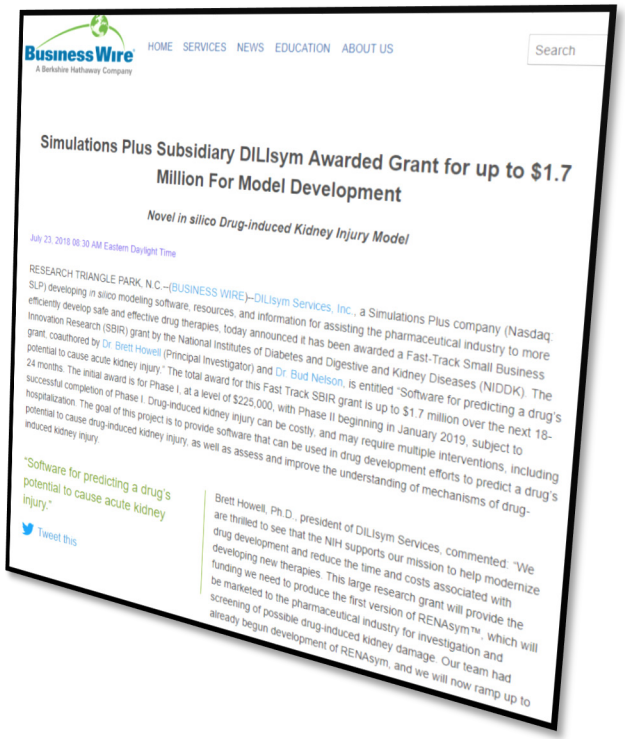
DILIsym Services Has Secured Funding to Leverage Its Experience to Provide a Predictive Software Tool for Drug-Induced Kidney Injury: **RENAsym**

- Drug-induced kidney injury, or Acute Kidney Injury (AKI), is a major reason drugs fail, behind cardiovascular and liver injuries
- The DILIsym development group has experience with:
  - Managing software development within the context of a consortium
  - Constructing QST frameworks focused on toxicity
- **DILIsym Services is now pursuing development of a new tool, RENAsym**
- **DILIsym Services awarded a Fast-Track Small Business Innovation Research (SBIR) grant by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) to develop RENAsym**
  - **Spanning Summer 2018 to Fall 2020**
  - **Total SBIR funding is up to $1.7M**
  - **Phase II of grant was approved in Dec of 2018**

RENAsym™



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Simulations Plus Subsidiary DILIsym Awarded Grant for up to $1.7 Million For Model Development

Novel in silico Drug-induced Kidney Injury Model

July 23, 2018 08:30 AM Eastern Daylight Time

RESEARCH TRIANGLE PARK, N.C.--(BUSINESS WIRE)--DILIsym Services, Inc., a Simulations Plus company (Nasdaq: SLP) developing *in silico* modeling software, resources, and information for assisting the pharmaceutical industry to more efficiently develop safe and effective drug therapies, today announced it has been awarded a Fast-Track Small Business Innovation Research (SBIR) grant by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK). The grant, coauthored by Dr. Brett Howell (Principal Investigator) and Dr. Bud Nelson, is entitled "Software for predicting a drug's potential to cause acute kidney injury." The total award for this Fast Track SBIR grant is up to $1.7 million over the next 18-24 months. The initial award is for Phase I, at a level of $225,000, with Phase II beginning in January 2019, subject to successful completion of Phase I. Drug-induced kidney injury can be costly, and may require multiple interventions, including hospitalization. The goal of this project is to provide software that can be used in drug development efforts to predict a drug's potential to cause drug-induced kidney injury, as well as assess and improve the understanding of mechanisms of drug-induced kidney injury.

"Software for predicting a drug's potential to cause acute kidney injury."

