Better Together: AI/ML, PBPK and QSP/QST Modeling in Drug Discovery & Development

Simulations Plus Inc.

July 25, 2023

Webinar Agenda

- Setting the Stage: Overview of Simulations Plus Inc.
- Exposure is the Key: PBPK Modeling in GastroPlus for Verdiperstat
- Safety First: the DILIsym Verdiperstat Application
- Panel Discussions and Q&A

Who We Are

NASDAQ: SLP

- AI/ML and PBPK/PBBM
  Software & Services
- Clinical Pharmacology & Pharmacometrics
  Software & Services
- QSP and QST
  Software & Services
- Regulatory and Strategic Consulting
  Services

190+

Employees Worldwide

>25 yrs.

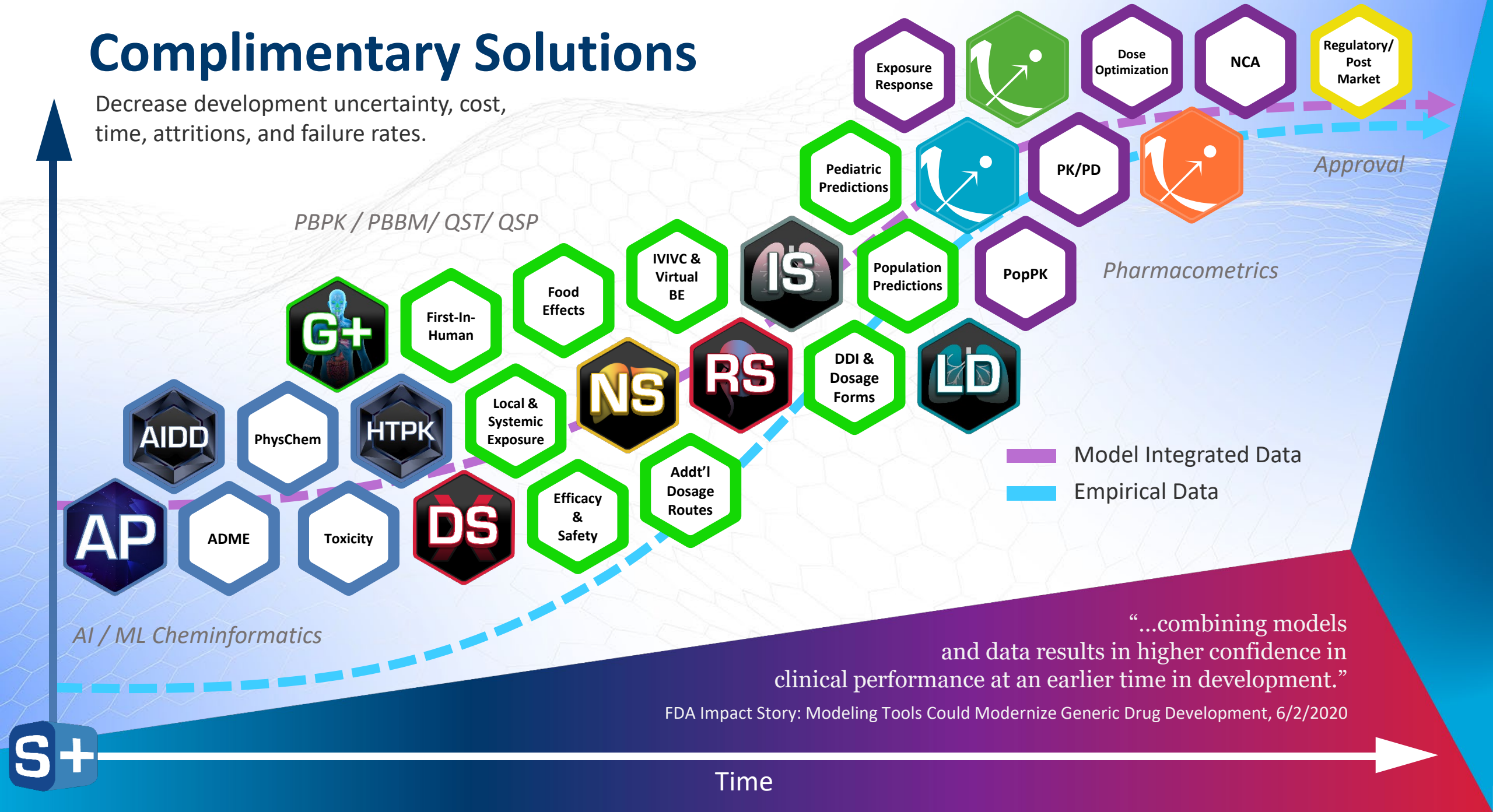
Established In 1996

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

SimulationsPlus

Complimentary Solutions

Decrease development uncertainty, cost, time, attritions, and failure rates.



"...combining models and data results in higher confidence in clinical performance at an earlier time in development."

FDA Impact Story: Modeling Tools Could Modernize Generic Drug Development, 6/2/2020

Multiple Quantitative Systems Pharmacology (QSP) and Toxicology (QST) Models to Address Your Questions

QSP: Inflammatory and Fibrotic Diseases

- Non-alcoholic fatty liver disease / steatohepatitis (NAFLD/NASH)
- Idiopathic pulmonary fibrosis (IPF)
- Interstitial lung disease (ILD) associated with systemic sclerosis
- Wound healing after myocardial infarction (MI)
- Uric acid disposition in gout
- Dysregulation of alternative and terminal pathways (AP, TP) of complement

QST: Liver and Kidney Safety

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

QSP: Immuno-Oncology

- Acute myeloid leukemia (AML)
- Multiple myeloma (MM)
- Solid tumor (NSCLC, melanoma)
- Diffuse large B-cell lymphoma (DLBCL)

QSP: Autoimmune Diseases

- Rheumatoid arthritis (RA)
- Psoriatic arthritis (PSA)
- Psoriasis (PSO)
- Atopic dermatitis (AD)
- Systemic lupus erythematosus (SLE)
- Ulcerative colitis (UC)
- Crohn's disease (CD)

QSP and QST models can also be newly developed for additional therapeutic areas

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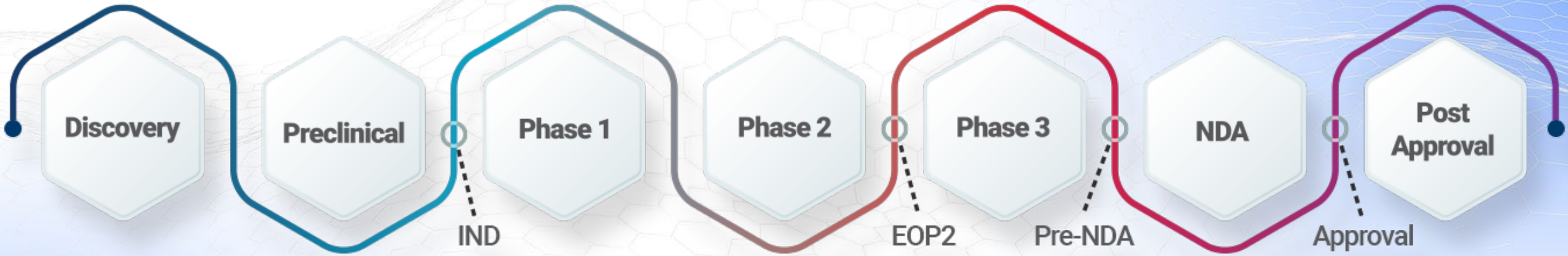
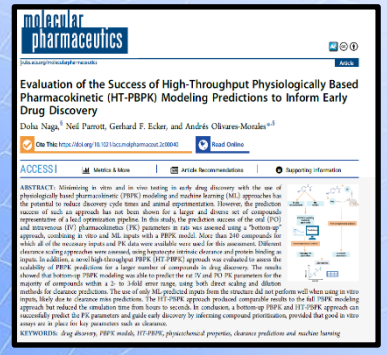
Our Biosimulation Solutions Are Validated Throughout Your Drug Product's Lifecycle

