DILIsym Services

A SIMULATIONS PLUS COMPANY

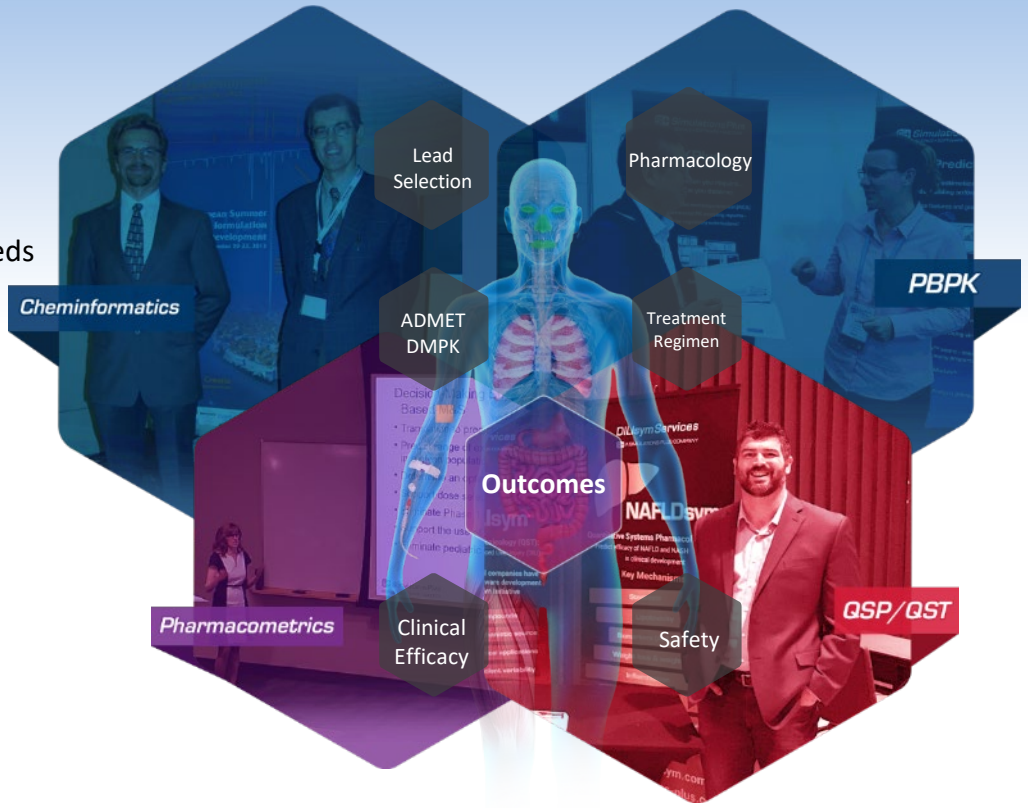
What's New in DSX®?

October 14, 2020

At *SimulationsPlus* We Put It All Together

Science

- Seamless collaboration
- Integrated, innovative solutions to meet <u>your</u> needs



Business

- Resources available to get the job done on time
- One-stop shopping – single vendor for all of your *in silico* drug development needs

We have the *Solutions* and the *People* to Address *<u>Your</u>* Drug Development Questions!

NASDAQ: SLP

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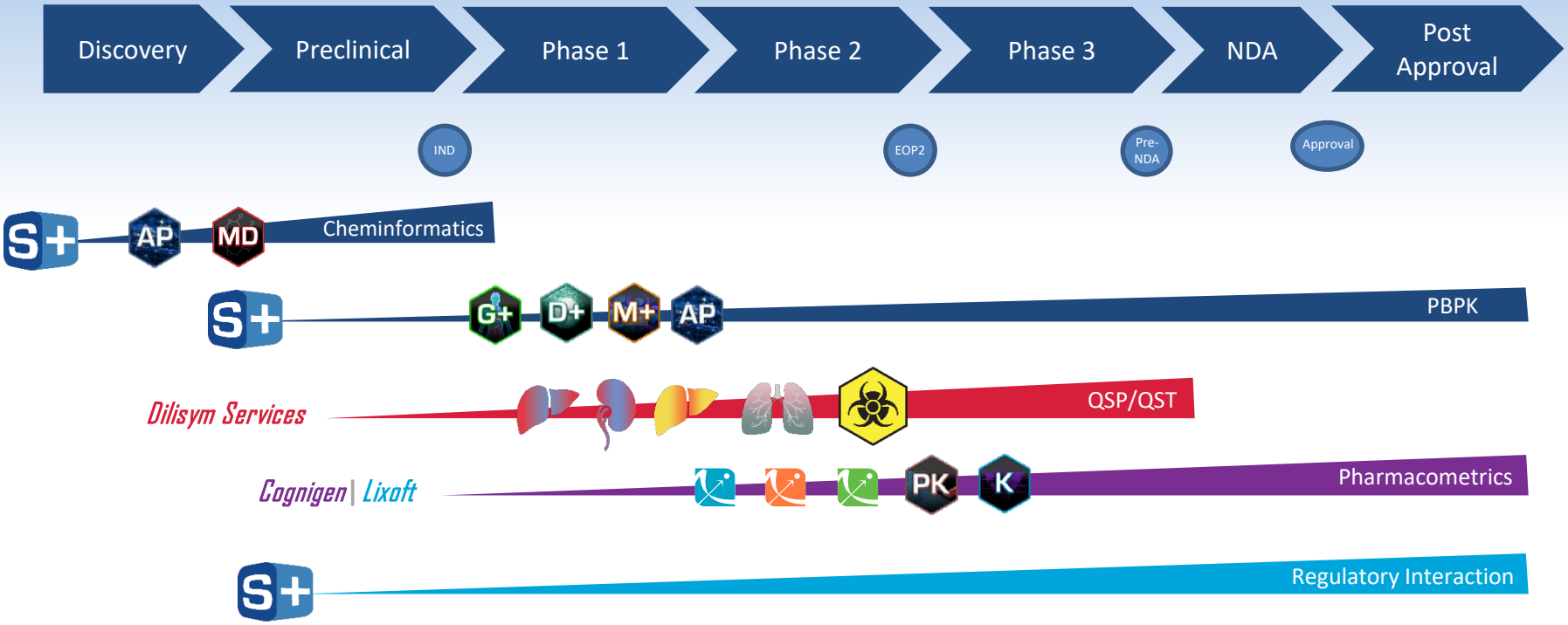
Our solutions inform the entire drug development process