Tweet this

Brett Howell, Ph.D., president of DILIsym Services, commented: "We are thrilled to see that the NIH supports our mission to help modernize drug development and reduce the time and costs associated with developing new therapies. This large research grant will provide the funding we need to produce the first version of RENAsym™, which will be marketed to the pharmaceutical industry for investigation and screening of possible drug-induced kidney damage. Our team had already begun development of RENAsym, and we will now ramp up to

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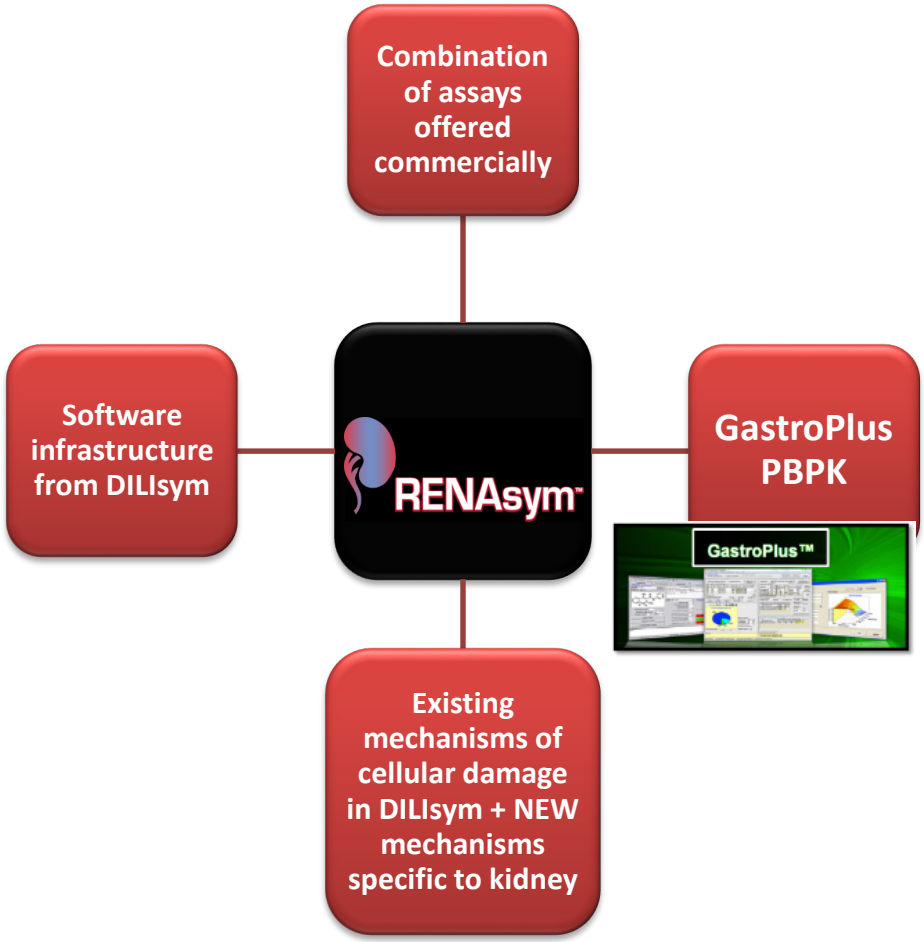
All Necessary Tools are Available to Develop the Initial Version of RENAsym

- Existing knowledge of tox mechanisms will be leveraged
- Commercially available assays have already identified to test predictivity
- GastroPlus PBPK module will drive predictions of kidney tissue concentrations
  - Kidney model will also eventually be updated
- Existing DILIsym software infrastructure will be utilized
  - Conversion to alternative code base will follow DILIsym software path eventually (including as GastroPlus module capability)



Prior Work in the Kidney Space

OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 2017, 1–12

doi: 10.1093/toxsci/kfx239
Advance Access Publication Date: November 6, 2017
Research Article

Multiscale Mathematical Model of Drug-Induced Proximal Tubule Injury: Linking Urinary Biomarkers to Epithelial Cell Injury and Renal Dysfunction

Yeshitila Gebremichael,* James Lu,† Harish Shankaran,‡ Gabriel Helmlinger,‡ Jerome Mettetal,‡ and K. Melissa Hallow*,1