(1000+ peer-reviewed journal articles reference GastroPlus® applications)



LEAD SELECTION | **FIRST-IN-HUMAN** | **FOOD EFFECTS** | **pH-DEPENDENT DDI** | **METABOLIC DDI** | **BIOEQUIVALENCE**


molecular pharmaceutics

Evaluation of the Success of High-Throughput Physiologically Based Pharmacokinetic (HT-PBPK) Modeling Predictions to Inform Early Drug Discovery

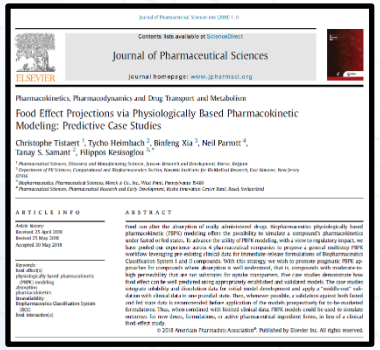
Doha Naga, Neil Parrott, Gerhard F. Ecker, and Andrés Olivares-Morales

Naga et al. (2022)
2800+ downloads!


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

Neil A. Miller · Micaela B. Reddy · Aki T. Heikkinen · Viera Lukacova · Neil Parrott

Miller et al. (2019)
80+ citations!


Journal of Pharmaceutical Sciences

Food Effect Projections via Physiologically Based Pharmacokinetic Modeling: Predictive Case Studies

Christophe Tistaert, Tycho Heimbach, Binfeng Xia, Neil Parrott, Tanay S. Samant, Filippos Kesisoglou

Tistaert et al. (2018)
40+ citations!


Journal of Pharmaceutical Sciences

Prediction of pH-Dependent Drug-Drug Interactions for Basic Drugs Using Physiologically Based Biopharmaceutics Modeling: Industry Case Studies

Amitava Mitra, Neil Parrott, Neil Miller, Richard Lloyd, Christophe Tistaert, Tycho Heimbach, Yan Ji, Filippos Kesisoglou

Mitra et al. (2020)
30+ citations!


Research Article

Predicting the Drug–Drug Interaction Mediated by CYP3A4 Inhibition: Method Development and Performance Evaluation

Hong-cun Ren · Yang Sai · Tao Chen · Chun Zhang · Lily Tang · Cheng-guang Yang

Ren et al. (2022)
1000+ downloads!

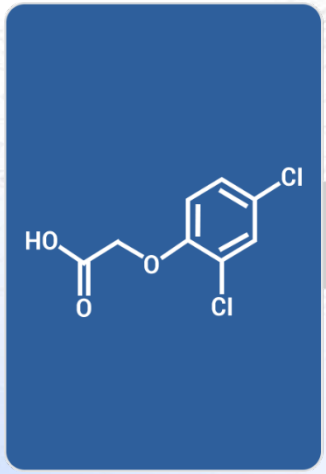

Journal of Pharmaceutical Sciences

Establishing the Bioequivalence Safe Space for Immediate-Release Oral Dosage Forms using Physiologically Based Biopharmaceutics Modeling (PBBM): Case Studies

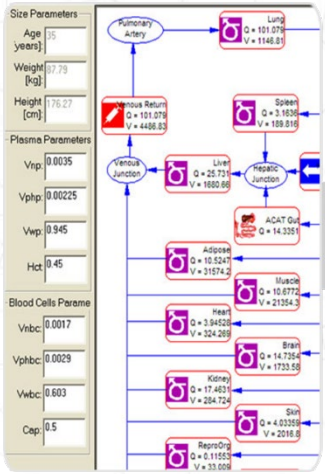
Tycho Heimbach, Filippos Kesisoglou, Jasmina Novakovic, Christophe Tistaert, Martin Mueller-Zsigmondy, Sivacharan Kolliparai, Tanuif Ahmed, Amitava Mitra, Sandra Suarez-Sharp

Heimbach et al. (2021)
20+ citations!

The Machine Learning / PBPK / QST(P) Marriage…



ADME Property Inputs



PK Levels + ML QST Inputs

Quantitative Structure Activity Relationships (QSAR)

ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™

Quantitative Systems Pharmacology/Toxicology (QSP/QST)

RENAsym™ DILIsym™ IPFsym™ NAFLDsym™

Immunetrics

Prediction of the Liver Safety Profile of a First-in-Class Myeloperoxidase Inhibitor Using Quantitative Systems Toxicology Modeling

Jeffrey L. Woodhead[1], Yeshi Gebremichael[1], Joyce Macwan[1], Irfan Qureshi[2], Richard Bertz[2], Victoria Wertz[2], Brett A. Howell[1]

[1]Simulations Plus, Inc., Lancaster, CA, USA; [2]Biohaven Pharmaceuticals, New Haven, CT, USA

CONTACT INFORMATION: jeff.woodhead@simulations-plus.com

SimulationsPlus

PURPOSE

The novel myeloperoxidase inhibitor verdiperstat was developed as a treatment for neuroinflammatory and neurodegenerative diseases. Phase 2 clinical studies had shown some promise for efficacy at the 600 mg BID dose; however, this is a large dose and verdiperstat had shown some *in vitro* signals suggesting possible liver toxicity. Mild liver signals had also been observed during Phase 1 trials, though it was unclear whether these were drug-related or not. In order to provide an added layer of confidence in the liver safety of verdiperstat before proceeding to Phase 3, a computational prediction of verdiperstat liver safety was performed using DILIsym v8A, a quantitative systems toxicology (QST) model of liver safety.

METHODS

A physiologically-based pharmacokinetic (PBPK) model of verdiperstat was constructed in GastroPlus 9.8, and the estimates for the liver and plasma time course of verdiperstat were input into DILIsym. *In vitro* experiments measured the likelihood that verdiperstat would inhibit mitochondrial function, inhibit bile acid transporters, and generate reactive oxygen species (ROS). Predictions of liver verdiperstat exposure from the PBPK model and parameters derived from the *in vitro* experimental results were used as inputs into DILIsym. Two alternate sets of parameters were used as inputs in order to fully explore the sensitivity of model predictions within the potential range of the *in vitro* data. Verdiperstat dosing protocols up to 600 mg BID were simulated for up to 48 weeks using a simulated population (SimPops) in DILIsym.

RESULTS

In vitro experiments were conducted in cell vesicles (for bile acid transport) and HepG2 cells (for ROS and ETC inhibition). These experiments showed verdiperstat to be a mild inhibitor of the bile acid transporter MRP4 (Figure 1), a mild generator of ROS (Figure 2), and a mild inhibitor of the mitochondrial electron transport chain (ETC, Figure 3). For ROS and ETC inhibition, the intracellular concentration was measured by mass spectrometry.

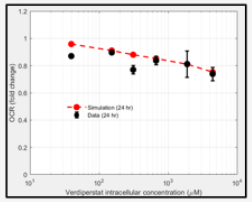


Figure 2. Relationship between measured intracellular verdiperstat and oxygen consumption rate, demonstrating a dose-dependent decrease in oxygen consumption and thus an inhibition of the electron transport chain.

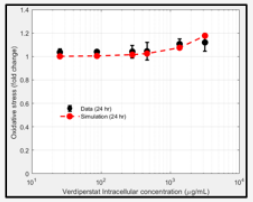


Figure 3. Relationship between measured intracellular verdiperstat and normalized TBARS generation, demonstrating a dose-dependent increase in oxidative stress.