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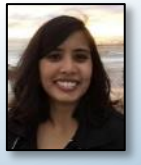

Lara Clemens
Postdoctoral Fellow
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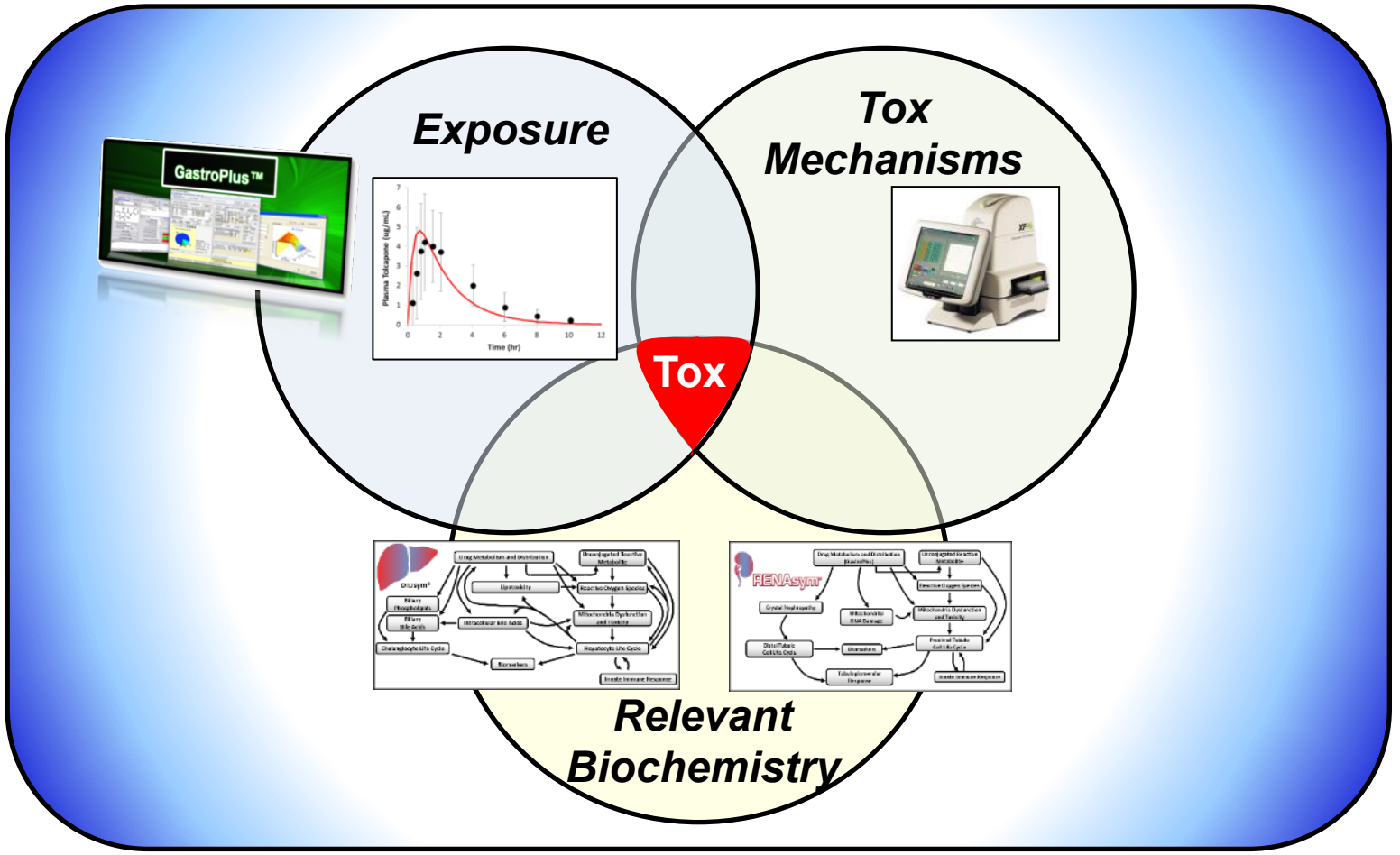
James Beaudoin
Scientist I
RTP, NC

