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SCIENCE + SOFTWARE = SUCCESS

DILIsym Services

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# Applying in silico-in vitro-in vivo extrapolation (IS-IVIVE) techniques to predict exposure and guide risk assessment


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Brett Howell, President – DILIsym Services, an SLP Company

Email: bhowell@dilisym.com

# Evolving relationship between *in silico* tools and R&D

- **Model "supported" (first questions 20 years ago)**: Do you think modeling and simulation might help?
- **Model "based" (current questions today)**: How can I maximize the value of modeling and simulation in my development program?
- **Model "driven" (future questions)**: How do I change the R&D process to reflect the availability of *in silico* tools and techniques?

U.S. Department of Health & Human Services

FDA U.S. FOOD & DRUG ADMINISTRATION

FDA Voice by Commissioner Scott Gottlieb, M.D.

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# How FDA Plans to Help Consumers Capitalize on Advances in Science

Posted on July 7, 2017 by FDA Voice

To build upon such opportunities, FDA will soon unveil a comprehensive Innovation Initiative. It will be aimed at making sure our regulatory processes are modern and efficient, so that safe and effective new technologies can reach patients in a timely fashion. We need to make sure that our regulatory principles are efficient and informed by the most up to date science. We don't want to present regulatory barriers to beneficial new medical innovations that add to the time, cost, and uncertainty of bringing these technologies forward if they don't add to our understanding of the product's safety and benefits.

Today we announced our detailed work plan for the steps we're taking to implement different aspects of Cures. I want to highlight one example of these steps, which we're investing in, and will be expanding on, as part of our broader Innovation Initiative. It's the use of in silico tools in clinical trials for improving drug development and making regulation more efficient.

FDA's Center for Drug Evaluation and Research (CDER) is currently using modeling and simulation to predict clinical outcomes, inform clinical trial designs, support evidence of effectiveness, optimize dosing, predict product safety, and evaluate potential adverse event mechanisms. We'll be putting out additional, updated guidance on how aspects of these in silico tools can be advanced and incorporated into different aspects of drug development.

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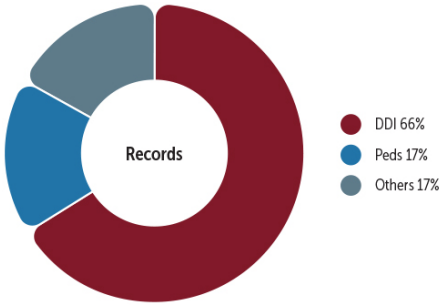
FDA Voice blog: July 7th, 2017

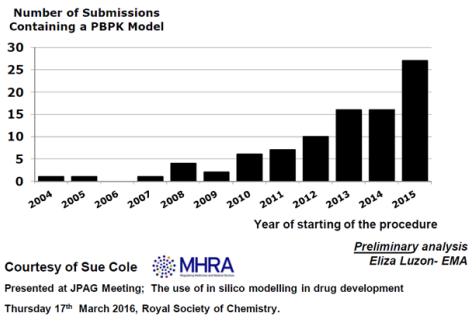
# Recent PBPK Modeling Trends: Regulatory Information

- 180 PBPK modeling citations in the FDA's Office of Clinical Pharmacology database (2008-15)
- 60 submissions received by EMA containing PBPK models (2013-15)
- 17 PBPK modeling citations at Japan PMDA (2014-16)

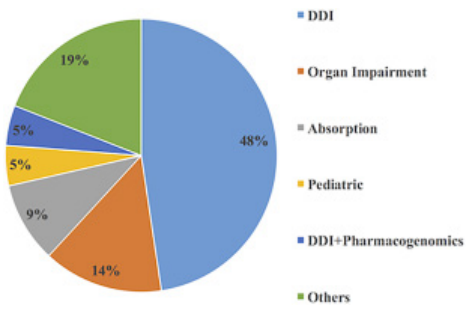
**FDA**




**EMA**




**PMDA**

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Mehrotra et al., DMD, 2016
Sato et al., CPT 2017
Cole et al., 2016 JPAG Meeting

SimulationsPlus | Cognigen | DILIsym Services

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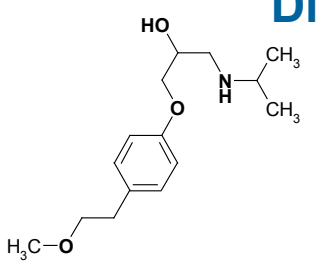
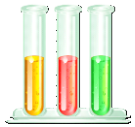
# (NASDAQ: SLP); total employees ~100

- Simulations Plus, Inc.
  - Software development, PBPK modeling & simulation, and QSAR modeling
- Cognigen Corporation, a Simulations Plus company
  - Software development, pharmacometric services, and population PK/PD data analyses
- DILIsym Services, a Simulations Plus company
  - Software development, systems pharmacology/toxicology modeling

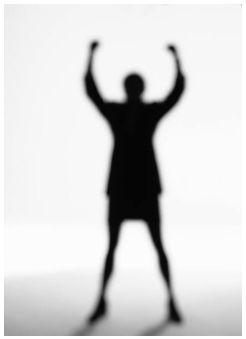
# Simulations Plus:
# Your end-to-end M&S solutions provider




**Discovery**



**Preclinical**



**Clinical**

**ADMET Predictor™** ⟷ **GastroPlus™**

- **MedChem Studio™**
  **MedChem Designer™**
- **DDDPlus™**
  **MembranePlus™**
- **PKPlus™**
- **KIWI™**
- **DILIsym®**
- **NAFLDsym®**

**Consulting Services and Collaborations**

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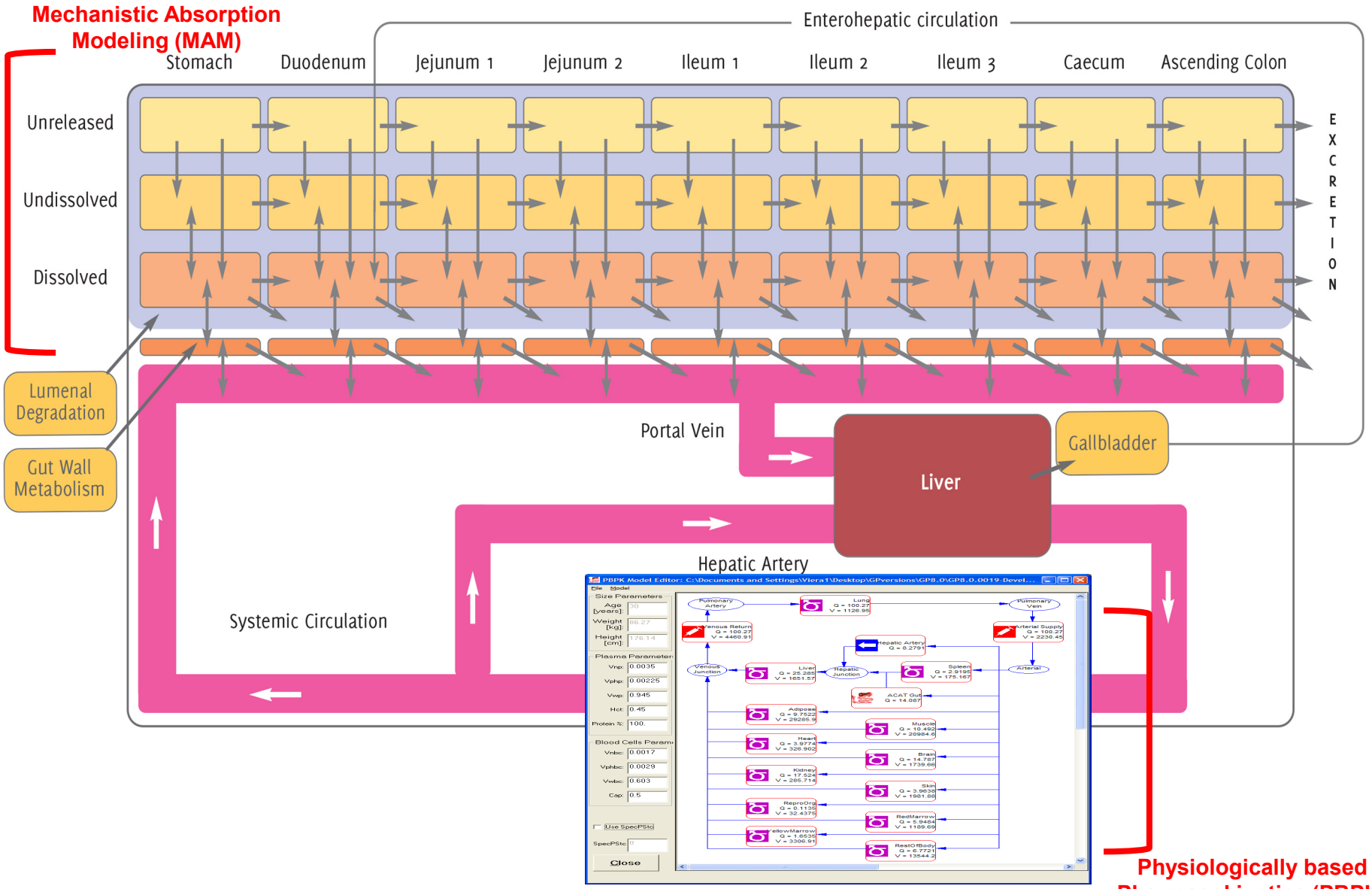
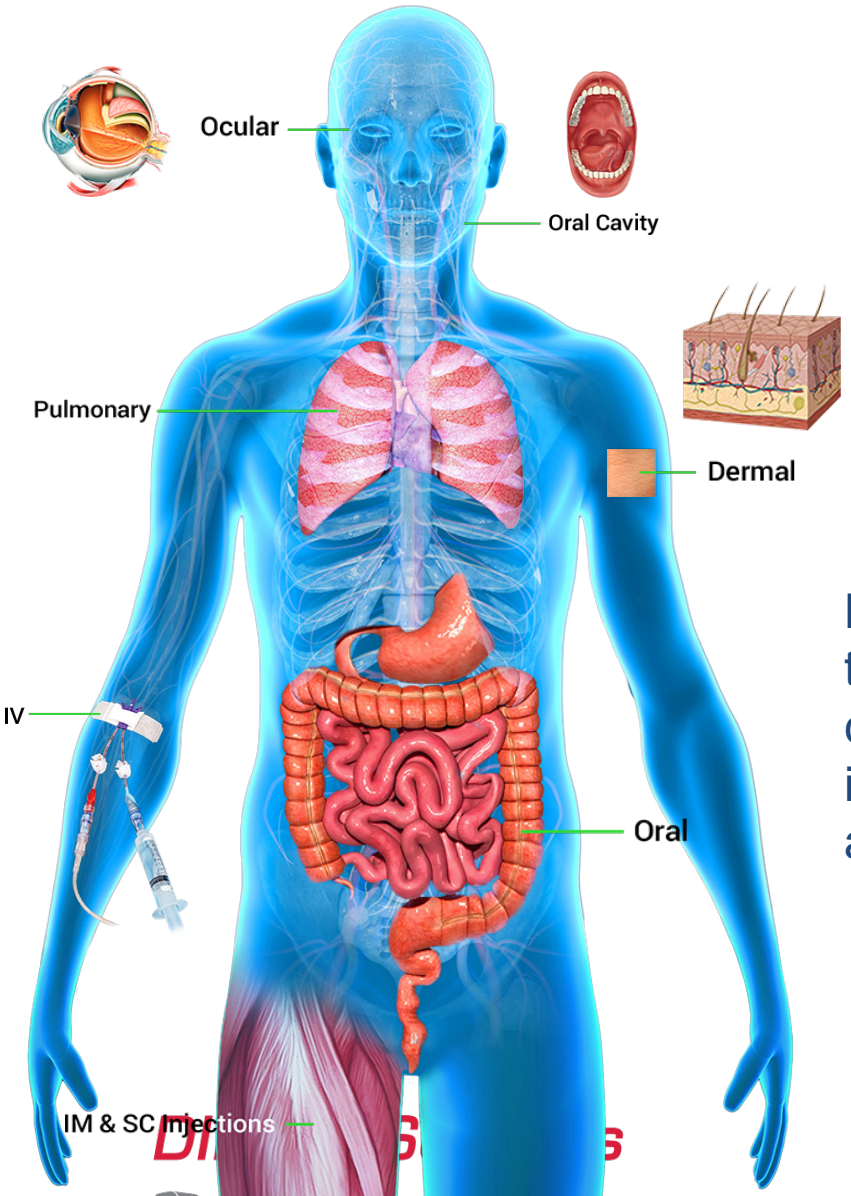
# Advanced Compartmental Absorption and Transit Model (ACAT™)