Results from the *in vitro* experiments were used to calculate input parameters into DILIsym v8A, in the table below. OCR consumption was modeled in MITOsym v3B, a QST model of *in vitro* mitochondria, and translated into DILIsym; ROS generation was modeled in an *in vitro*-like parameterization in DILIsym (red lines in Figures 2 and 3). An alternate, conservative parameterization using an estimate of intracellular concentration as equal to the nominal concentration, which was suggested by the liver partition coefficient of 1 used in the PBPK model, was also developed; these parameters are also in the table below.

Mechanism	DILIsym Parameter	Unit	Alternate Verdiperstat Value	Primary Verdiperstat Value
BA Transport Inhibition	Inhibition constant for BSEP	µM	No inhibition	No inhibition
	Inhibition constant for basolateral efflux (MRP3/4)	µM	32.55**	32.55**
	Inhibition constant for NTCP	µM	No Inhibition	No Inhibition
Oxidative Stress	Liver RNS/ROS production rate constant 1	mL/nmol/hour	1.7×10^{-4}	1.15×10^{-6}
Mitochondrial Dysfunction	Coefficient for ETC Inhibition 1	µM	6.94×10^{5}	6.94×10^{5}
	Coefficient for ETC Inhibition 3	µM	2.43	243
	Max inhibitory effect for ETC inhibition 3	Dimensionless	0.39	0.39

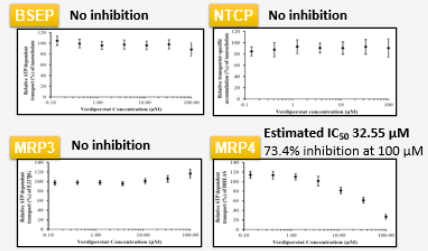
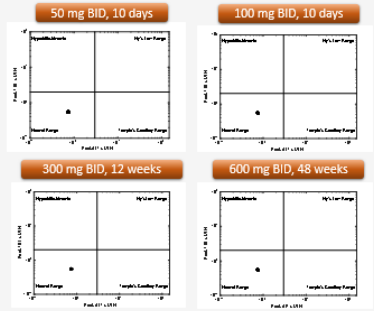
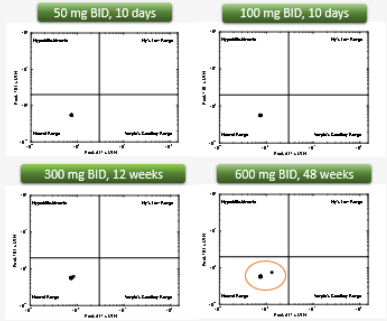


Figure 1. Inhibition of bile acid transporters by verdiperstat



In SimPops simulations (n = 285), no ALT elevations over 3x ULN were predicted using either the primary (above) or alternate (below) parameterizations. Mild ALT elevations (less than 3x ULN) occurred at the 600 mg BID dose in the alternate parameterization.



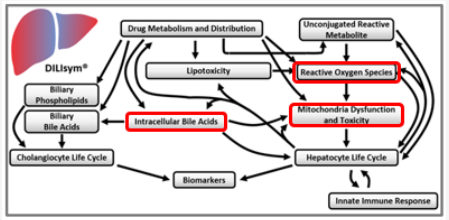


Diagram of the interactions between submodels in DILIsym v8A. In vitro *measurements of oxidative stress, mitochondrial dysfunction, and bile acid transport inhibition are used as inputs, and the DILIsym model of liver physiology computes the likelihood that those mechanisms will affect the hepatocyte life cycle, which will in turn affect biomarker release and immune system activation.*

CONCLUSION

Verdiperstat was predicted to be safe, with only rare, mild liver enzyme increases as a potential possibility in very highly sensitive individuals. Subsequent Phase 3 clinical trials conducted after the conclusion of this modeling work found that ALT elevations in the verdiperstat treatment group were generally similar to those in the placebo group. This validates the DILIsym simulation results and demonstrates the power of QST modeling to predict the liver safety profile of novel therapeutics.

ACKNOWLEDGEMENTS

- Biohaven Pharmaceuticals, Inc.
- The members of the DILI-sim and RENAsym Initiatives

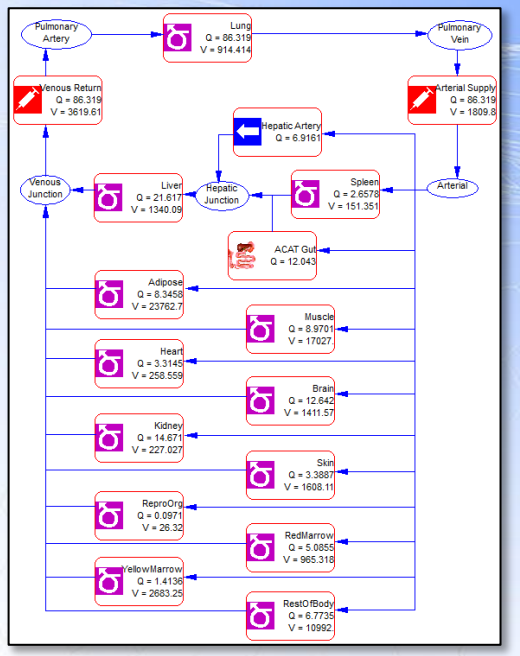
www.simulations-plus.com

ulationsPlus

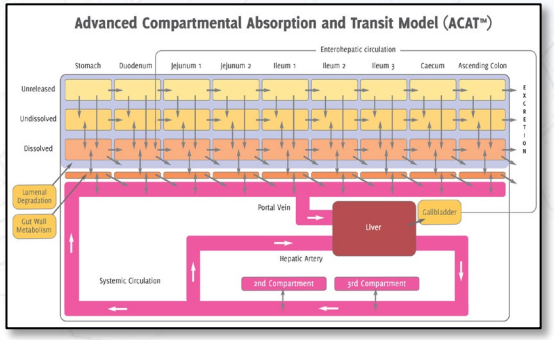
GastroPlus PBPK Framework for Verdiperstat

- Metabolism of Verdiperstat by CYP1A2 (liver) and CYP3A4 (gut and liver) included
- Verdiperstat also eliminated via renal clearance (minor pathway) – included in the model
- All tissues treated as perfusion-limited models
  - Tissue:plasma partition coefficients (Kps) calculated using the Lukacova (default) method in GastroPlus using physicochemical properties
- Plasma and liver exposure simulated using GastroPlus model imported into DILIsym to simulate liver safety of Verdiperstat