QST Predicts Tox via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



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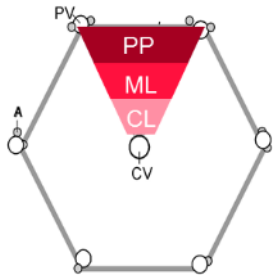
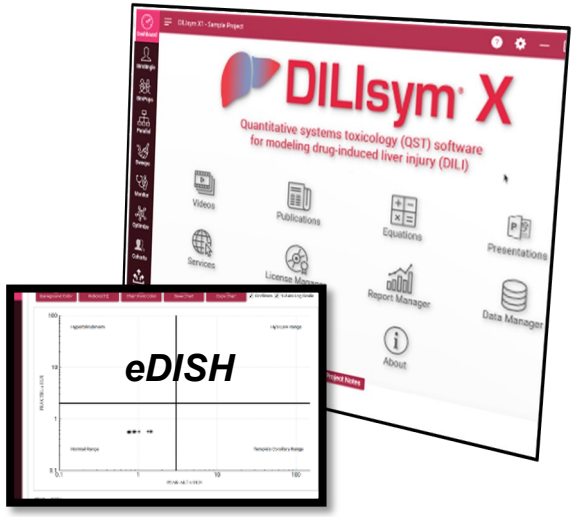
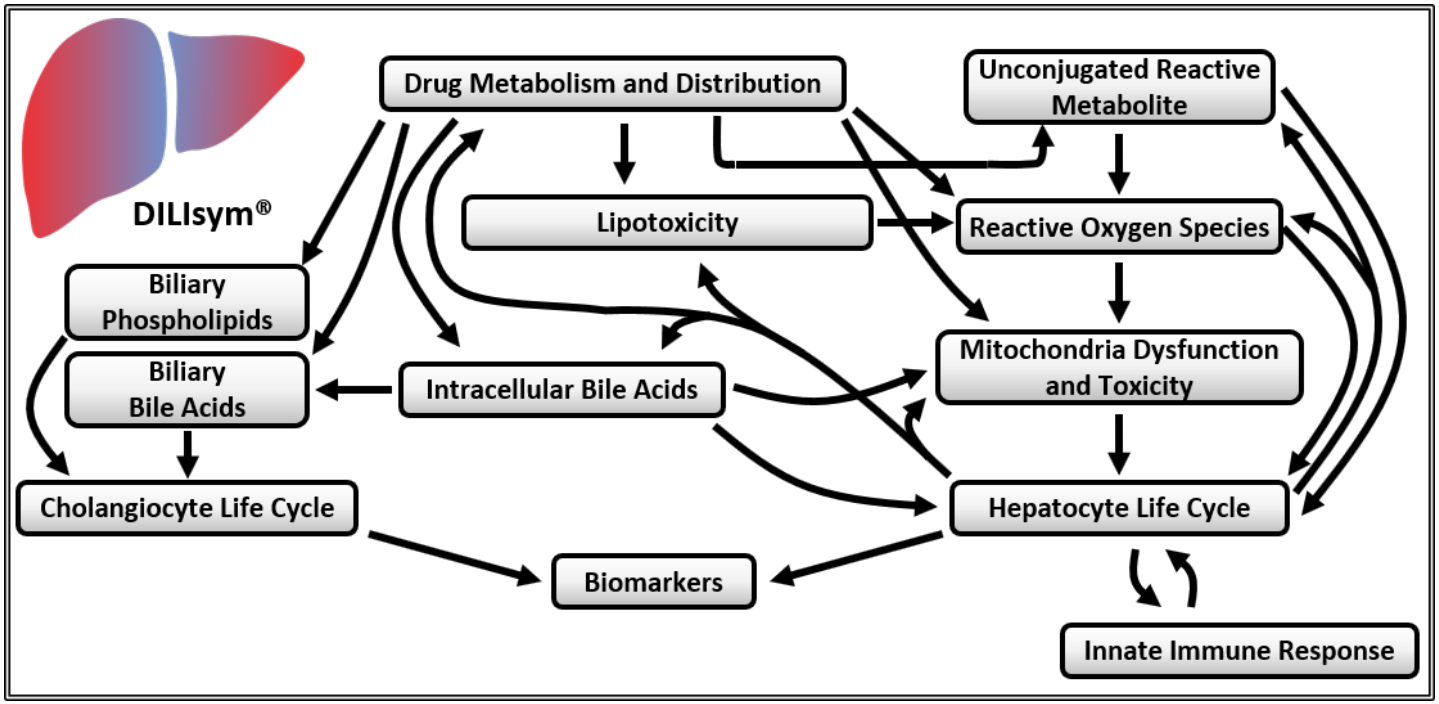
DILIsym Overview

- **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**
- **<u>Over 80</u> detailed representations of optimization or validation compounds with ~80% success**
- **Single and <u>combination drug</u> therapies**







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DILIsym Utilizes Various Data Types to Inform Decisions

Exposure Data

PBPK Modeling

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

Modeling & Simulation

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*

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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DILI-sim Initiative

Consortium Distributing and Developing Software for
Predicting and Preventing Drug-Induced Liver Injury (DILI)

Dr. Paul B. Watkins
Director, DILI-sim Initiative;
Chair, Scientific Advisory Board

Join Today and Support Cutting Edge Research to Make Patients Safer!