*School of Chemical, Materials and Biomedical Engineering, College of Engineering, University of Georgia, Athens, Georgia; †IMED Biotech Unit, Astrazeneca Pharmaceuticals, Cambridge, UK; and ‡IMED Biotech Unit, Astrazeneca Pharmaceuticals, Waltham, Massachusetts

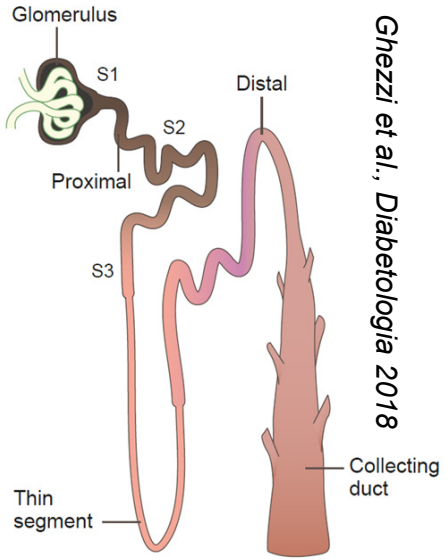
1To whom correspondence should be addressed at School of Chemical, Materials and Biomedical Engineering, College of Engineering, University o Georgia, 597 D. W. Brooks Dr., Athens, GA 30602. Fax: 706-542-2861; E-mail: hallowkm@uga.edu.

ABSTRACT

Drug-induced nephrotoxicity is a major cause of acute kidney injury, and thus detecting the potential for nephrotoxicity early in the drug development process is critical. Various urinary biomarkers exhibit different patterns following drug-induced injury, which may provide greater information than traditional biomarkers like serum creatinine. In this study, we