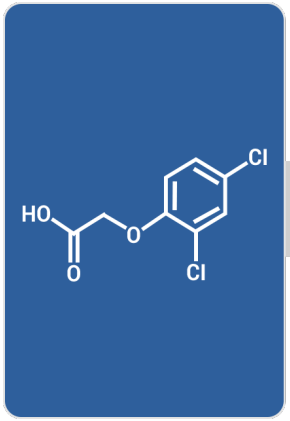


# Mechanistic Absorption Models in GastroPlus™

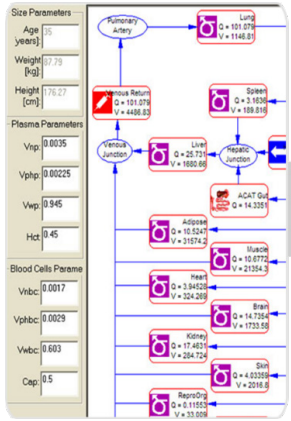



Note: all developed through funded collaborations with industry companies and/or the U.S. FDA

# Saying “I do” to the QSAR/PBPK marriage…




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

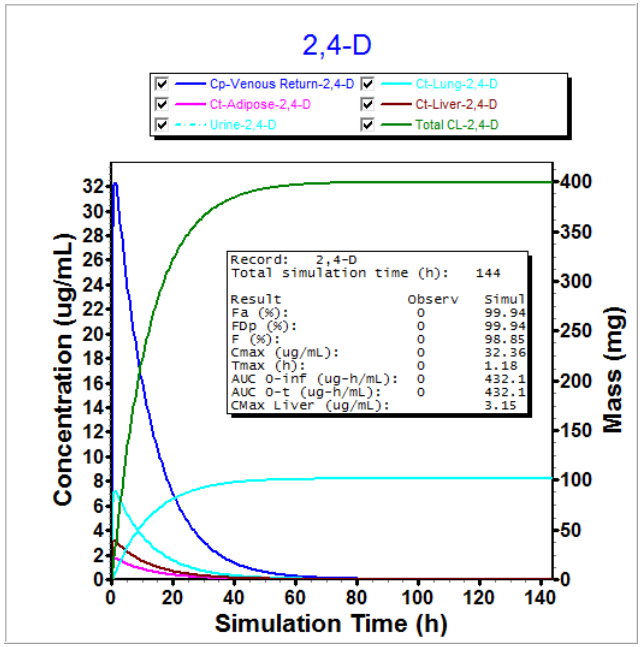


Quantitative Structure Activity Relationships (QSAR)

ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™




Goal: **reliably** and **efficiently** utilize PBPK modeling to reduce animal/human testing

# Are the models any good?

Independent comparison of aqueous solubility predictors (Dearden JC. Exper. Opin. Drug Discovery 2006 1:31)

Comparison of first-in-human (FIH) PBPK prediction accuracy in a 2-year study of 21 compounds (Cole et al., ISSX 2008)

**Table 4. Predictive abilities of some commercially available software for aqueous solubility prediction, based on 122-compound test-set of drugs.**

| Software | % Compounds predicted within ~0.5 log unit | % Compounds predicted within ~1.0 log unit | $r^2$ | $q^2$ | s | Ref. |
|---|---|---|---|---|---|---|
| SimulationsPlus | 64.8 | 91.0 | 0.82 | 0.82 | 0.47 | [203] |
| Admensa | 72.1 | 88.5 | 0.76 | 0.74 | 0.65 | [205] |
| Pharma Algorithms ADME Boxes | 59.0 | 86.9 | 0.74 | 0.73 | 0.62 | [206] |
| ChemSilico | 59.8 | 86.0 | 0.67 | 0.65 | 0.73 | [202] |
| ACDLabs | 59.0 | 85.2 | 0.73 | 0.72 | 0.66 | [204] |
| AlogS | 51.6 | 81.1 | 0.67 | 0.66 | 0.73 | [207] |
| PredictionBase | 46.7 | 81.1 | 0.48 | 0.46 | 1.07 | [208] |
| ESOL | 54.9 | 78.7 | 0.60 | 0.59 | 0.84 | [209] |
| MOLPRO | 62.3 | 77.9 | 0.44 | 0.42 | 1.22 | [210] |
| Absolv 2 | 44.3 | 74.6 | 0.53 | 0.51 | 0.95 | [206] |
| QikProp | 47.6 | 73.8 | 0.57 | 0.57 | 0.97 | [201] |
| SPARC* | 42.9 | 73.1 | 0.73 | 0.72 | 0.96 | [211] |
| Cerius²ADME | 37.7 | 72.9 | 0.61 | 0.60 | 1.02 | [212] |
| WSKOWWIN | 41.0 | 67.2 | 0.51 | 0.49 | 1.17 | [213] |
| ADMEWORKS Predictor | 34.4 | 66.4 | 0.42 | 0.39 | 1.24 | [214] |
| AlogP98 | 38.5 | 62.3 | 0.42 | 0.40 | 0.77 | [85,212] |
| CHEMICALC‡ | 23.3 | 45.7 | 0.35 | 0.34 | 1.96 | [215] |

*Based on 119 compounds; SPARC could not calculate solubilities of 3 compounds.
‡Based on 116 compounds, using log P method with calculated melting point, which was not available for 6 compounds; kindly calculated by Prof. G. Schüürmann.



**Summary of IV profile prediction accuracy**

| APPROACH | PROFILE | Vss | | CL | |
|---|---|---|---|---|---|
| | Weighted sum of squares (RANK) | AFE | % within 2-fold error (3-fold error) | AFE | % within 2-fold error (3-fold error) |
| GastroPlus | -11.7 (1) | 1.4 | 90 (100) | 1.6 | 80 (85) |
| PKSim | -6.4 (2) | 1.7 | 70 (90) | 1.6 | 80 (85) |
| Current Pfizer Approach | -3.8 (3) | 1.6 | 75 (85) | 1.6 | 80 (85) |
| SimCYP – hlm | 5.6 (4)* | 1.5 | 80 (95) | 2.5 | 58 (74) |
| SimCYP – rhCYP | 7.8 (5)* | 1.5 | 80 (95) | 2.4 | 55 (65) |
| ChloePK | 8.5 (6)* | - | - | 1.7 | 70 (80) |

**Summary of Oral profile prediction accuracy**

AFE➔ Average Fold Error

| APPROACH | PROFILE | AUC | | Cmax | |
|---|---|---|---|---|---|
| | Weighted sum of squares (RANK) | AFE | % within 2-fold error (3-fold error) | AFE | % within 2-fold error (3-fold error) |
| GastroPlus | -9.8 (1) | 2.7 | 50 (72) | 2.0 | 67 (72) |
| Current Pfizer Approach | -5.3 (2) | 3.9 | 33 (56) | 2.5 | 44 (61) |
| SimCYP - rhCYP | -3.7 (3) | 3.0 | 56 (67) | 2.2 | 61 (72) |
| SimCYP - hlm | 5.7 (4)* | 3.6 | 41 (53) | 2.7 | 53 (59) |
| PKSim | 6.1 (5)* | 4.7 | 22 (39) | 5.0 | 17 (33) |
| ChloePK | 7.0 (6)* | 2.8 | 39 (50) | 2.4 | 50 (61) |

**Table 2.** Performance of algorithms

| | Star (234) | | Nostar (50) | | Zwitterions (18) | | Other (266) |
|---|---|---|---|---|---|---|---|
| Method | *MAE* | Rank | *MAE* | Rank | *MAE* | *AE* | *MAE* |
| A_S+logP | 0.33 | I | 0.7 | I | 0.4 | -0.01 | 0.4 |
| ALOGPS[3] | 0.39 | I | 0.7 | I | 0.64 | -0.51 | 0.44 |
| VLOGP[4] | 0.50(0.41) | II | 0.95(0.84) | I,III | 0.87(0.69) | -0.8(-0.62) | 0.56(0.47) |
| SLIPPER | 0.58 | II | 0.91 | I,III | 1.2 | -1.14 | 0.6 |
| QikProp | 0.58 | II | 1.01 | III | 0.83 | -0.48 | 0.64 |
| CSlogP | 0.61 | II | 0.95 | I,III | 0.54 | -0.06 | 0.68 |
| TLOGP[5] | 0.64 | II | 1.01 | III | 1.26 | -0.97 | 0.69 |
| Absolv | 0.65 | II | 0.94 | I,III | 1.98 | -1.97 | 0.61 |
| QuantlogP[3] | 0.7 | II | 1.03 | III | 1.91 | -1.9 | 0.68 |
| QLOGP | | | | | | | |
| VEGA[6] | | | | | | | |
| CLIP[7] | | | | | | | |
| LSER | | | | | | | |
| MLOGP | | | | | | | |
| SPARC[8,9] | 0.93 | III | 1.17 | III | 0.72 | 0.06 | 0.99 |
| COSMOFrag[3] | 1.13 | III | 1.38 | IV | 2.48 | -2.47 | 1.09 |
| LSER UFZ[8] | 1.19 | IV | 2.15 | IV | 2.32 | -1.75 | 1.29 |
| GBLOGP[7] | 1.25 | IV | 1.76 | IV | 2.51 | 2.46 | 1.26 |
| HINT | 1.38 | IV | 2.14 | IV | 3.25 | -3.24 | 1.39 |
| AAM | 1.37 | IV | 1.87 | IV | 2.96 | | 1.36 |

Independent comparison of logP predictors (Tetko & Poda, 2007)