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Benefits of Stage 4 DILI-sim Membership

- Two global, floating end-user licenses to the current version of the DILIsym® software package
- Includes integrated GastroPlus® version, when available
- Licenses to an add-on feature of DILIsym that enables use of server/cloud parallel computing with unlimited nodes (upcharge for non-members)
- 31% discount on consulting services related to DILIsym
- 10 total hours of private training for employees of the Member company related to DILIsym use
- The right to vote on DILIsym software development items going forward
- Attendance at DILI-sim research, development, and software update meetings/discussions (typically held quarterly)
- Access to the DILIsym Discovery Support Program (DDSP), a Members-only, lower cost program for enabling internal software use

*Now includes **RENAsym™ Consortium** membership at no additional cost!*

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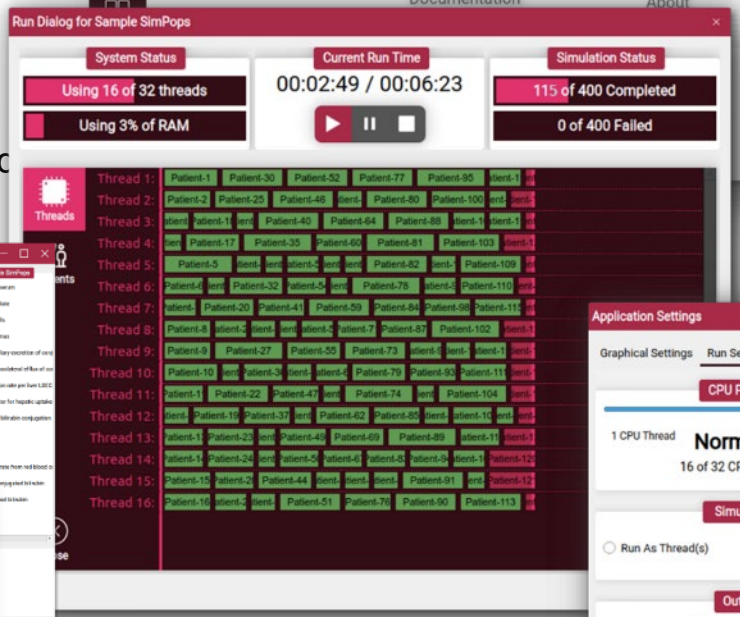
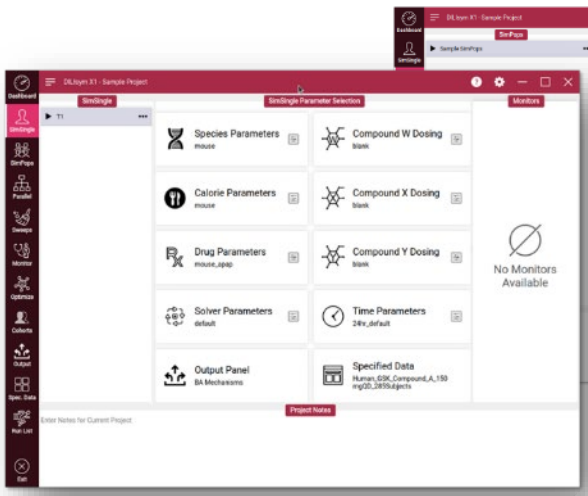
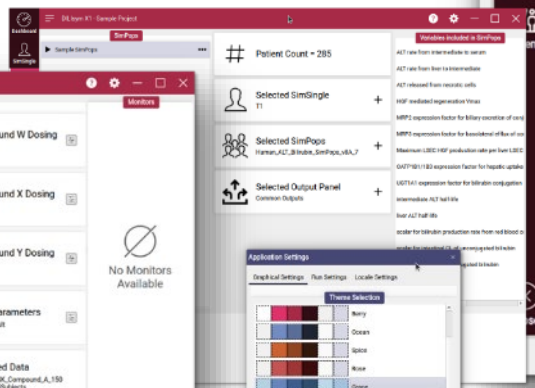
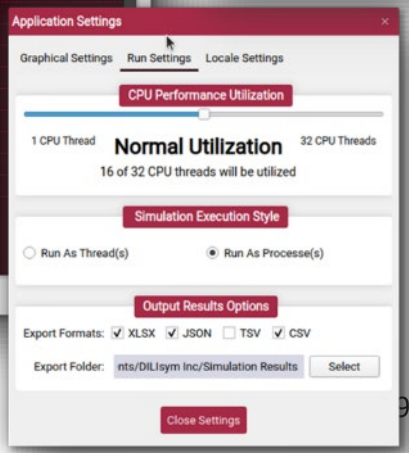
Highlights of DILIsym Version X (DSX)

- 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 (HIV/AIDS)
  - Anastrozole (breast cancer)
  - Tamoxifen (breast cancer and advanced-stage or metastatic hormone-receptor-positive disease)
- 2 New SimCohorts that include variability in susceptibility to liver injury and biomarker-related parameters (ALT and bilirubin)









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T1230-09-58

Synergy Between Two Mechanisms of Action Contributes to Species Differences in the Liver Safety Profile for PF-04895162

Vinal V. Lakhani[1†], Grant Generaux[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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Advancing Pharmaceutical Sciences, Careers, and Community

PURPOSE

PF-04895162 (ICA-105665), a drug in development for the treatment of epilepsy, was terminated after transaminase elevations (up to grade 3) were observed in healthy volunteers (NCT01691274). The human hepatotoxicity was unexpected because liver safety concerns had not been raised in preclinical safety studies (Aleo et al. 2019).