GastroPlus PBPK Model Structure



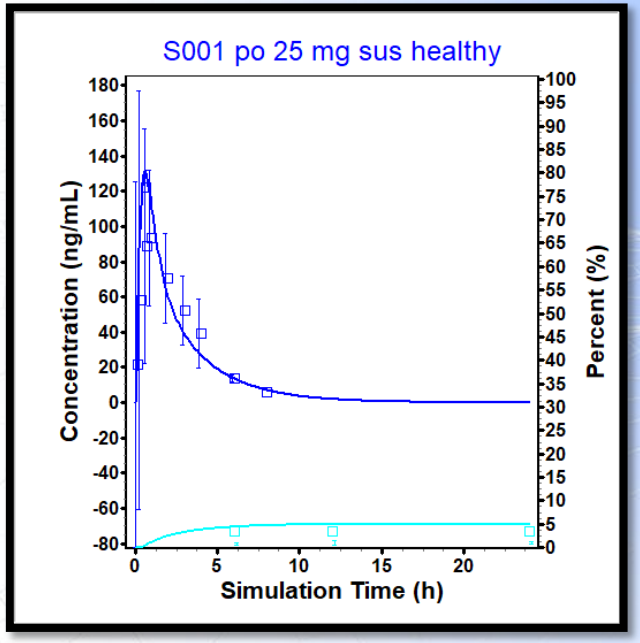
GastroPlus Gut Absorption Model

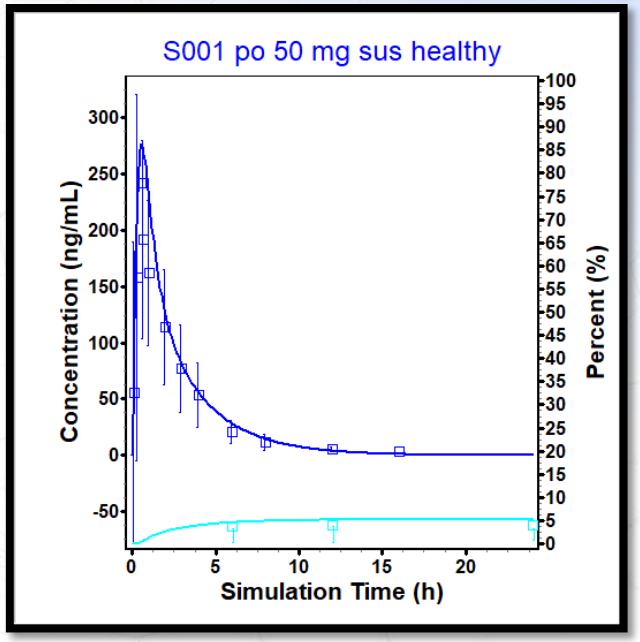


Verdiperstat Representation for PBPK Calibrated to Clinical Data

- Observed PK profiles following a single dose of 7.5, 25, and 50 mg of Verdiperstat were used to calibrate the PBPK representation
- The model utilized experimental properties of the compound
- ***The model captured well the observed PK data***

Dose (mg)	Cmax (sim/obs)	AUC_{0-t} (sim/obs)
25	1.08	0.97
50	1.14	1.17

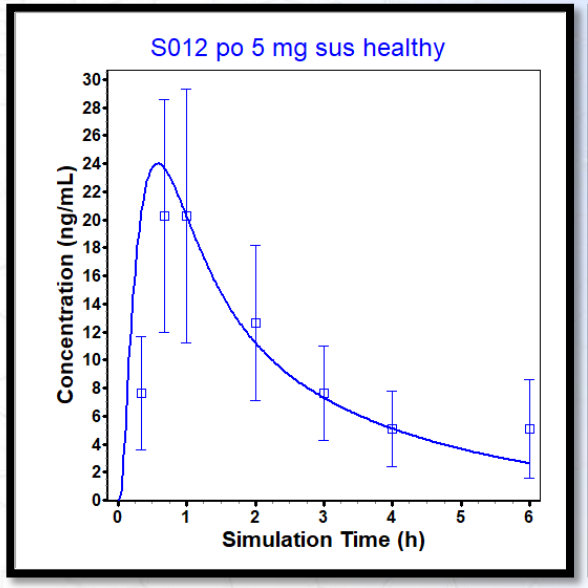


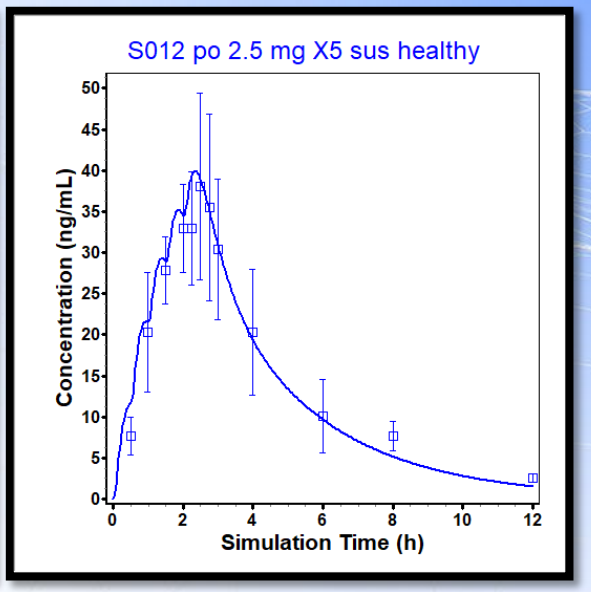


- Simulated plasma concentrations of verdiperstat
- Mean observed plasma concentrations of verdiperstat
- Mean observed amount of unchanged verdiperstat in urine
- Simulated amount of verdiperstat excreted in urine

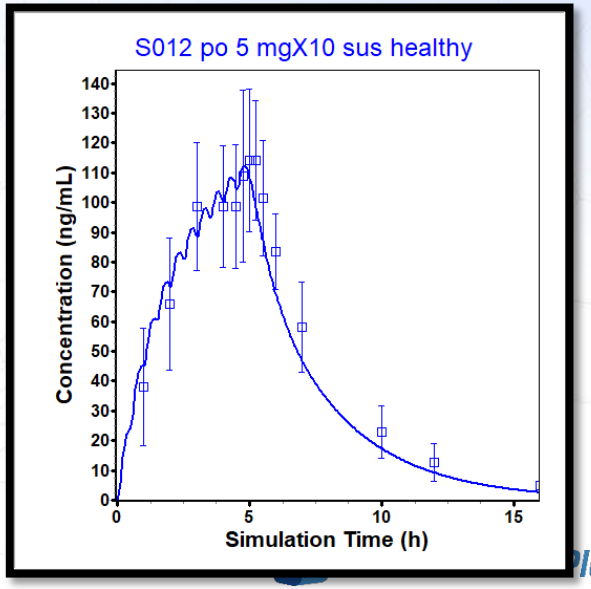
Verdiperstat Representation for PBPK Validated to Clinical Data

- PK profiles following single ascending (Part A: 1, 2.5, and 5 mg) and fractionated ascending (Part B:1 mg x 10, 2.5 mg x 5, 2.5 mg x 10, 5 mg x 5, 5 mg x 10; Doses administered every 30 minutes)
- ***The model captured observed PK profiles well***





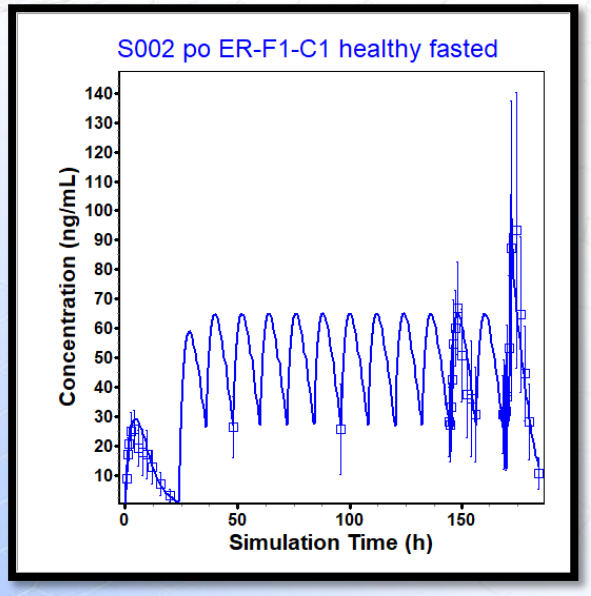
Dose (mg)	Cmax (sim/obs)	AUC_{0-t} (sim/obs)
5	1.19	1.01
2.5 mg x 5	1.05	0.96
5 mg x 10	0.99	0.91