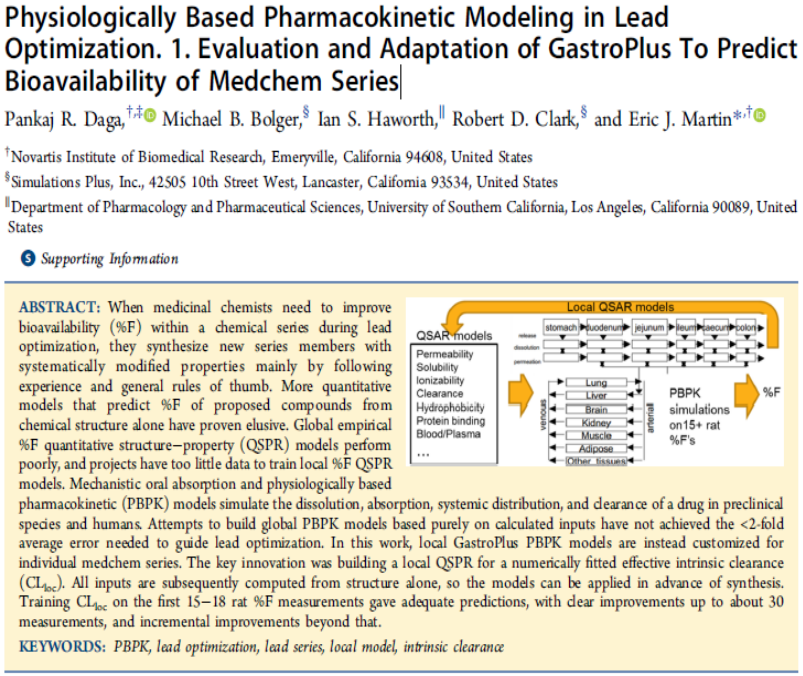
| Predicted by | Trained with | MAE | RMSE | $R^2$ |
|---|---|---|---|---|
| ACD/Percepta v. 12 | 15932 lit $pK_a$ | 0.77 | 1.05 | 0.84 |
| ADMET Predictor v. 6.1 | 14147 lit $pK_a$ | 0.73 | 0.95 | 0.86 |
| **ADMET Predictor v. 7.0** | **14149 lit $pK_a$ + 19467 Bayer $pK_a$** | **0.51** | **0.67** | **0.93** |

Fraczkiewicz et al. (2015) J. Chem. Inf. Model. 55(2):389

# Recent Validation

Daga et al. (2018) Mol. Pharm.
>75% of compounds predicted within 2-fold

Lawless et al. (2016) ASCPT Annual Meeting
Using QSAR & PBPK to predict human F%:
70% of compounds predicted within 2-fold



**Physiologically Based Pharmacokinetic Modeling in Lead Optimization. 1. Evaluation and Adaptation of GastroPlus To Predict Bioavailability of Medchem Series**

Pankaj R. Daga,[†,‡] Michael B. Bolger,[§] Ian S. Haworth,[∥] Robert D. Clark,[§] and Eric J. Martin*[,†]

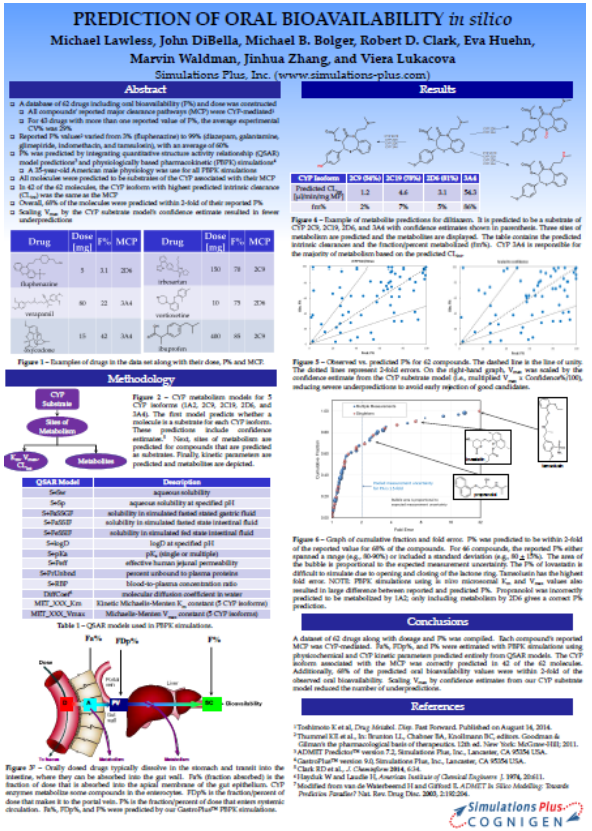
[†]Novartis Institute of Biomedical Research, Emeryville, California 94608, United States
[§]Simulations Plus, Inc., 42505 10th Street West, Lancaster, California 93534, United States
[∥]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, California 90089, United States

Supporting Information

**ABSTRACT:** When medicinal chemists need to improve bioavailability (%F) within a chemical series during lead optimization, they synthesize new series members with systematically modified properties mainly by following experience and general rules of thumb. More quantitative models that predict %F of proposed compounds from chemical structure alone have proven elusive. Global empirical %F quantitative structure−property (QSPR) models perform poorly, and projects have too little data to train local %F QSPR models. Mechanistic oral absorption and physiologically based pharmacokinetic (PBPK) models simulate the dissolution, absorption, systemic distribution, and clearance of a drug in preclinical species and humans. Attempts to build global PBPK models based purely on calculated inputs have not achieved the <2-fold average error needed to guide lead optimization. In this work, local GastroPlus PBPK models are instead customized for individual medchem series. The key innovation was building a local QSPR for a numerically fitted effective intrinsic clearance ($CL_{loc}$). All inputs are subsequently computed from structure alone, so the models can be applied in advance of synthesis. Training $CL_{loc}$ on the first 15−18 rat %F measurements gave adequate predictions, with clear improvements up to about 30 measurements, and incremental improvements beyond that.

**KEYWORDS:** *PBPK, lead optimization, lead series, local model, intrinsic clearance*



**PREDICTION OF ORAL BIOAVAILABILITY *in silico***

Michael Lawless, John DiBella, Michael B. Bolger, Robert D. Clark, Eva Huehn, Marvin Waldman, Jinhua Zhang, and Viera Lukacova
Simulations Plus, Inc. (www.simulations-plus.com)

Abstract

Results

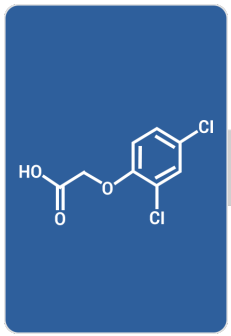
Methodology

Conclusions

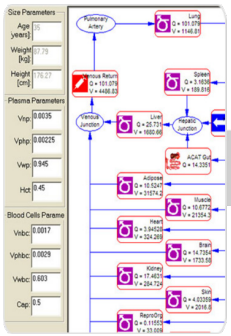
References

DILIsym Services

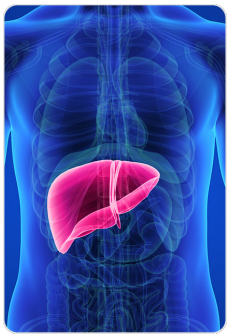
# Saying “I do” to the QSAR/PBPK marriage…




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)

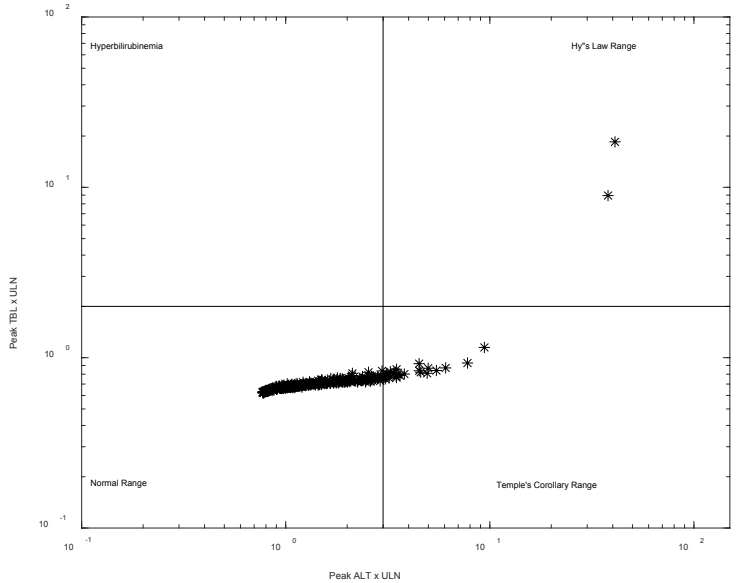
ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™

Quantitative Systems Pharmacology/Toxicology (QSP/QST)

DILIsym®

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# DILIsym Services, Inc. – Our Vision

***"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)
  - **DILIsym** and **NAFLDsym** simulation consulting projects
  - Consulting and data interpretation
  - *in vitro* assay experimental design and management

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


***Sample of Some Current Consortium Members***

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

FDA

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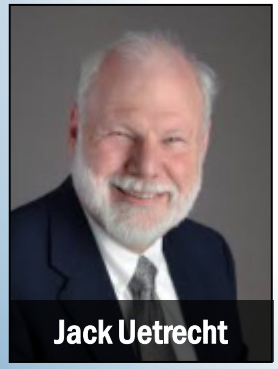
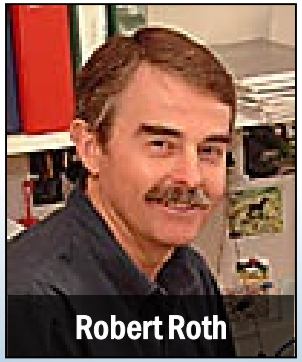
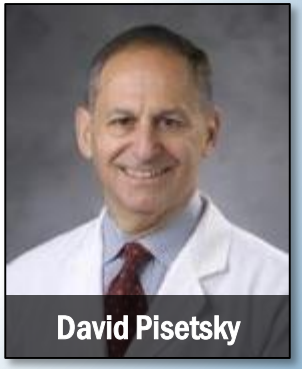
# The DILI-sim Scientific Advisory Board Includes World Class Scientists




DILIsym®

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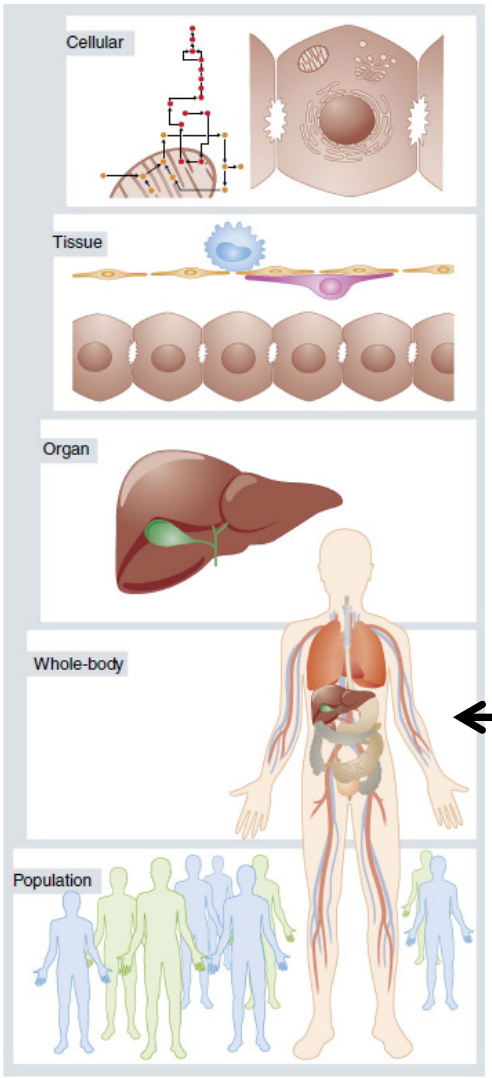
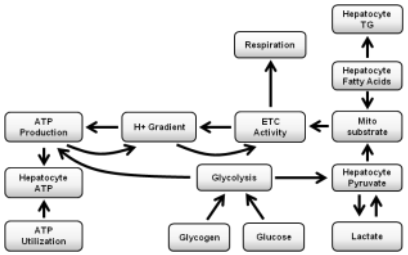
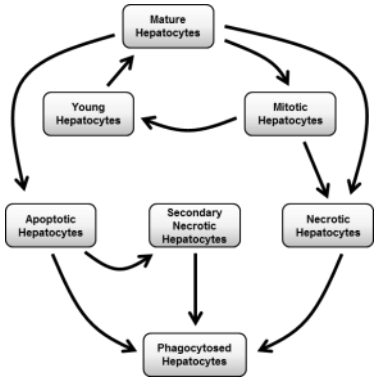
# DILIsym: Quantitative Systems Toxicology