The Purpose is to better understand the mechanisms underlying the apparent species differences, between rat and human, when evaluating the liver safety of compound PF-04895162. This case study of PF-04895162 fits into the broader, pharmacology goal of improving the detection of potential liver liabilities prior to the introduction of compounds to the clinic.

CONCLUSIONS

This investigative study shows the ability of DILIsym to reproduce species differences in hepatotoxicity by integrating PK and *in vitro* data. Additionally, this study supports the contention that combined *in vitro* and *in silico* screening methods have the potential to identify latent hepatotoxic risks.

Reference:
Aleo MD, Aubrecht J, D Bonin P, et al. Phase I study of PF-04895162, a Kv7 channel opener, reveals unexpected hepatotoxicity in healthy subjects, but not rats or monkeys: clinical evid

METHODS

We retrospectively analyzed PF-04895162 using a computational representation of drug induced liver injury, DILIsym, which integrates *in vitro* data of hepatotoxic mechanisms with *in vivo* predictions of liver exposure.

The *in vitro* data included bile acid transporter inhibition and measuring mitochondrial dysfunction.

- Specifically, IC_{50} values were measured using standard vesicular transport assays for human BSEP, NTCP, MRP3 and MRP4, as well as, for rat Bsep, Mrp3 and Ntcp.
- Mitochondrial dysfunction was determined by measuring the oxygen consumption rate in human and rat hepatocytes using the Seahorse XF Analyzer.

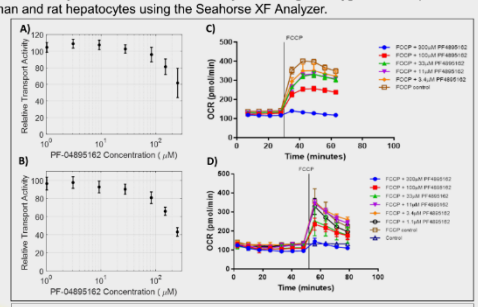

FIGURE 1. Examples of *in vitro* data collected. (A – B) Assessment of PF-04895162 inhibiting (A) human BSEP and (B) rat Bsep. (C – D) Oxygen consumption rate (OCR) of primary (C) human and (D) rat hepatocytes after incubation with PF-04895162, followed by addition of FCCP.

In vivo predictions of liver exposure are based on physiologically based pharmacokinetic (PBPK) models; these models were fit using pre-clinical and clinical measurements.

- The rat PBPK model included compartments for blood, liver, muscle, gut, and other tissue. This model was fit using rat plasma concentration measurements following IV and PO dosing.
- The human PBPK model included two compartments: blood and liver; the remaining tissues were aggregated into a general representation of systemic volume of distribution. This model was fit using clinical plasma concentration measurements following a single PO dose.

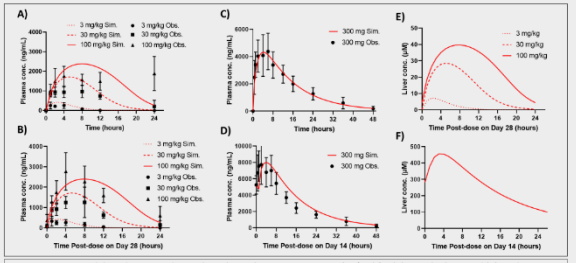

FIGURE 2. PBPK Models and Measured Data. (A – B) Rat plasma PF-04895162 (ng/mL) for (A) a single dose, and (B) on day 28, following daily dosing. (C – D) Human plasma PF-04895162 (ng/mL) for (C) a single dose, and (D) on day 14, following 300 mg BID dosing. (E – F) Liver concentrations in (E) rat on day 28 following daily dosing, and (F) human on day 14 following 300 mg BID.

These *in vitro* data (Fig 1) and PBPK models (based on *in vivo* data) (Fig 2) were integrated in DILIsym to predict hepatotoxicity in both species. Furthermore, simulations were conducted in SimPops, a simulated population where sensitivity to hepatotoxic mechanisms varies with variability in physiologic parameters.

RESULTS

Simulation results reproduced lack of rat hepatotoxicity and presence of clinical hepatotoxicity.