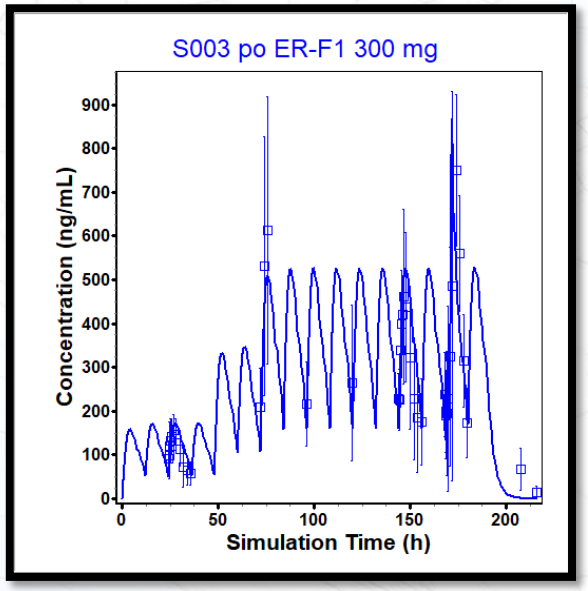
– Simulated plasma concentrations of verdiperstat
□ Mean observed plasma concentrations of verdiperstat

Verdiperstat Representation for PBPK to Clinical Data of Controlled Release (CR) Formulation

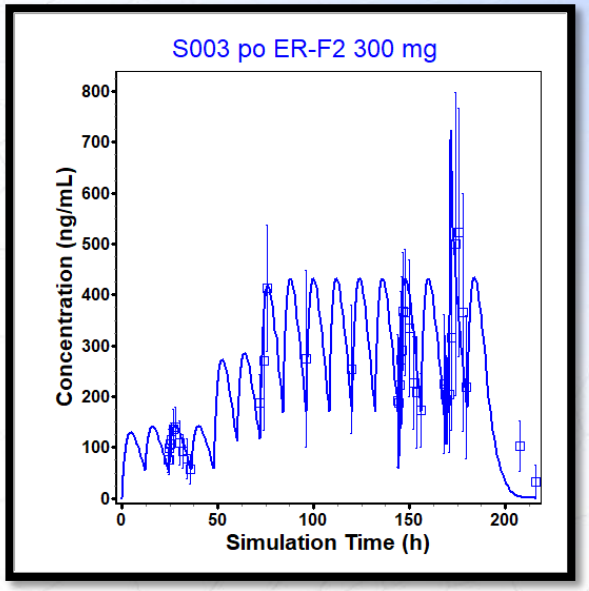
- PK profiles following multiple dose administrations of different ER formulations were simulated using the validated PBPK model
  - Deconvoluted *in vivo* dissolution was used in the simulations



Day 1	Day 2-7	Day 8
25 mg qd F1	50 mg bid F1	50 mg qd + fat breakfast



Day 1-2	Day 3	Days 4-7	Day 8
100 mg bid F4	200 mg bid F4	300 mg bid F1	300 mg bid F1 + fat breakfast

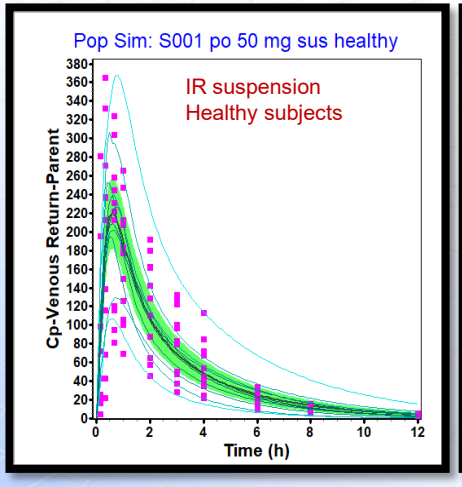


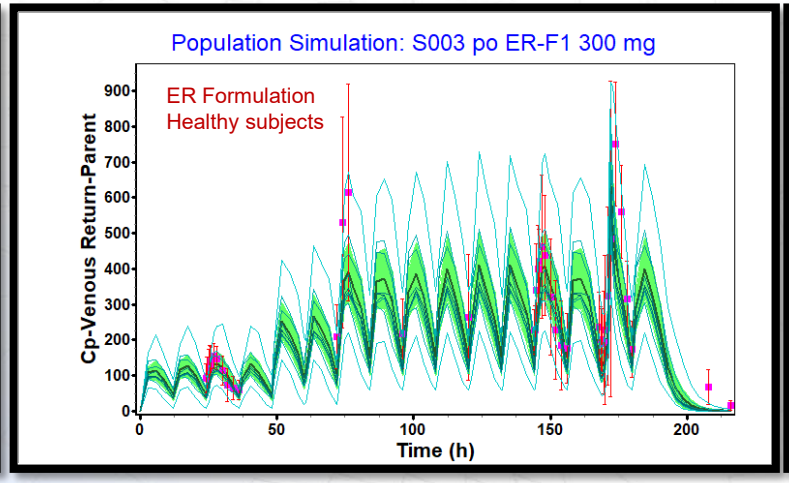
Day 1-2	Day 3	Days 4-7	Day 8
100 mg bid F4	200 mg bid F4	300 mg bid F2	300 mg bid F2 + fat breakfast

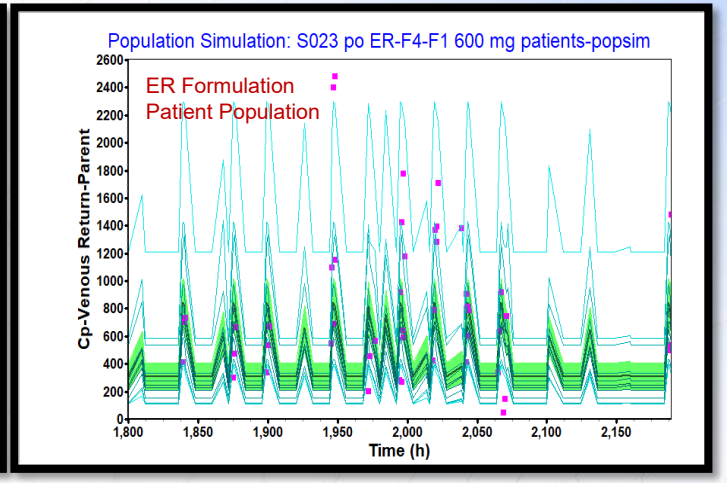
– Simulated plasma concentrations of verdiperstat
□ Mean observed plasma concentrations of verdiperstat

Verdiperstat Representation for PBPK: Population Simulation Captured Clinical Variability

- High and low PK profiles of IR formulation from individual subjects were modeled to identify key sources of variability
- Parameters such as **permeability**, **gastric emptying time** and **clearance** were most impactful
- ***Default %CV distribution in GastroPlus for other parameters were able to capture observed variability***







Day 1
A single dose of 50 mg IR suspension

Day 1-2	Day 3	Days 4-7	Day 8
100 mg bid F4	200 mg bid F4	300 mg bid F1	300 mg bid F1 + fat breakfast

Week 1	Week 2	Week 3-12
100 mg bid F4	300 mg bid F1	600 mg bid F1

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DILIsym Services QST Software Aids Decisions

- Predicts drug-induced liver disease
- v8A released Q1 2019
- Includes mechanistic representation of normal hepatic biochemistry
- Evaluated >80 compounds with 40+ companies

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….***

Relevant Recent DILIsym News / Publications

U.S. FDA Renews Annual DILIsym Software Licenses

FDA Maintains Access to Leading Liver Injury Software Program

May 06, 2020 08:30 AM Eastern Daylight Time

RESEARCH TRIANGLE PARK, N.C.--(BUSINESS WIRE)--DILIsym Services, Inc., a Simulations Plus company (Nasdaq: SLP) and a leading provider of simulation and modeling software for pharmaceutical safety and efficacy, today announced that the U.S. Food and