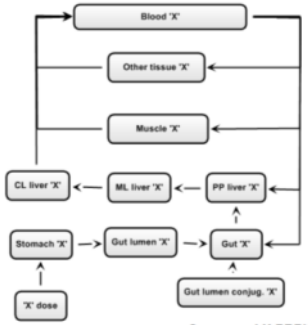

Mitochondrial dysfunction



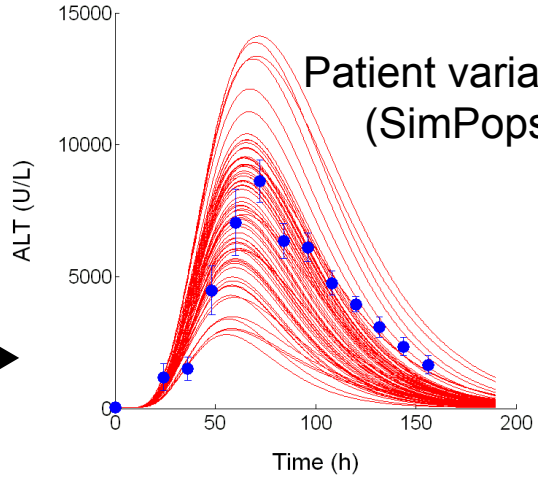

Cellular life-cycle




Drug distribution & metabolism




Patient variability (SimPops)




*Kuepfer 2010, Molecular Systems Biology*

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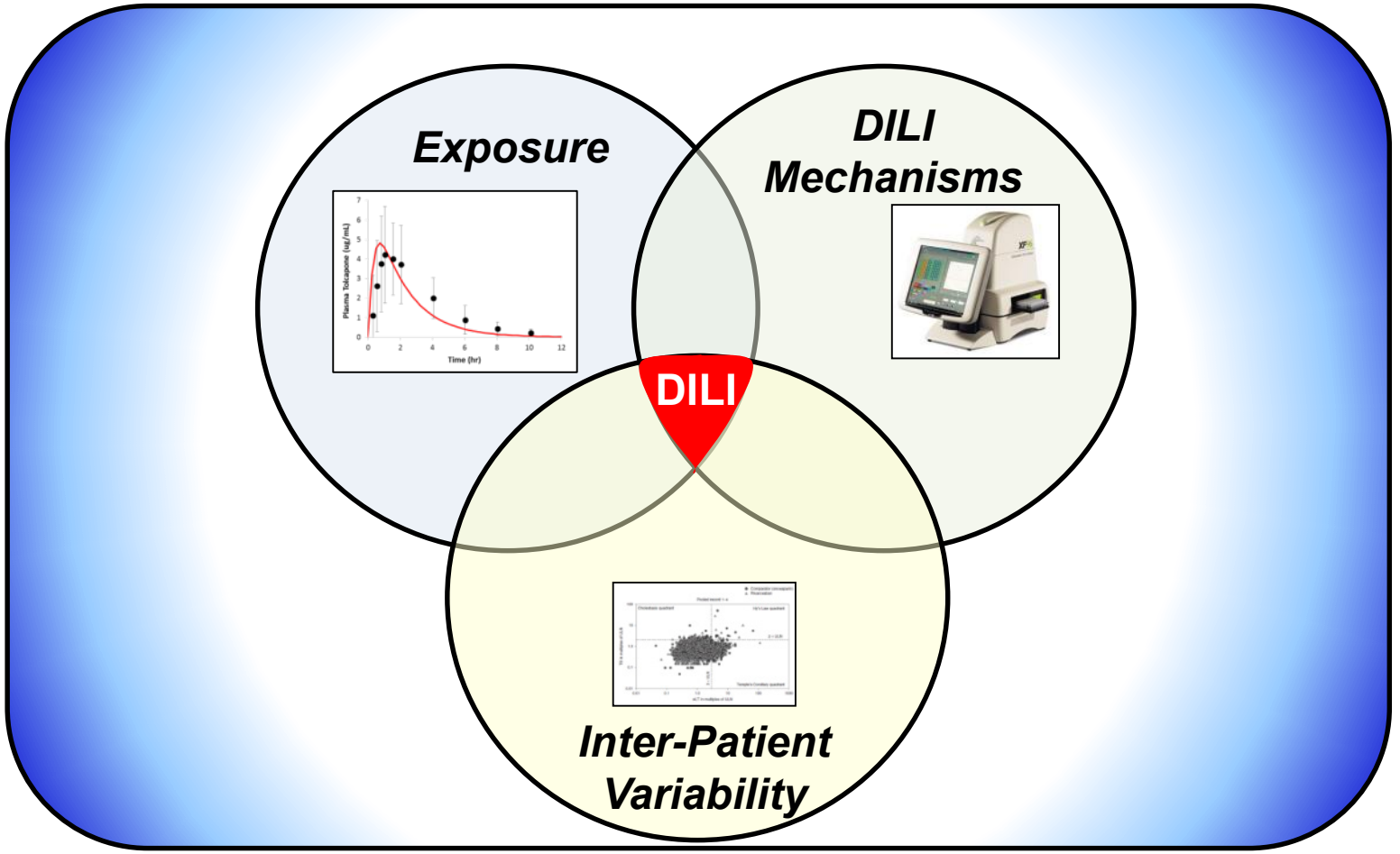
# DILIsym Predicts DILI via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability

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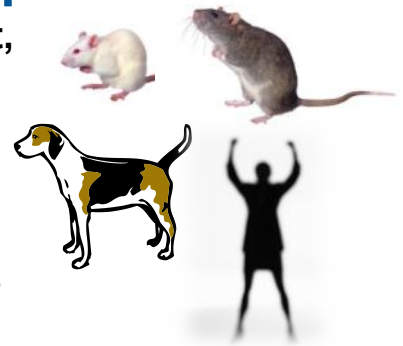
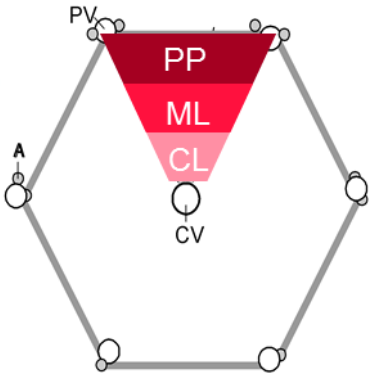
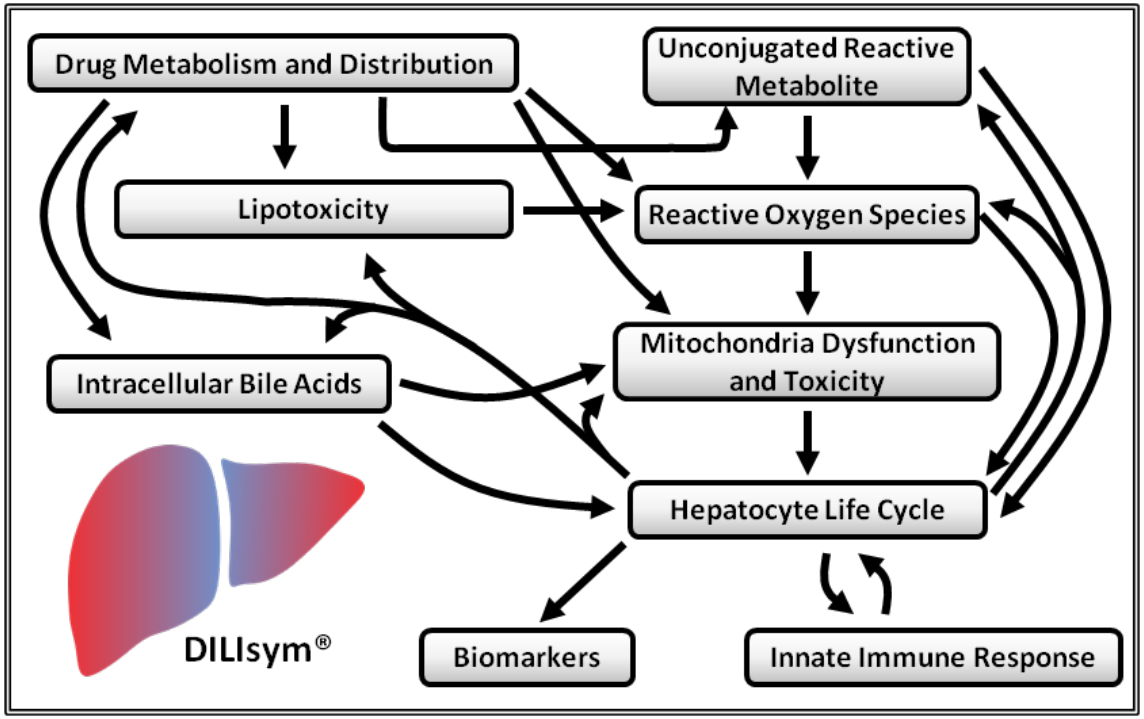
# DILIsym Overview: Multiple Sub-models Combined

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential cellular processes represented to multiple scales in interacting sub-models**
  - Pharmacokinetics
  - Dosing (IP, IV, Oral)
  - Transporter Inhibition
  - Drug metabolism
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Bile acid mediated toxicity
  - Steatosis and lipotoxicity
  - Cellular energy balance
  - Hepatocyte apoptosis and necrosis, and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers






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

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# Applications of DILIsym Along the Drug Development Pipeline