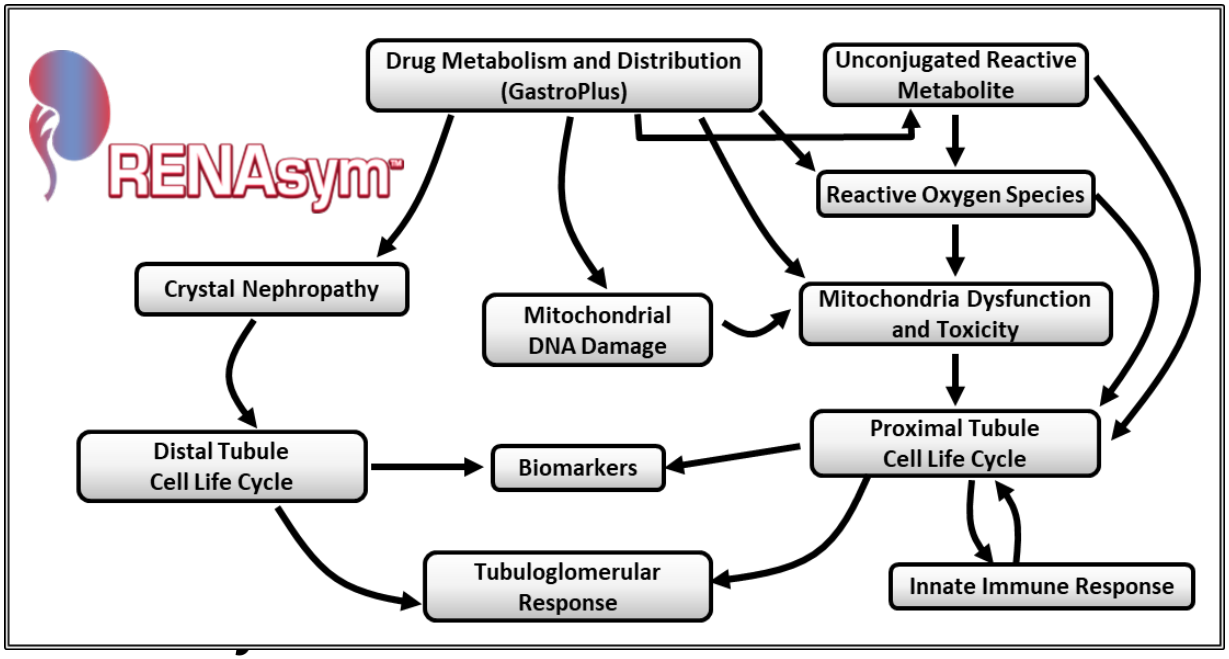
RENAsym Version 1A Preview

- **Species: human and rat**
  - Population variability
- **The three primary zones of the renal proximal tubule represented**
- **Some of the key cellular processes represented in multiple-scale, interacting sub-models**
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Cellular energy balance
  - Crystal nephropathy
  - PTC and DTC apoptosis and necrosis, and proliferation
  - Immune cells contribution
  - Immune mediators
  - Caloric intake
  - Biomarkers of cell death and function
  - Renal function (tubuloglomerular response)



Ghezzi et al., Diabetologia 2018

- **Starting with well known kidney toxicants plus negative controls, such as cisplatin, gentamycin, and APAP**
- **Single and combination drug therapies to be examined**



RENAsym Will Utilize Various Data Types to Inform Decisions

Exposure Information

PBPK Modeling within GastroPlus

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

In vitro Mechanistic Tox Data

Assays performed to determine quantitative aspects of tox mechanisms

- **Oxidative stress**
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity**
  - *ETC inhibition*
  - *Uncoupling*
- **Other assays to be added as well**

Modeling & Simulation

RENAsym™

Simulations and Assays inform:

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

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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Summary of Phase I Model Development Milestones Accomplishments - Structure

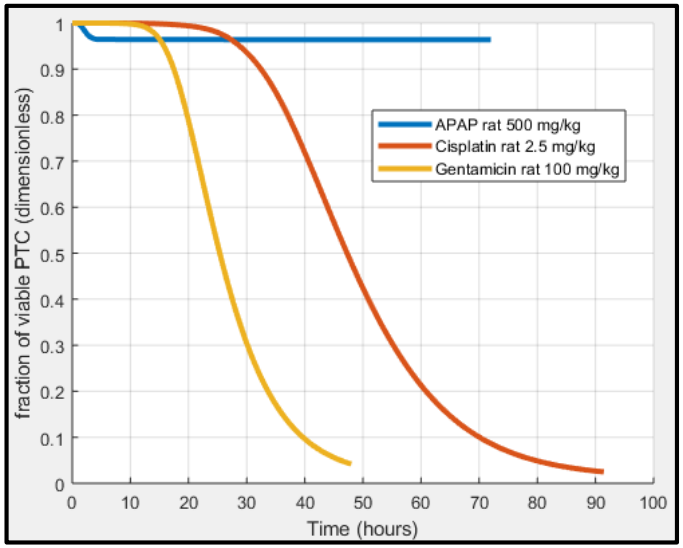
Model Structural Representation		
RENAsym Component	**Proposed**	**Completed**
Cellular life cycle	Yes	Yes
Bioenergetics	Yes	Yes
Biomarker (αGST)	Yes	Yes
Reactive oxygen species production and clearance kinetics	Yes	Yes
Two cellular injury mechanisms (Apoptosis, Necrosis)	Yes	Yes
Two cellular death pathways (Oxidative stress, Mitochondrial Toxicity)	Yes	Yes
Cellular Regeneration*	Yes	Yes (homeostasis condition)

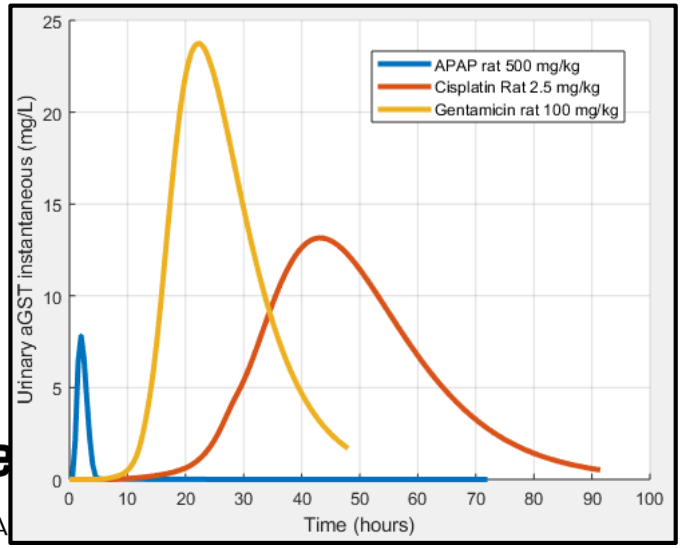
* The model structure for cellular regeneration has been implemented and parameterized under homeostasis condition. The model has yet to be fully optimized to account for PTC dedifferentiation and proliferation occurring during acute kidney injury.