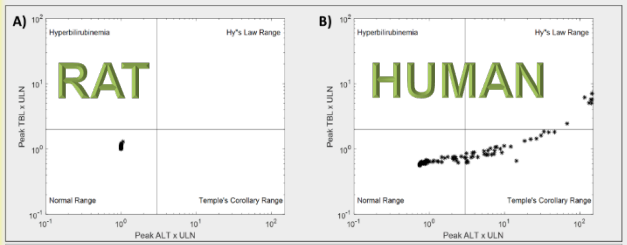

FIGURE 3. Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) Plots. Simulation of PF-04895162 (A) 100 mg/kg/day for 28 days in rat SimPops (n=294). (B) 300 mg BID for 14 days and 14 day follow-up in human SimPops (n=285).

While simulations reproduce species differences, ALT elevations in simulated humans were less frequent than observed clinically, 59/285 (21%) *vs.* 6/8 (75%) respectively.

Simulated human liver injury was sometimes more severe than observed clinically. ALT >10x

he simulated human hepatotoxicity was demonstrated to be due to interaction between these two mechanisms; elimination of either mechanism from the model abrogated injury (Table 1).

Table 1. Sensitivity Analysis of Toxicity Mechanisms

Simulations	Mechanisms On	Mechanisms Off	ALT Elevations ≥3x ULN
300 mg po BID for 14 days in Multi16[‡]	ETCi, BAi	-	8/16
	ETCi	BAi	0/16
	BAi	ETCi	0/16

[‡] Multi16 is a Human SimCohort (n = 16), which includes individuals sensitive to different mechanisms of toxicity. ETCi = electron transport chain inhibition. BAi = bile acid transporter inhibition.

Alt h the IC_{50} for BSEP inhibition by PF-04895162 was higher (311 μM) than has been generally thought to ibute to hepatotoxicity, toxicity from the bile acid mechanism still occurred. Analysis of the modeling re hus indicated multiple contributors to the simulated species differences. Additionally, the simulated h liver exposure was greater than the simulated rat liver exposure, which allowed PF-04895162 to ge both mitochondrial toxicity and inhibition of bile acid transporters. Modeling even higher PF-95162 liver exposures than were measured in the rat safety studies aggravated mitochondrial toxicity but did not result in rat hepatotoxicity due to insufficient accumulation of cytotoxic bile acid species.

Simulated individuals' time to peak ALT is similar to clinical observations.

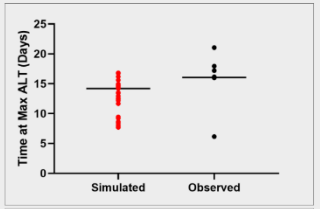

FIGURE 4. Comparison of time to peak ALT in human SimPops versus measured clinical data. Simulated individuals are represented in red. Clinical measurements for subjects in the Phase I study are represented in black. The median value is indicated by a line.

300 mg BID dosing of PF-04895162 for 14d was associated with delayed ALT elevations. Delayed timing is often considered indicative of a putative adaptive immune response. Simulations demonstrate that delayed ALT elevations due to intrinsic mechanisms, where the more gradual bile acid accumulation accounts for the delayed response.

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Representation of Efavirenz-mediated Drug-Induced Liver Injury (DILI) Using Quantitative Systems Toxicology (QST)

Diane M. Longo[1], Kyunghee Yang[1],, Brett A. Howell[1], Jeffrey L. Woodhead[1]

[1]DILIsym Services, Inc., Research Triangle Park, NC, USA

Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with other agents to treat human immunodeficiency virus (HIV) infection. Efavirenz treatment is associated with a low frequency of serum enzyme elevations[1]. To understand the hepatotoxicity mechanisms underlying clinically observed liver signals, efavirenz was represented in DILIsym®, a QST model of DILI.

Methods

- The potential for efavirenz to inhibit bile acid transporters was assessed using transporter-overexpressing vesicles and cells.
- The potential for efavirenz to induce mitochondrial dysfunction or oxidative stress was assessed in HepG2 cells.
- Mechanistic *in vitro* data were used to define DILIsym hepatotoxicity parameters for efavirenz.
- A previously constructed GastroPlus physiologically based pharmacokinetic (PBPK) representation[2] was used to predict efavirenz exposure.
- Simulated populations (SimPops) that include variability in hepatotoxicity mechanisms and in efavirenz exposure were used to simulate the *in vivo* response in humans to efavirenz in DILIsym.

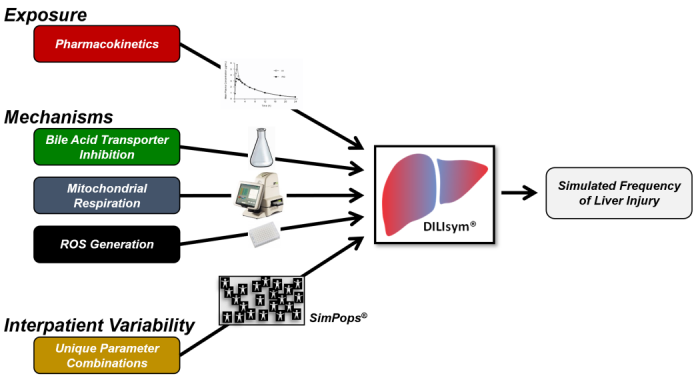

Figure 1. Diagram of the overall workflow for this study, including the processes of determining compound exposure, determining toxicity parameters from *in vitro* data, and incorporating interpatient variability to simulate the frequency of liver injury