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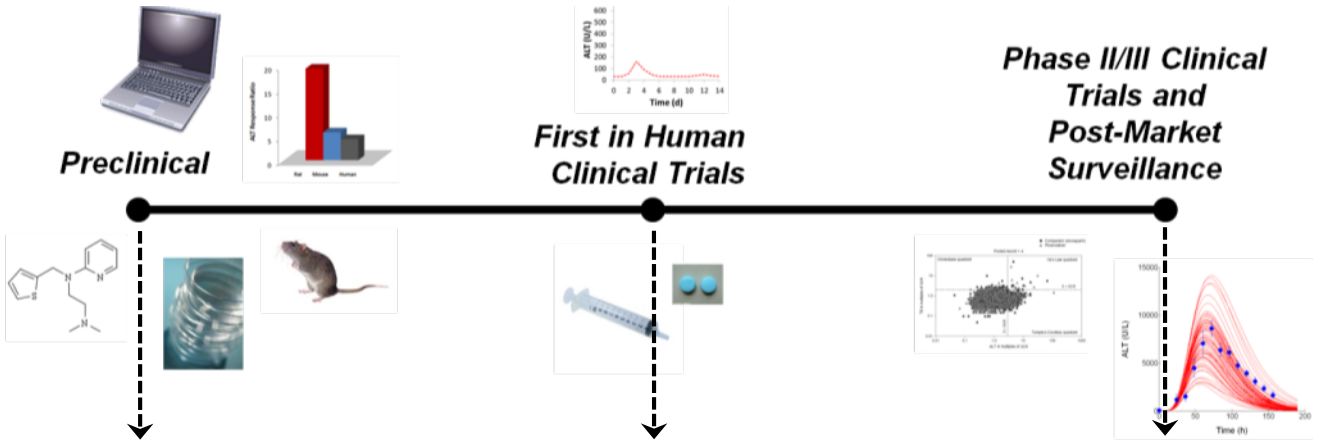
Predictions of hepatotoxicity for humans and preclinical animal models




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

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# Known DILIsym Applications Submitted to or Intended for Regulatory Agencies

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

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

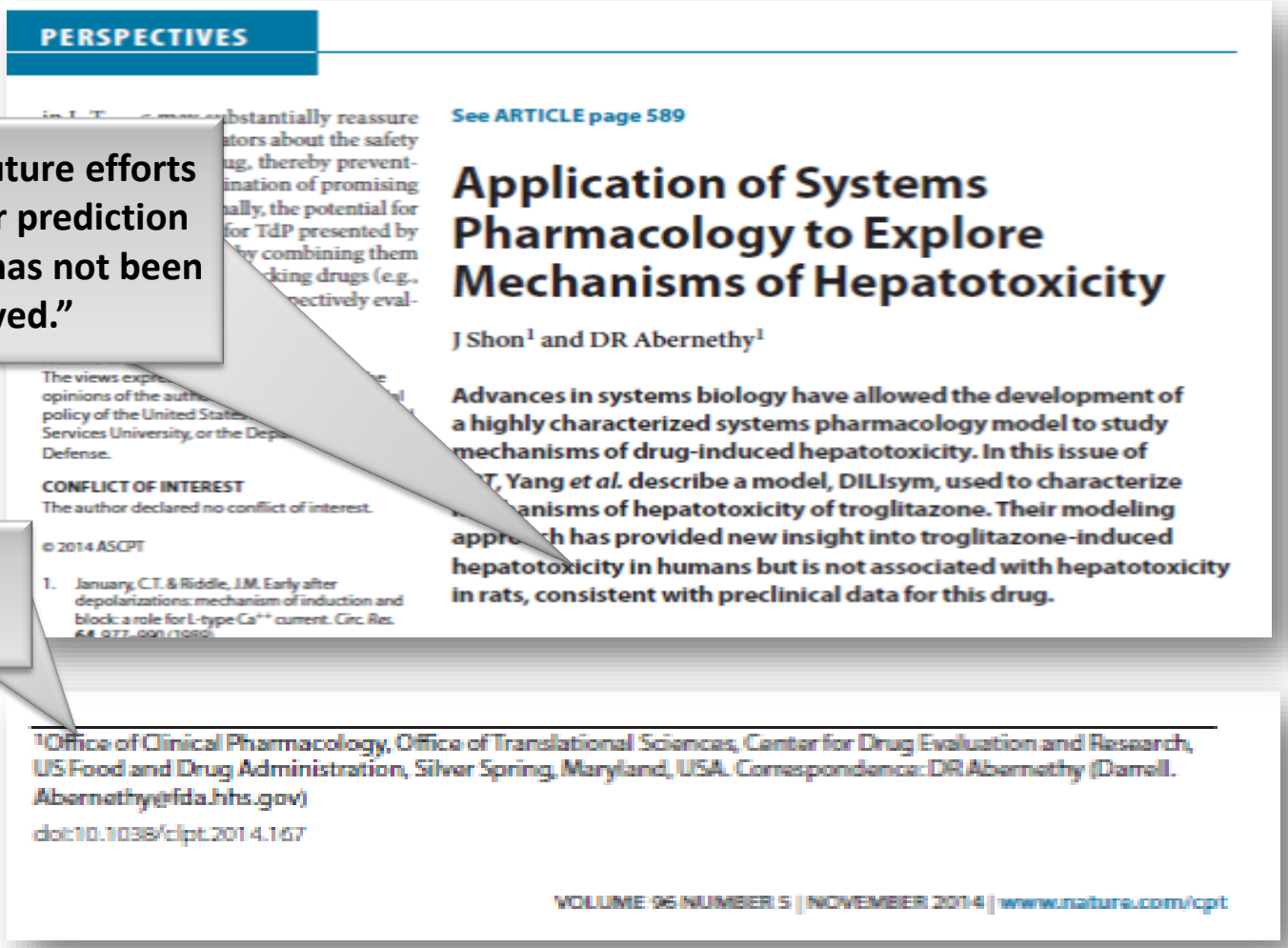
**Several additional sponsors have declared intent to include results in regulatory communications in the future

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

# Scientists at the FDA Have Expressed a Strong Interest in DILIsym Results

**"We look forward to future efforts to apply this model for prediction of hepatotoxicity that has not been clinically observed."**

***FDA Office of Clinical Pharmacology***



PERSPECTIVES

...substantially reassure ...tors about the safety ...ug, thereby prevent- ...ination of promising ...ally, the potential for ...for TdP presented by ...y combining them ...king drugs (e.g., ...pectively eval-

The views expr... opinions of the auth... policy of the United State... Services University, or the Dep... Defense.

CONFLICT OF INTEREST
The author declared no conflict of interest.

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1. January, C.T. & Riddle, J.M. Early after depolarizations: mechanism of induction and block: a role for L-type $Ca^{++}$ current. Circ. Res. 64, 977–990 (1989).

See ARTICLE page 589

## Application of Systems Pharmacology to Explore Mechanisms of Hepatotoxicity

J Shon[1] and DR Abernethy[1]

**Advances in systems biology have allowed the development of a highly characterized systems pharmacology model to study mechanisms of drug-induced hepatotoxicity. In this issue of ...T, Yang *et al.* describe a model, DILIsym, used to characterize ...anisms of hepatotoxicity of troglitazone. Their modeling appr...h has provided new insight into troglitazone-induced hepatotoxicity in humans but is not associated with hepatotoxicity in rats, consistent with preclinical data for this drug.**

[1]Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA. Correspondence: DR Abernethy (Darrell.Abernethy@fda.hhs.gov)

doi:10.1038/clpt.2014.167



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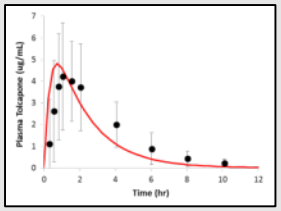
# DILIsym Utilizes Various Data Types to Inform Decisions

## 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

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**Simulations and Assays inform:**

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

## *In vitro* Mechanistic DILI Data

**Assays performed to determine quantitative aspects of DILI mechanisms**

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

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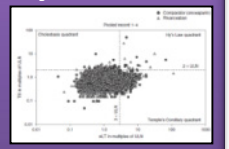
## Clinical Data

- **Biomarkers**
  - **Timing and magnitude of injury**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**



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# Example Project Executive 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

# Example Project Outline

- Goals
- Summary of mechanistic DILI *in vitro* assay results and DILIsym parameters for Compound Y and Compound X
  - Mitochondrial dysfunction
  - Oxidative stress
  - Bile acid transport inhibition
- Physiologically-based pharmacokinetic (PBPK) modeling in GastroPlus to represent *in vivo* exposure for Compound X and Compound Y
- Simulation results in the full simulated population (SimPops) for Compound X and Compound Y
- Analysis of DILI mechanisms for Compound X and Compound Y

# Example Project Goal – Assess Compound X and Compound Y

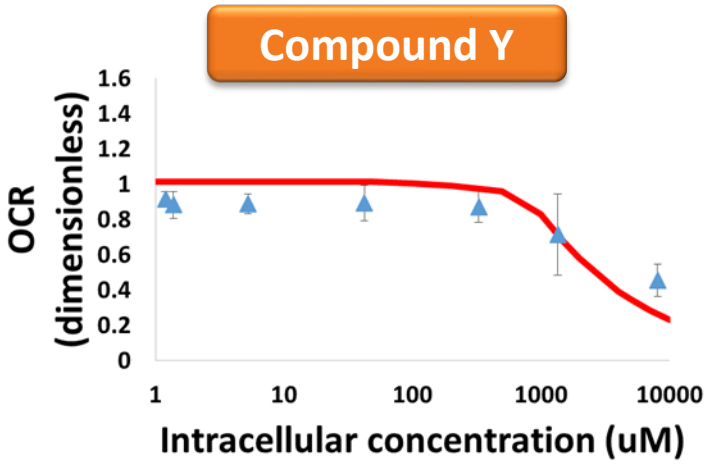
- The primary goal of this simulation work within the DILIsym software was to:
  - quantitatively and mechanistically assess the liver toxicity potential of Compound X and Compound Y combining clinical and mechanistic *in vitro* data with DILIsym and GastroPlus software simulations of previous or prospective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

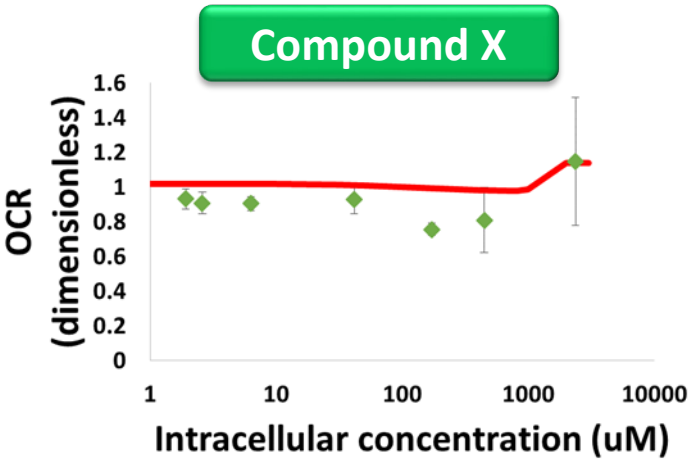
# Example Project Outline

- Goals
- Summary of mechanistic DILI *in vitro* assay results and DILIsym parameters for Compound Y and Compound X
  - Mitochondrial dysfunction
  - Oxidative stress
  - Bile acid transport inhibition
- Physiologically-based pharmacokinetic (PBPK) modeling in GastroPlus to represent *in vivo* exposure for Compound X and Compound Y
- Simulation results in the full simulated population (SimPops) for Compound X and Compound Y
- Analysis of DILI mechanisms for Compound X and Compound Y

# Mitochondrial Toxicity Parameters Determined for Compound Y and Compound X

- Parameter values were fit to mitochondrial data for Compound Y and Compound X
  - Electron transport chain inhibition for Compound Y
  - Both electron transport chain inhibition and uncoupling for Compound X
  - 24 hour data used
- MITOsym and DILIsym used to parameterize both compounds




| DILIsym Parameter | Compound Y Value | Compound X Value | Units |
|---|---|---|---|
| Coefficient for ETC inhibition 1 | 38,000 | Not used | µM |
| Coefficient for ETC Inhibition 3 | 0.1 | 4,200 | µM |
| Max inhibitory effect for ETC inhibition 3 | 0.2 | 0.4 (max effect) | dimensionless |
| Uncoupler 1 effect Km | No effect | 15,000 | µM |
| Uncoupler 1 effect Vmax | No effect | 22 | dimensionless |
| Uncoupler 1 effect Hill | No effect | 4 | dimensionless |