Pharm Res (2020) 37:24
https://doi.org/10.1007/s11095-019-2726-0

RESEARCH PAPER

Comparison of the Hepatotoxic Potent Treatments for Autosomal-Dominant P Using Quantitative Systems Toxicology

First Approved Cancer Treatment for TGCT Included DILIsym Simulations in FDA Review

FDA Review Cites DILIsym Results as Part of Turalio® Submission

October 27, 2020 08:30 AM Eastern Daylight Time

RESEARCH TRIANGLE PARK, N.C.--(BUSINESS WIRE)--DILIsym Services, Inc., a Simulations Plus company (Nasdaq: SLP ding provider of modeling and simulation software for pharmaceutical safety and efficacy, today announced that ns using their DILIsym® software were noted in a U.S. Food and Drug Administration (FDA) review of the New Drug

Application of the DILIsym® Quantitative Systems Toxicology drug-induced liver injury model to evaluate the carcinogenic hazard potential of acetaminophen

Gary Eichenbaum[a,*], Kyunghee Yang[b], Yeshitila Gebremichael[b], Brett A. Howell[b], F. Jay Murray[c], David Jacobson-Kram[d], Hartmut Jaeschke[e], Edwin Kuffner[a], Cathy K. G John C.K. Lai[f], Daniele Wikoff[g], Evren Atillasoy[f]

[a] Johnson & Johnson, New Brunswick, NJ, 08901, USA
[b] DILIsy
[c] Murra

OXFORD | SOT Society of Toxicology academic.oup.com/toxsci

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

https://doi.org/10.1093/toxsci/kfac051
Advance Access Publication Date: 12 May 2022
Research article

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

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

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

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Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

DILIsym: Quantitative systems toxicology impacting drug development

Paul B. Watkins

Clinical Pharmacology & Therapeutics

Article

Quantitative Systems Toxicology Modeling Predicts th Biliary Efflux Contributes to Tolvaptan Hepatotoxicity

James J. Beaudoin, William J. Brock, Paul B. Watkins, Kim L. R. Brouwer

First published: 03 August 2020 | https://doi.org/10.1002/cpt.2007

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RESEARCH PAPER

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

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Assessment of the Mechanism for Remdesivir-Associated Clinical ALT Elevations Using DILIsym Quantitative Systems Toxicology Modeling

Kyunghee Yang[1], Brett A Howell[1], Joy Y. Feng[2], Darius Babusis[2], Tomas Cihlar[2], Scott Q Siler[1]

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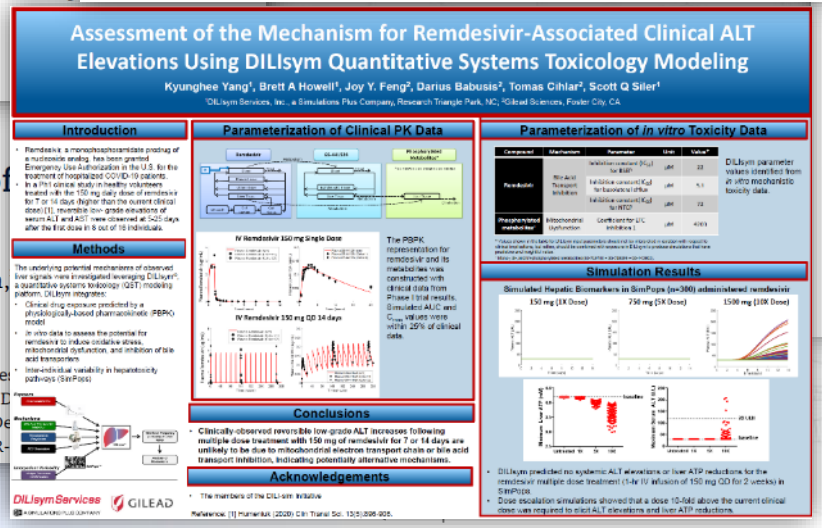

Mechanistic Investigations Support Liver Safety of Ubrogepant

Brenda Smith,* Josh Rowe,*[,1] Paul B. Watkins,[†] Messoud Ashina, Jeffrey L. Woodhead,[§] Frank D. Sistare,[¶] and Peter J. Goadsby[‖]

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Quantitative systems toxicology (QST) reproduces species differences in PF-04895162 liver safety due to combined mitochondrial and bile acid toxicity

Grant Generaux[1] | Vinal V. Lakhani[1] | Yuching Yang[1] | Sashi Nadanaciva[2] | Luping Qiu[3] | Keith Riccardi[4] | Li Di[4] | Brett A. Howell[1] | Scott Q. Siler[1] | Paul B. Watkins[5,6] | Hugh A. Barton[7] | Michael D. Aleo[3] | Lisl K. M. Shoda[1]

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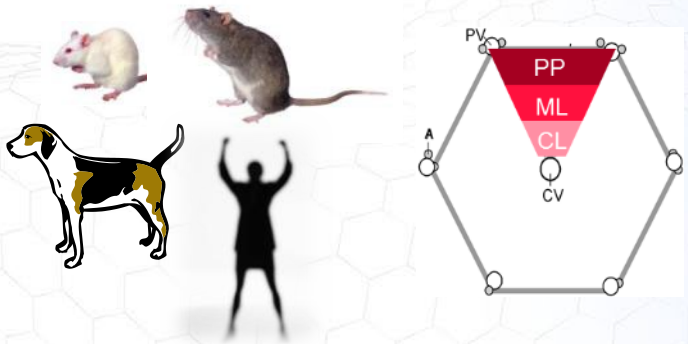
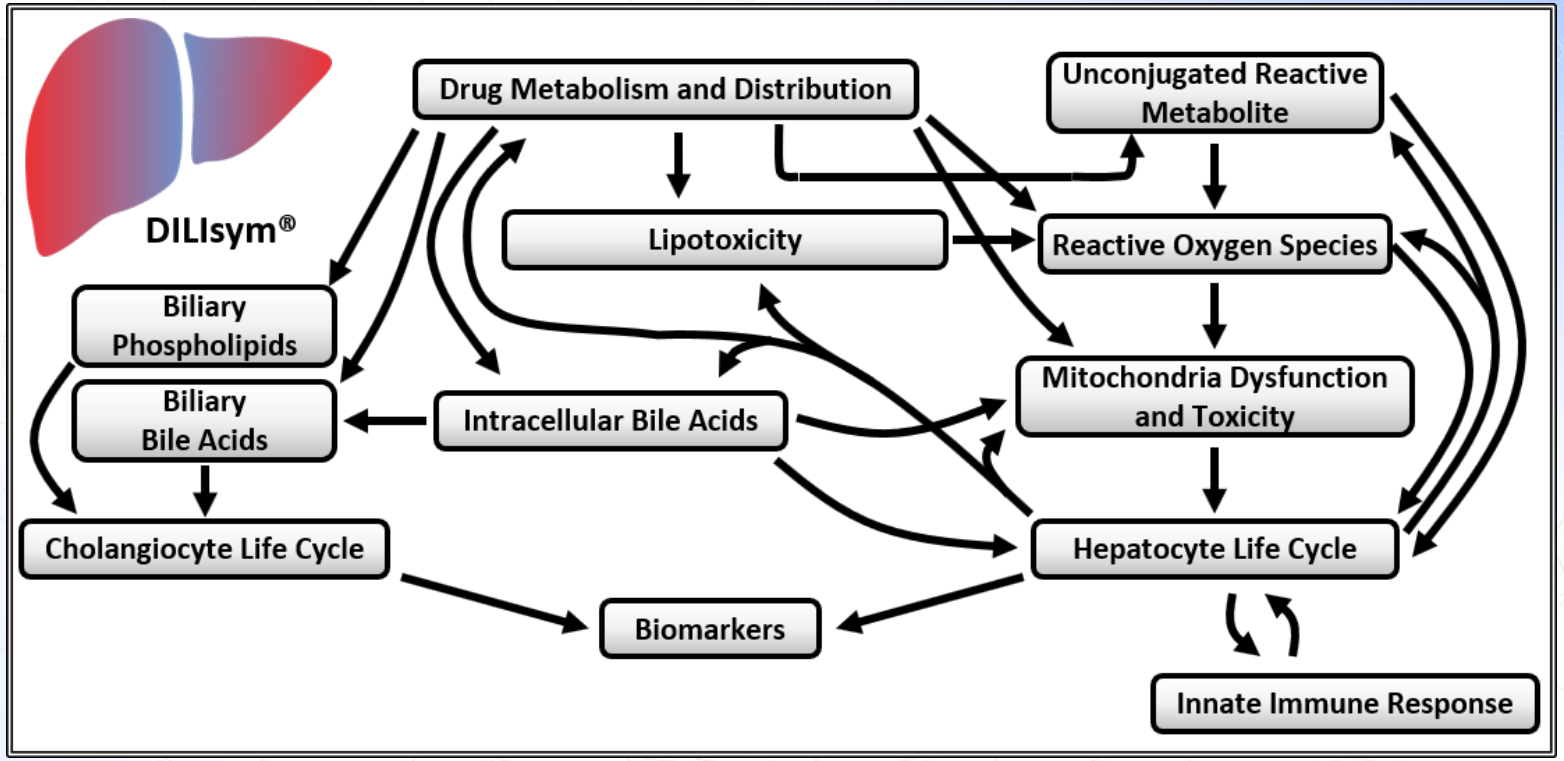
DILIsym Software Overview