Results

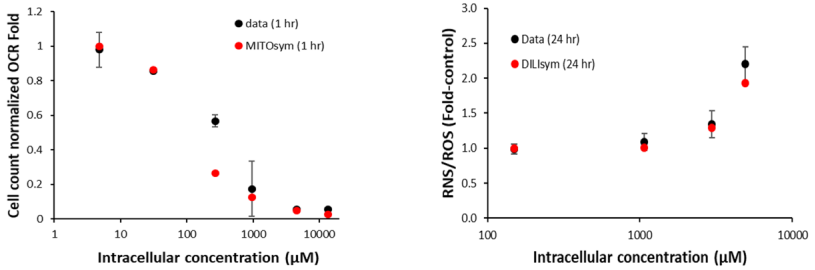

Figure 2. Comparison of simulation results and *in vitro* assay data to identify DILIsym parameter values that reproduce the efavirenz-mediated OCR response at 1 hr (left) and the efavirenz-mediated ROS response at 24 h (right).

- Mechanistic *in vitro* assays indicated that efavirenz inhibits the mitochondrial electron transport chain (ETC), increases oxidative stress, and inhibits bile acid transporters (BSEP, NTCP, and MRP4).
- Combining *in vitro* hepatotoxicity data with predicted exposure, DILIsym predicted infrequent ALT elevations (1-2%) in SimPops following administration of efavirenz (600 mg QD) for 12 weeks.

Dosing Regimen	Observed ALT >5X ULN[1]	Simulated ALT >5X ULN
Efavirenz 600 mg QD oral	1% to 8%	1% to 2%

Table 1. Comparison of observed vs. simulated hepatotoxicity for efavirenz

Conclusions

DILIsym correctly predicted infrequent hepatotoxicity for efavirenz, consistent with the low rate of ALT elevations seen clinically. This study demonstrates the ability of DILIsym to combine *in vitro* mechanistic data, predicted liver compound exposure, and biological variability to predict the incidence of liver injury. Thus, DILIsym represents a powerful tool to assess the potential DILI liabilities of new compounds. In addition, the efavirenz representation developed in the current study can be used to predict DILI drug-drug interactions between efavirenz and co-administered drug(s).

References

[1] Efavirenz. URL: https://livertox.nlm.nih.gov/Efavirenz.htm.

[2] Inger Darling. Efavirenz physiologically based pharmacokinetic model development and validation as a moderate cyp3a4 inducer for drug-drug interaction predictions. URL: https://www.simulations-plus.com/assets/M1430-13-87_aaps_efavirenz_poster_2019-10-18.pdf.