*Preclinical Data and Simulation Results*

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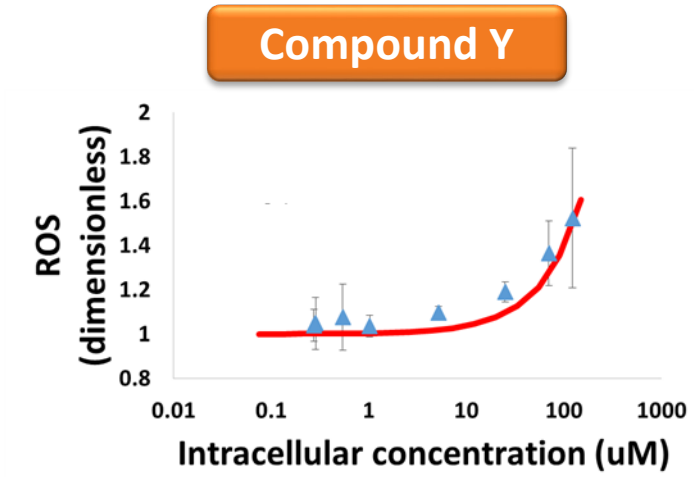
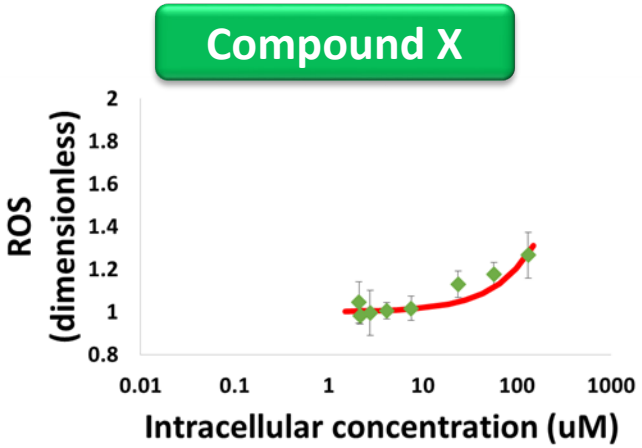
# Oxidative Stress Parameters Determined for Compound Y and Compound X

- Parameter values were fit to 24-hour ROS data for Compound Y and Compound X
- DILIsym representation of *in vitro* environment used to parameterize both compounds
- Saturable model explored but did not lead to better fit

| DILIsym Parameter | Compound Y Value | Compound X Value | Units |
|---|---|---|---|
| RNS/ROS production rate constant 1 | $3.4 \times 10^{-4}$ | $1.7 \times 10^{-4}$ | mL/nmol/hr |

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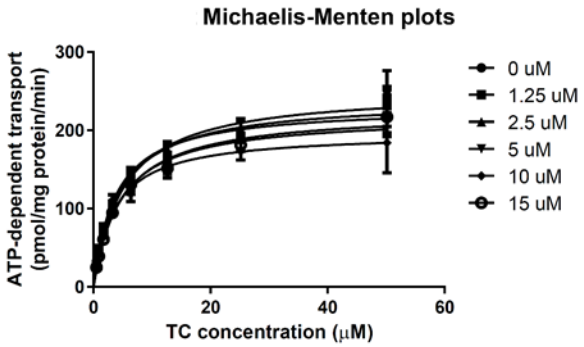
*Preclinical Data and Simulation Results*

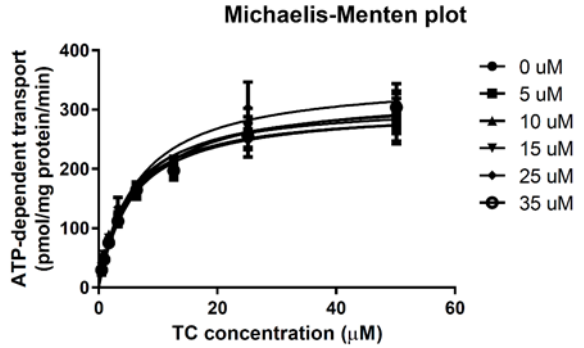
# Compound Y Weakly Inhibits BSEP; Compound X Does Not

- Compound Y is a weak but noncompetitive/uncompetitive inhibitor of BSEP
- Compound X does not inhibit BSEP
  - No changes to $V_{max}$ or $K_m$ of transporters observed over course of assay

**Compound Y; $K_i$ = 140 μM, α = 0.6**




**Compound X; no inhibition**




*Preclinical Data*

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# DILIsym Toxicity Parameters for Compound Y and X

| Mechanism | Parameter | Unit | DILIsym Parameter Value* | |
|---|---|---|---|---|
| | | | Compound Y | Compound X |
| Mitochondrial Dysfunction | Coefficient for ETC inhibition 1 | µM | 38,000 | Not used |
| | Coefficient for ETC Inhibition 3 | µM | 0.1 | 4,200 |
| | Max inhibitory effect for ETC inhibition 3 | dimensionless | 0.2 | 0.4 |
| | Uncoupler 1 effect Km | µM | No effect | 15,000 |
| | Uncoupler 1 effect Vmax | dimensionless | No effect | 22 |
| | Uncoupler 1 effect Hill | dimensionless | No effect | 4 |
| Oxidative Stress | RNS/ROS production rate constant 1 | mL/nmol/hr | $3.4 \times 10^{-4}$ | $1.7 \times 10^{-4}$ |
| Bile Acid Transporter Inhibition | BSEP inhibition constant | µM | 140 | No inhibition |
| | BSEP inhibition alpha value | dimensionless | 0.6 | No inhibition |
| | NTCP inhibition constant | µM | No inhibition | No inhibition |
| | MRP4 inhibition constant | µM | 40 | 75 |

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

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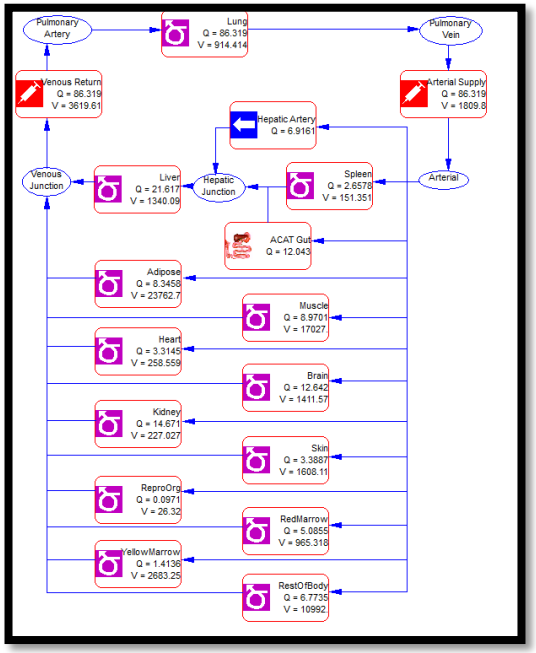
# Example Project Outline

- Goals
- Summary of mechanistic DILI *in vitro* assay results and DILIsym parameters for Compound Y and Compound X
  - Mitochondrial dysfunction
  - Oxidative stress
  - Bile acid transport inhibition
- Physiologically-based pharmacokinetic (PBPK) modeling in GastroPlus to represent *in vivo* exposure for Compound X and Compound Y
- Simulation results in the full simulated population (SimPops) for Compound X and Compound Y
- Analysis of DILI mechanisms for Compound X and Compound Y

# 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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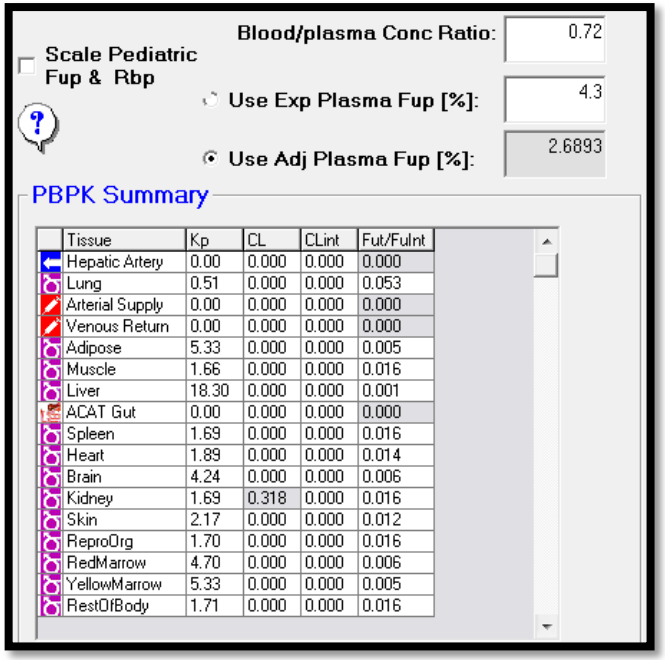
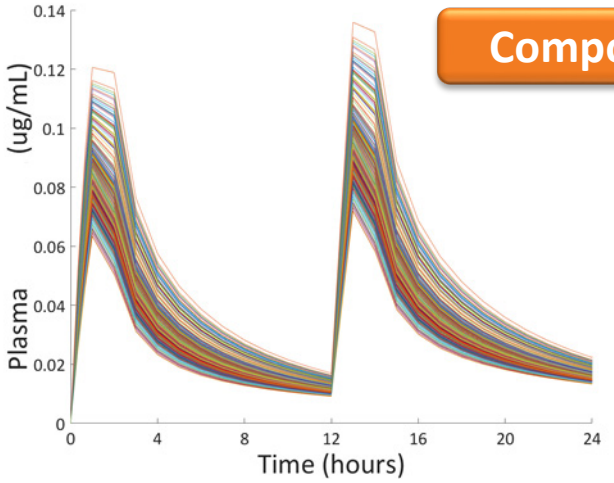
# Compound Y PBPK Representation Calculated at Clinical Dose