Comparison of the Toxicity Effect of Positive and Negative Control Drugs in Rats

RATS

- Toxic response comparisons of simulated rats administered at single doses of 500 mg/kg APAP, 100 mg/kg gentamicin and 2.5 mg/kg cisplatin
- Rat treated with 500 mg/kg APAP shows mild/negligible cell death
- Gentamicin and cisplatin show significant cell death
  - Cell proliferation is not yet included in the current in-progress version
  - **However, the model clearly captures the expected qualitative responses of positive and negative control compounds**





Simulation Results

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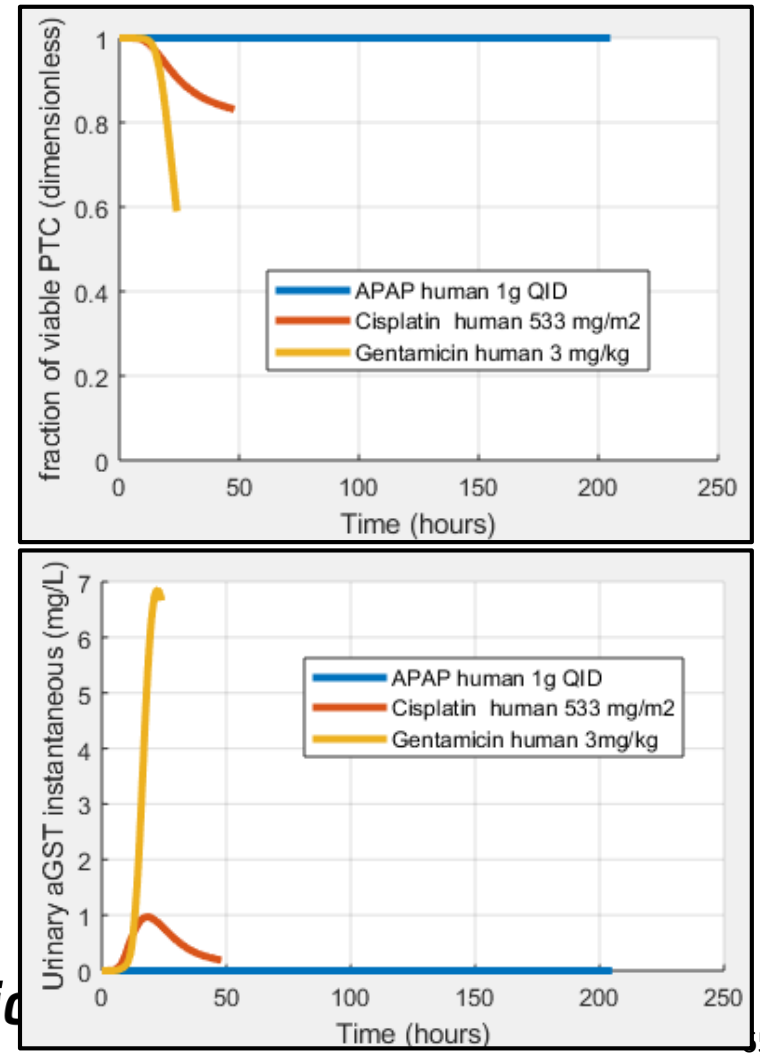
Comparison of the Toxicity Effect of Positive and Negative Control Drugs in Humans