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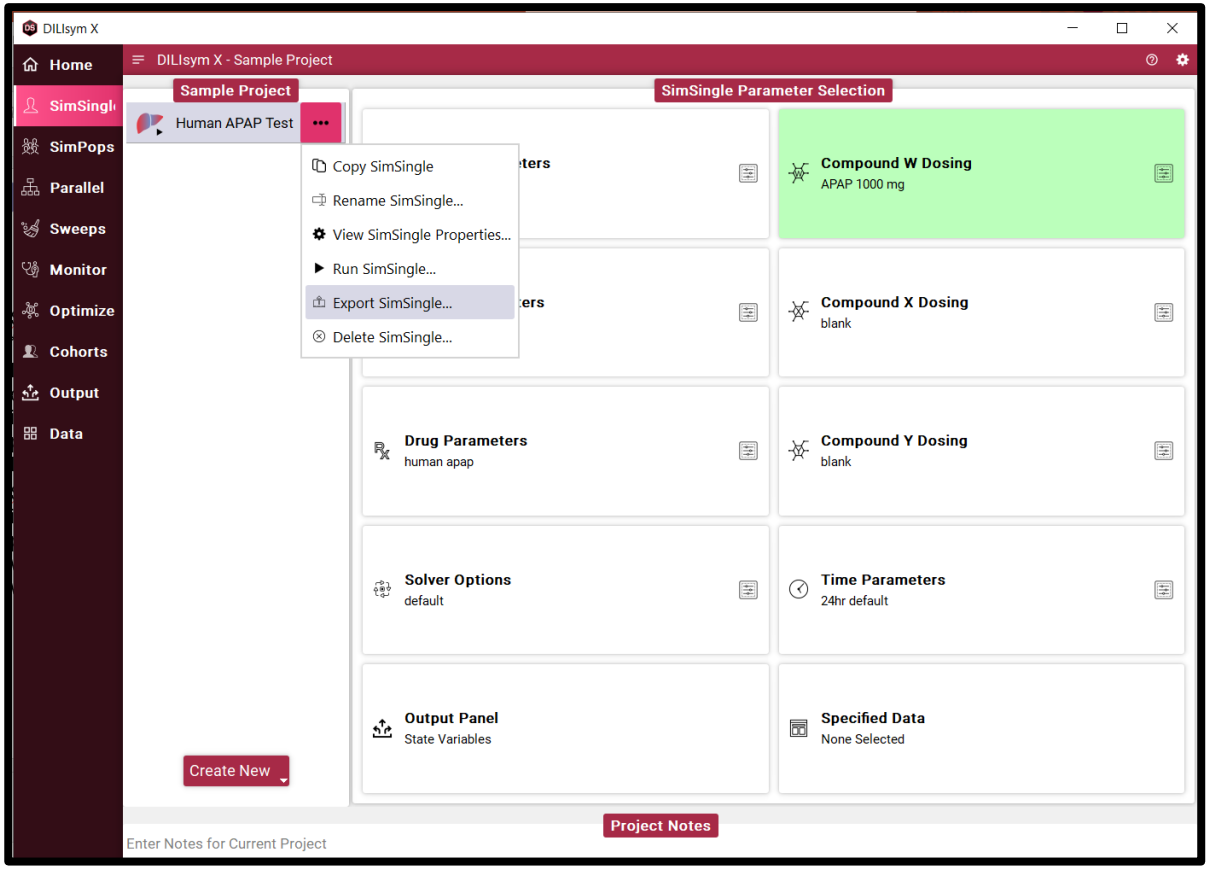
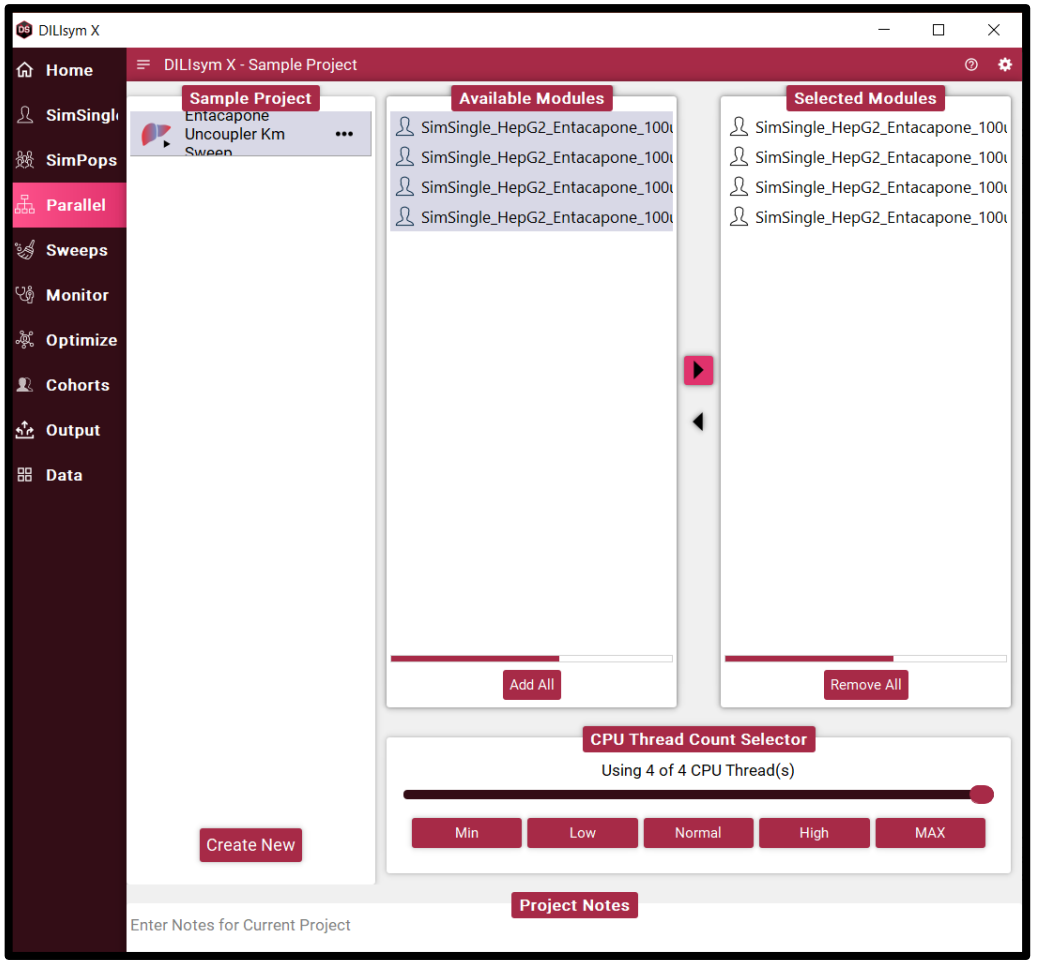
The DILIsym X Workspace is Organized According to Projects, Not SimSingles

- Projects are started or opened which contain one or more SimSingles, but also other settings files
- SimSingles are created within a given project
- This organization allows for improved overall project organization and management compared to DILIsym version 8A



Running Parallel Simulations in DILIsym

- The Parallel tab allows users to run several SimSingles, SimPops, or Sweeps in parallel
- Utilizes selected number of threads
- Excellent feature for collection of simulations that need to get completed over an extended period of time (e.g. overnight)



Live DSX Demonstration

DILI-sim Initiative

Consortium Distributing and Developing Software for Predicting and Preventing Drug-Induced Liver Injury (DILI)

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- 10 total hours of private training for employees of t
- The right to vote on DILIsym software development
- Attendance at DILI-sim research, development, and so
  quarterly)
- Access to the DILIsym Discovery Support Program (DDSP), a
  internal software use

QUESTIONS?

https://www.simulations-plus.com/contact/

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