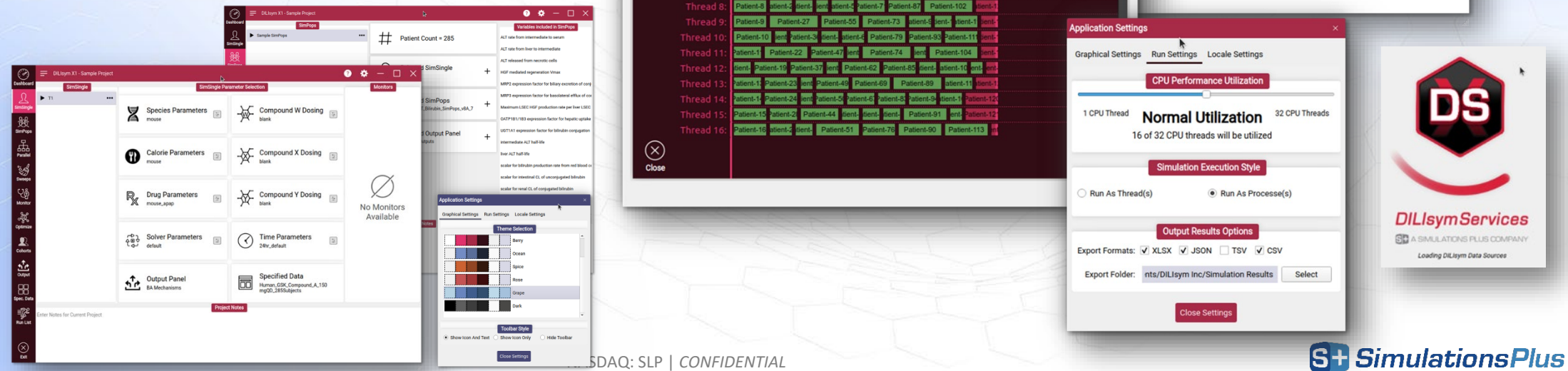
- Multiple species: human, rat, mouse, and dog
  - Population variability
- The three primary acinar zones of liver represented
- Essential cellular processes represented to multiple scales in interacting sub-models
- ~90 detailed representations of validation compounds with >80% success and **zero false positive predictions**
- Single and combination drug therapies





Highlights of DILIsym® Version X (DSX)

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



DILIsym Utilizes Various Data Types to Inform Decisions

DMPK and 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*

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*

Modeling & Simulation

Simulations and Assays inform:

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

Clinical Data / Protocol Information

Client specified protocols

- **Dosing protocols, fasting/fed state, meal times**
- **Patient types (NHV, disease, etc.)**
- **Anthropometric data**
  - *Body weight, age, ethnicity*

Prediction of the Liver Safety Profile of a First-in-Class Myeloperoxidase Inhibitor Using Quantitative Systems Toxicology Modeling

Jeffrey L. Woodhead[1], Yeshi Gebremichael[1], Joyce Macwan[1], Irfan Qureshi[2], Richard Bertz[2], Victoria Wertz[2], Brett A. Howell[1]

[1]Simulations Plus, Inc., Lancaster, CA, USA; [2]Biohaven Pharmaceuticals, New Haven, CT, USA

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SimulationsPlus

PURPOSE

The novel myeloperoxidase inhibitor verdiperstat was developed as a treatment for neuroinflammatory and neurodegenerative diseases. Phase 2 clinical studies had shown some promise for efficacy at the 600 mg BID dose; however, this is a large dose and verdiperstat had shown some *in vitro* signals suggesting possible liver toxicity. Mild liver signals had also been observed during Phase 1 trials, though it was unclear whether these were drug-related or not. In order to provide an added layer of confidence in the liver safety of verdiperstat before proceeding to Phase 3, a computational prediction of verdiperstat liver safety was performed using DILIsym v8A, a quantitative systems toxicology (QST) model of liver safety.

METHODS

A physiologically-based pharmacokinetic (PBPK) model of verdiperstat was constructed in GastroPlus 9.8, and the estimates for the liver and plasma time course of verdiperstat were input into DILIsym. *In vitro* experiments measured the likelihood that verdiperstat would inhibit mitochondrial function, inhibit bile acid transporters, and generate reactive oxygen species (ROS). Predictions of liver verdiperstat exposure from the PBPK model and parameters derived from the *in vitro* experimental results were used as inputs into DILIsym. Two alternate sets of parameters were used as inputs in order to fully explore the sensitivity of model predictions within the potential range of the *in vitro* data. Verdiperstat dosing protocols up to 600 mg BID were simulated for up to 48 weeks using a simulated population (SimPops) in DILIsym.

RESULTS

In vitro experiments were conducted in cell vesicles (for bile acid transport) and HepG2 cells (for ROS and ETC inhibition). These experiments showed verdiperstat to be a mild inhibitor of the bile acid transporter MRP4 (Figure 1), a mild generator of ROS (Figure 2), and a mild inhibitor of the mitochondrial electron transport chain (ETC, Figure 3). For ROS and ETC inhibition, the intracellular concentration was measured by mass spectrometry.

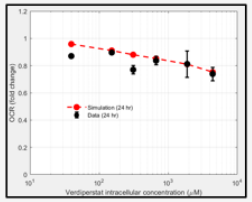


Figure 2. Relationship between measured intracellular verdiperstat and oxygen consumption rate, demonstrating a dose-dependent decrease in oxygen consumption and thus an inhibition of the electron transport chain.

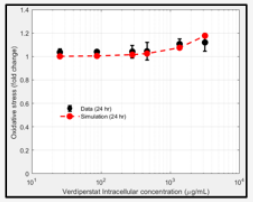


Figure 3. Relationship between measured intracellular verdiperstat and normalized TBARS generation, demonstrating a dose-dependent increase in oxidative stress.