HUMANS

- Toxic response comparisons of simulated human administered at multiple dose of 1 g QID APAP, single doses of 3 mg/kg gentamicin and 533 mg/m2 cisplatin
  - Simulation results show no cell death or αGST elevations from APAP exposure
  - Significant cell death and αGST elevations were observed with gentamicin
  - Mild cell death and GST elevation were observed with cisplatin

- **The in-progress model reproduces the expected qualitative behavior for the positive and negative control compounds**



Simulation Results

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The RENAsym Consortium is Being Formed for Gathering Development Direction and Sharing of Important Compounds and Data

- ***Please contact us today to join the consortium!***
- Consortium of partners who will be privy to the progress being made on RENAsym along the way, in addition to the following:
  - Chance to offer advice and steer direction at multiple meetings each year
  - Chance to vote on certain RENAsym development items for prioritization
  - Stellar RENAsym SAB who will help guide software progress and overall design and goals
  - Discount on renewal of membership or license in Fall of 2020 when product is available as part of membership (and/or within GastroPlus as a module)
- Membership cost is low, only to cover administrative costs
- Discounts on RENAsym access will be given when RENAsym is released in Fall of 2020 (and included in the membership agreement) depending on membership fees paid and timeframe for joining

RENAsym™

DILIsym Services

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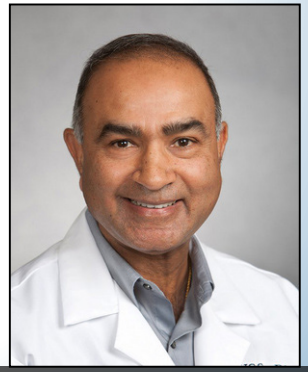
Experts Who Have Agreed to Serve on the RENAsym Scientific Advisory Board

Dr. Paul B. Watkins
Director, Institute for Drug Safety Sciences
Howard Q. Ferguson Distinguished Professor Of Medicine
UNC Eshelman School of Pharmacy

Dr. K Melissa Hallow
Assistant Professor
School of Chemical, Materials, and Biomedical Engineering
University of Georgia

Dr. Zheng Dong
Leon H. Charbonnier Endowed Chair, Regents Professor
Medical College of Georgia
Senior Career Scientist, Director of Research
Charlie Norwood VA Medical Center

Lauren Aleksunes, PharmD, PhD, DABT
Associate Professor, Graduate Director
Pharmacology and Toxicology
Rutgers University



Dr. Ravinder L Mehta
Professor of Medicine in the Division of Nephrology and
Associate Chair for Clinical Affairs
Department of Medicine
University of California, San Diego (UCSD)

SimulationsPlus

SCIENCE + SOFTWARE = SUCCESS

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Visit us in booth #3736 to discuss your projects

SOT presentations:

Michael Lawless
Sr. Scientist, Simulations Plus
Applying In Silico-In Vitro-In Vivo Extrapolation (IS-IVIVE) Techniques to Predict Exposure and Guide Risk Assessment
March 12 @ 8:39 AM - 9:08 AM Room 309

Guncha Taneja
Post-Doc Researcher, DILIsym Services, a Simulations Plus company
Development of a Quantitative Systems Toxicology Model of Drug-Induced Cholangiocyte Injury in DILIsym
March 12 @ 9:15 AM - 10:45 AM Board no. P658

Biological Modeling Specialty Section Meeting/Reception
1-year License Awards to GastroPlus & DILIsym Software
March 12 @ 6:00 PM - 7:30 PM Hilton Baltimore Key 11

Brett Howell
President, DILIsym Services, a Simulations Plus company
Assessing Effects of BHV-0223 40 mg Zydis Sublingual Formulation and Riluzole 50 mg Oral Tablet on Liver Function Test Parameters Utilizing DILIsym
March 13 @ 8:00 AM - 8:15 AM Room 321

John DiBella
President - Lancaster Division, Simulations Plus
Modeling Platforms Validation Process
March 13 @ 4:35 PM - 4:40 PM Room 314

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www.simulations-plus.com

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www.RENAsym.com

Emails: john.dibella@simulations-plus.com
bhowell@DILIsym.com

Phone: 661-723-7723

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