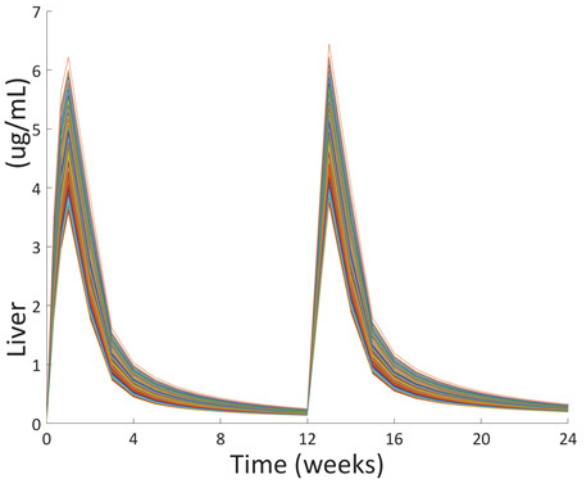
- GastroPlus predictions for liver and plasma at clinical dose shown at right
  - PBPK model specific predictions shown below
  - Dose escalation was simulated




| 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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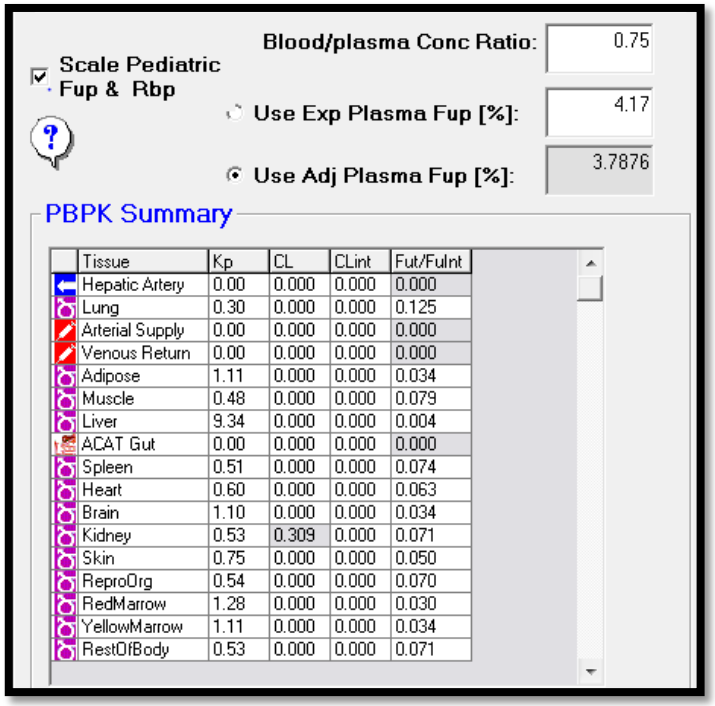
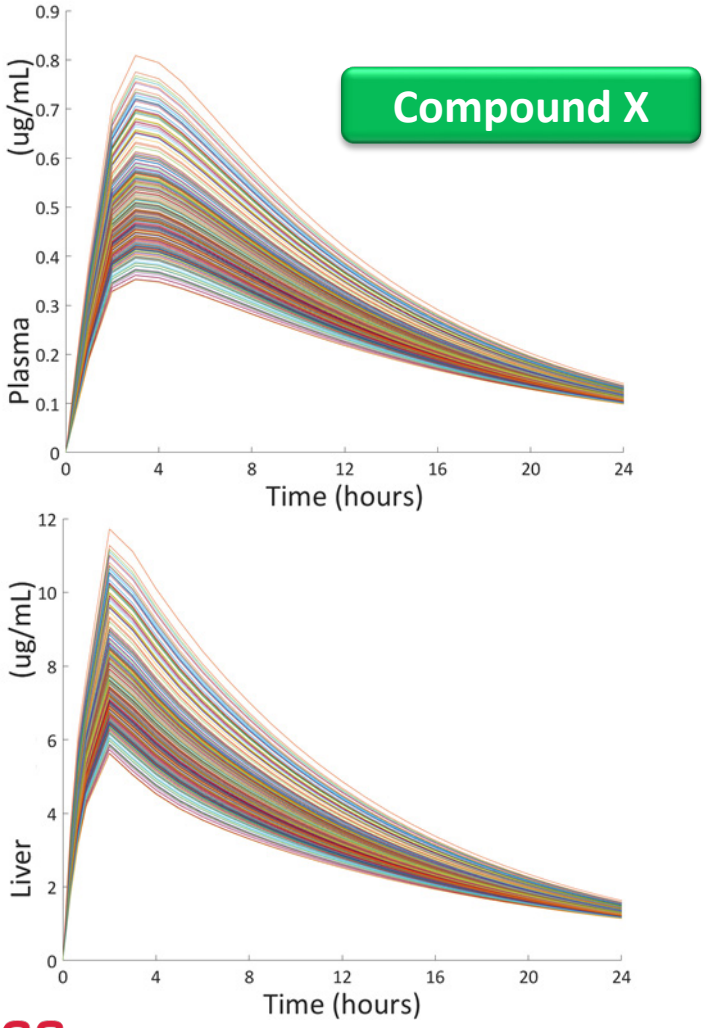
# Compound X PBPK Representation Calculated at Clinical Dose

- 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/Fulnt |
|---|---|---|---|---|
| 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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# Example Project Outline

- Goals
- Summary of mechanistic DILI *in vitro* assay results and DILIsym parameters for Compound Y and Compound X
  - Mitochondrial dysfunction
  - Oxidative stress
  - Bile acid transport inhibition
- Physiologically-based pharmacokinetic (PBPK) modeling in GastroPlus to represent *in vivo* exposure for Compound X and Compound Y
- Simulation results in the full simulated population (SimPops) for Compound X and Compound Y
- Analysis of DILI mechanisms for Compound X and Compound Y

# SimPops Results Show Compound X and Compound Y to be Safe at Clinical Doses; ALT Elevations Occur at Higher Doses for Both Compounds

Compound Y | Compound X

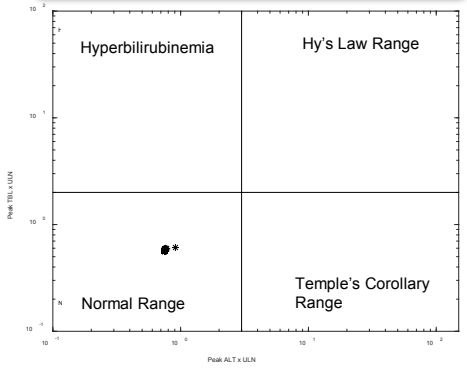
- Neither Compound Y nor Compound X are predicted to cause toxicity at the highest clinical dose
  - Some exposure variability included in these predictions due to GastroPlus population generation
- Both Compound Y and Compound X are predicted to cause mild ALT elevations at supratherapeutic doses
  - No bilirubin elevations or Hy's Law cases occurred in simulations with Compound X
  - 2 Hy's Law cases occurred at 10x clinical dose simulations with Compound Y

| | Compound | Dosing Protocol | Simulated* ALT > 3X ULN** |
|---|---|---|---|
| Compound Y | Compound Y | 1X Dose, 12 weeks | 0% (0/285) |
| | | 2X Dose, 12 weeks | 0% (0/285) |
| | | 5X Dose, 12 weeks | 0.3% (1/285) |
| | | 10X Dose, 12 weeks | 10.2% (29/285) |
| Compound X | Compound X | 1X Dose, 15 days | 0% (0/285) |
| | | 2X Dose, 15 days | 0% (0/285) |
| | | 5X Dose, 15 days | 1.1% (3/285) |
| | | 10X Dose, 15 days | 11.6% (33/285) |

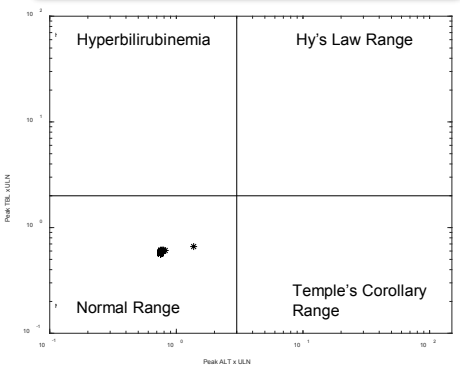
*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used
**Upper limit of normal (ULN) in DILIsym is 40 U/L