Results from the *in vitro* experiments were used to calculate input parameters into DILIsym v8A, in the table below. OCR consumption was modeled in MITOsym v3B, a QST model of *in vitro* mitochondria, and translated into DILIsym; ROS generation was modeled in an *in vitro*-like parameterization in DILIsym (red lines in Figures 2 and 3). An alternate, conservative parameterization using an estimate of intracellular concentration as equal to the nominal concentration, which was suggested by the liver partition coefficient of 1 used in the PBPK model, was also developed; these parameters are also in the table below.

Mechanism	DILIsym Parameter	Unit	Alternate Verdiperstat Value	Primary Verdiperstat Value
BA Transport Inhibition	Inhibition constant for BSEP	µM	No inhibition	No inhibition
	Inhibition constant for basolateral efflux (MRP3/4)	µM	32.55**	32.55**
	Inhibition constant for NTCP	µM	No Inhibition	No Inhibition
Oxidative Stress	Liver RNS/ROS production rate constant 1	mL/nmol/hour	1.7×10^{-4}	1.15×10^{-6}
Mitochondrial Dysfunction	Coefficient for ETC Inhibition 1	µM	6.94×10^{5}	6.94×10^{5}
	Coefficient for ETC Inhibition 3	µM	2.43	243
	Max inhibitory effect for ETC inhibition 3	Dimensionless	0.39	0.39

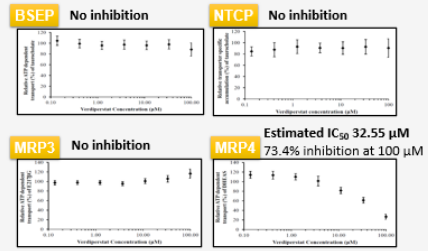
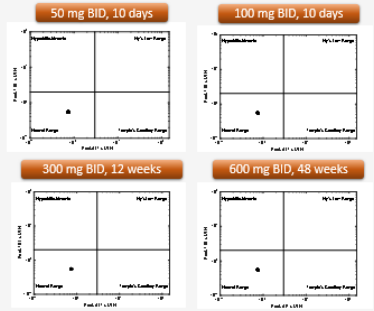
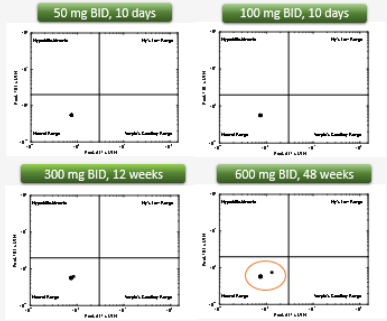


Figure 1. Inhibition of bile acid transporters by verdiperstat



In SimPops simulations (n = 285), no ALT elevations over 3x ULN were predicted using either the primary (above) or alternate (below) parameterizations. Mild ALT elevations (less than 3x ULN) occurred at the 600 mg BID dose in the alternate parameterization.



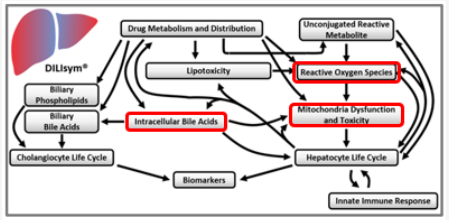


Diagram of the interactions between submodels in DILIsym v8A. In vitro *measurements of oxidative stress, mitochondrial dysfunction, and bile acid transport inhibition are used as inputs, and the DILIsym model of liver physiology computes the likelihood that those mechanisms will affect the hepatocyte life cycle, which will in turn affect biomarker release and immune system activation.*

CONCLUSION

Verdiperstat was predicted to be safe, with only rare, mild liver enzyme increases as a potential possibility in very highly sensitive individuals. Subsequent Phase 3 clinical trials conducted after the conclusion of this modeling work found that ALT elevations in the verdiperstat treatment group were generally similar to those in the placebo group. This validates the DILIsym simulation results and demonstrates the power of QST modeling to predict the liver safety profile of novel therapeutics.

ACKNOWLEDGEMENTS

- Biohaven Pharmaceuticals, Inc.
- The members of the DILI-sim and RENAsym Initiatives

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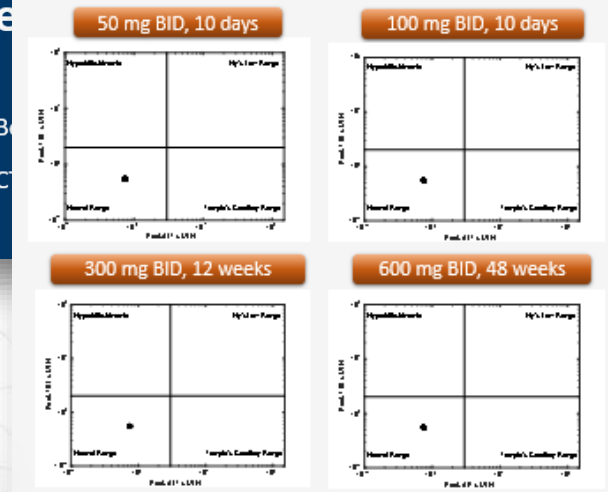

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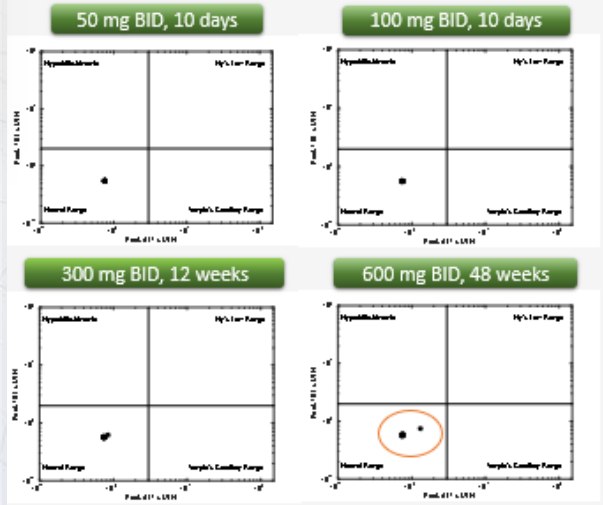

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QST "Discovery" and "Regulatory" Project Options

Discovery / Preclinical Approach

Description:
A streamlined QST analysis using necessary but cost-effective input data

Application:
Determination of potential injury for high level compound screening, lead optimization and risk/benefit analysis

Model Input:
- *Streamlined* compliment of in vitro data; oxidative stress, mitochondrial toxicity, and transporter data; specified PK/exposure information

Deliverable:
- Summary Report (Microsoft PowerPoint)

Regulatory Approach

Description:
A fully featured QST analysis using a full compliment of in vitro data coupled with PBPK modelling for model input

Application:
Regulatory submission ready and publication worthy approach for informing the highest confidence of candidate selection and safety evaluation

Model Inputs:
- *Full* compliment of in *vitro data*; oxidative stress, mitochondrial toxicity, and transporter data
- GastroPlus® ACAT/PBPK Model

Deliverables:
- Full Report (Microsoft Word)
- GastroPlus® model input, output, and database files; DILIsym input files

Mechanistic Modeling Saves Resources Today in R&D and Regulatory Interactions

- Prioritize and **make better investments**
- Integrate data to **tell a compelling story**
- Eliminate **unnecessary animal/human studies**
- Improve productivity to be the **first to market**
- Reduce **regulatory burden**
- Improve **patient lives**

Webinar Agenda

- Setting the Stage: Overview of Simulations Plus Inc.
- Exposure is the Key: PBPK Modeling in GastroPlus for Verdiperstat
- Safety First: the DILIsym Verdiperstat Application
- Panel Discussions and Q&A