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

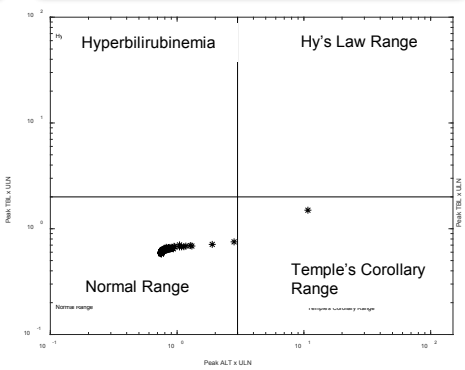
**Compound Y; 1X Dose, 12 weeks**



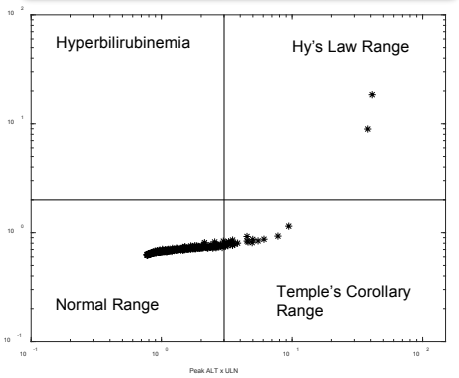

**Compound Y; 2X Dose, 12 weeks**



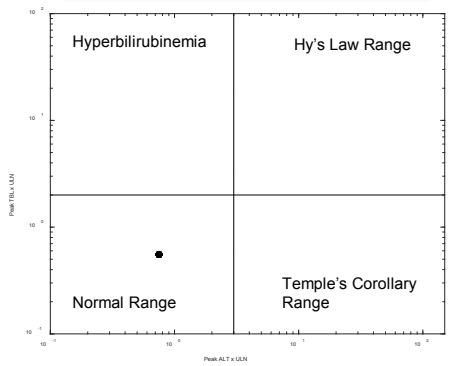

**Compound Y; 5X Dose, 12 weeks**



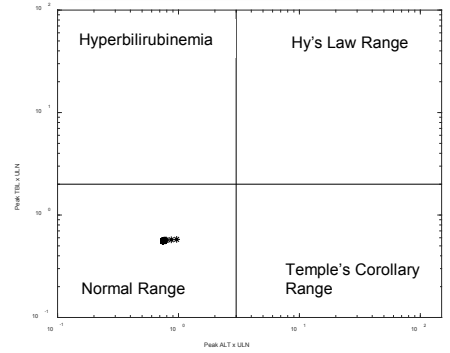

**Compound Y; 10X Dose, 12 weeks**



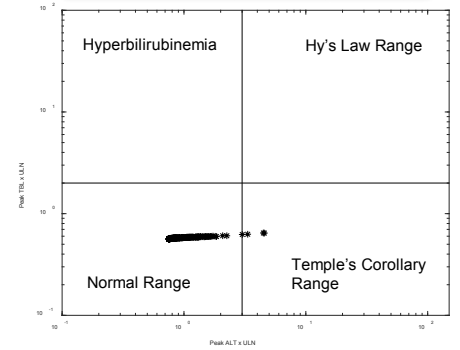

**Compound X; 1X Dose**



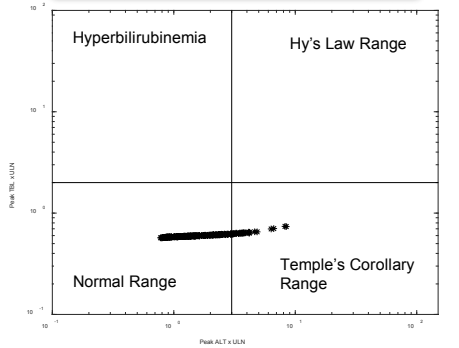

**Compound X; 2X Dose**




**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*

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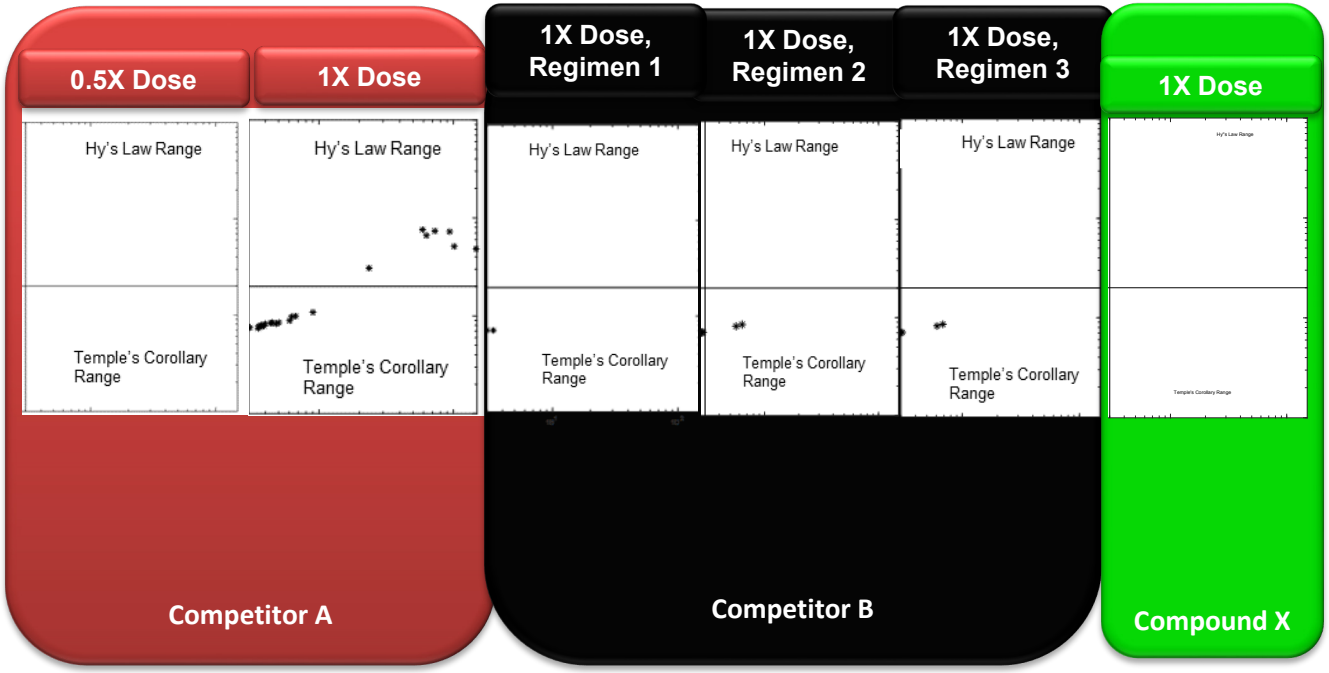
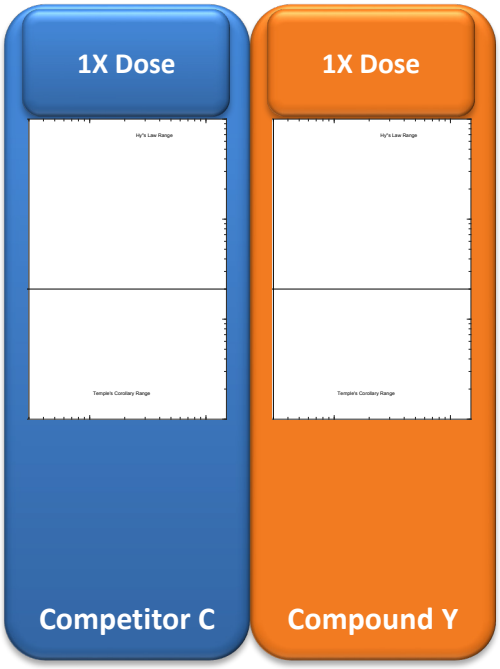
# Focus on Hy's Law Side of eDISH Plot – Comparison of Competitors and Compound X at Clinical Doses (285 Simulated Individuals in All Cases)



# Focus on Hy's Law Side of eDISH Plot – Comparison of Competitor and Compound Y at Predicted Clinical Doses (285 Simulated Individuals in All Cases)

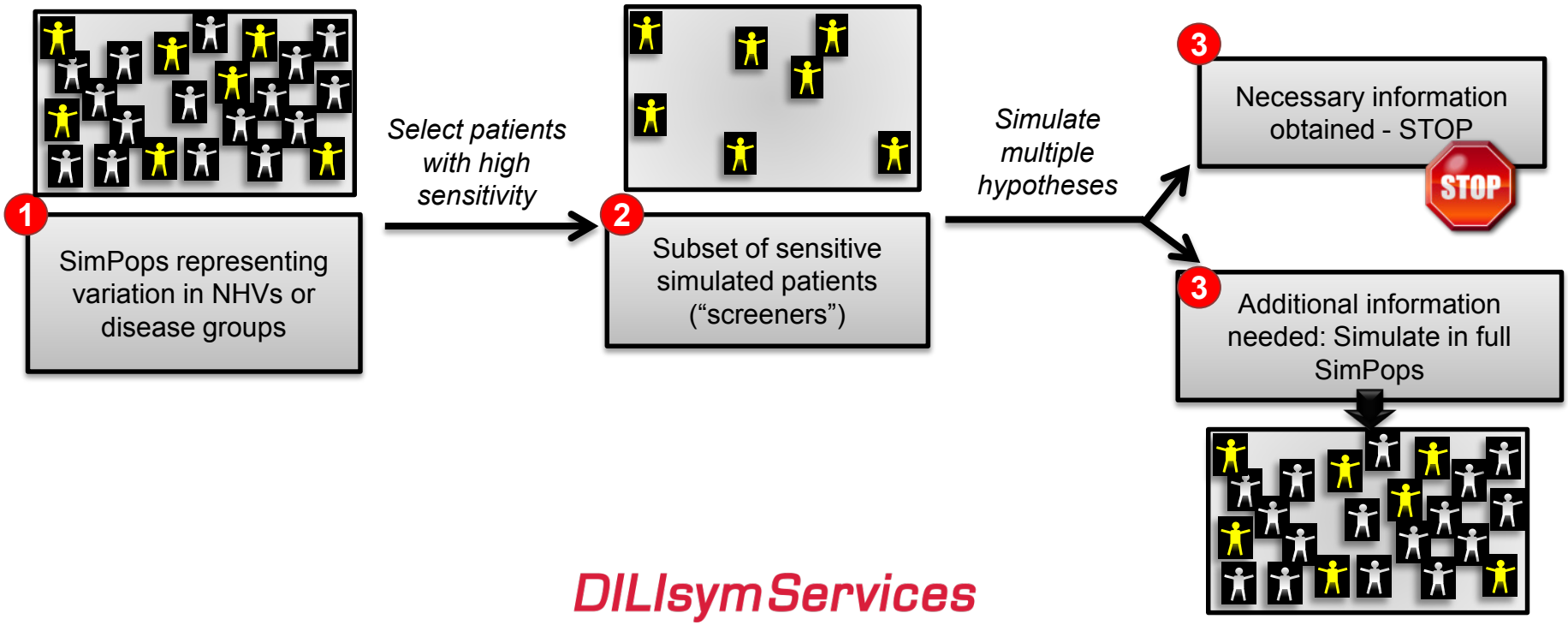


# Example Project Outline

- Goals
- Summary of mechanistic DILI *in vitro* assay results and DILIsym parameters for Compound Y and Compound X
  - Mitochondrial dysfunction
  - Oxidative stress
  - Bile acid transport inhibition
- Physiologically-based pharmacokinetic (PBPK) modeling in GastroPlus to represent *in vivo* exposure for Compound X and Compound Y
- Simulation results in the full simulated population (SimPops) for Compound X and Compound Y
- Analysis of DILI mechanisms for Compound X and Compound Y

# Subsets of Simulated Humans (SimCohorts) are Used for Preliminary Simulations in DILIsym

- SimPops include variability in toxicity mechanisms for healthy human volunteers and some disease populations
- SimCohorts consisting of a subset of sensitive individuals to specific DILI mechanisms from existing SimPops are used for screening and preliminary simulation purposes
- Simulations were conducted in a SimCohorts sensitive to specific DILI mechanisms, along with the baseline human simulations to produce preliminary results
- SimCohorts used: **Human_ROS_apop_mito_BA_v4A_1_Multi16**; includes some sensitive individuals from v4A_1 SimPops for bile acid, mitochondrial, ROS mechanisms and combinations (BA + mito) plus some insensitive individuals and the baseline individual

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# Mechanistic Investigation Simulation Results Show ROS as Main Driver of Compound X and Compound Y ALT Elevations

- Mechanistic investigation simulations for Compound Y and Compound X on a subset of susceptible individuals at 10x clinical dose
  - One mechanism turned off at a time; if turning off a mechanism leads to lower frequency of ALT elevations, that mechanism contributes to the simulated toxicity
- Compound Y and Compound X ALT elevations at simulated supratherapeutic doses are mostly due to ROS (oxidative stress) generation

| | Compound and Protocol | Mechanisms On | Mechanisms Off | Simulated* ALT > 3X ULN** |
|---|---|---|---|---|
| Compound Y | Compound Y | All | None | 29/29 |
| | | BA inhibition, ETC inhibition | ROS generation | 2/29 |
| | | BA inhibition, ROS generation | ETC inhibition | 24/29 |
| | | ETC inhibition, ROS generation | BA inhibition | 24/29 |
| Compound X | Compound X | All | None | 33/33 |
| | | BA inhibition, ETC inhibition, Uncoupling | ROS generation | 0/33 |
| | | BA inhibition, Uncoupling, ROS generation | ETC inhibition | 33/33 |
| | | BA inhibition, ETC inhibition, ROS generation | Uncoupling | 33/33 |
| | | BA inhibition, ROS generation | ETC inhibition, Uncoupling | 33/33 |
| | | ETC inhibition, Uncoupling, ROS generation | BA inhibition | 33/33 |

Compound Y Compound X

*The full v4A-1 SimPops (n=285) of normal healthy volunteers was used
**Upper limit of normal (ULN) in DILIsym is 40 U/L

# Summary of Key Points

- Combining QSAR, PBPK, and QST models is a powerful approach to getting more information out of your data investments early in product development
- The Simulations Plus family has extensive experience with these approaches and can help
- ADMET Predictor / GastroPlus / DILIsym is a winning software package combination for predicting the liver safety of your molecules and those of your competitors prior to clinical trial surprises

SimulationsPlus

SCIENCE + SOFTWARE = SUCCESS

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## Visit us in booth #442 for product demonstrations

## SOT presentations:

Altered bile acid homeostasis and mitochondrial function: potential mechanisms for BMS-986020-induced human hepatobiliary toxicity
**Monday @ 1:30 PM; Poster board #P814**

*In silico-in vitro* extrapolation for dermal exposure
**Tuesday @ 10:10 AM; Hemisfair Ballroom C3**

Mechanistic modeling of mitochondrial biogenesis within DILIsym could explain clinically observed adaptation of ALT elevations
**Tuesday @ 3:00 PM; Poster board #P294**

Prediction of the liver toxicity of the endothein receptor antagonsists sitaxsentan and ambrisentan for the treatment of pulmonary arterial hypertension with a QST tool
**Wednesday @ 1:30 PM; Poster board #P836**

## For Company News & Events, Visit:
## www.simulations-plus.com