DILIsym Services

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DILIsym User Training –

SimPops in DILIsym - Background, Construction and Current Patient Groups Represented

DILIsym Development Team

Goals for This Training Session

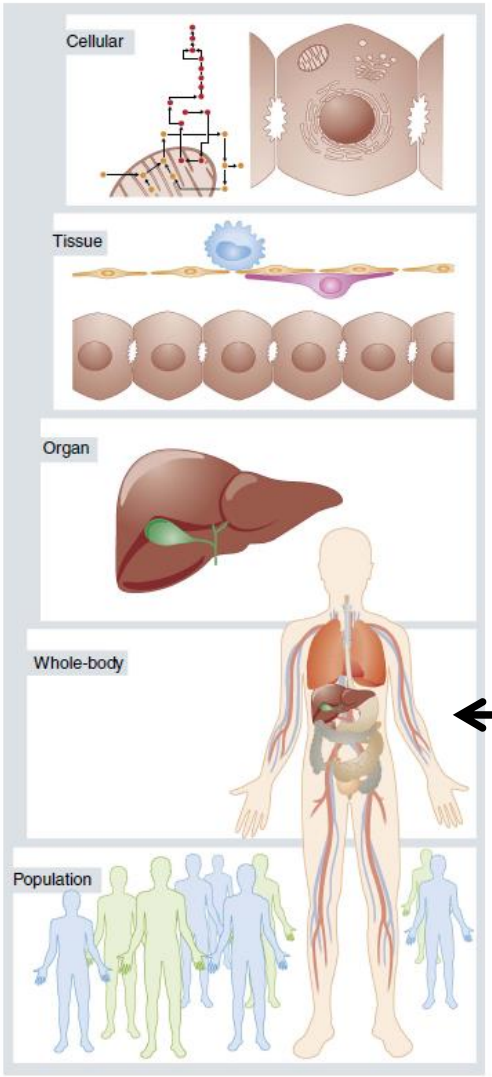
Participants should understand the following general concepts:

- How to apply SimPops and SimCohorts within DILIsym
- The approaches used to generate SimPops within DILIsym
- Current SimPops included within DILIsym

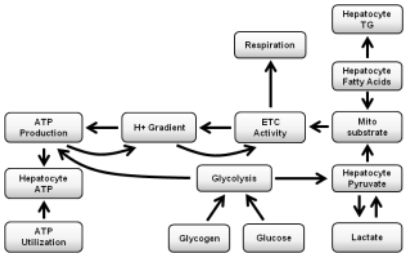
Overview of SimPops Training Session

- General introduction to SimPops concepts
- Processes for constructing SimPops
- SimPops included in DILIsym v7A
- Snapshots of real world examples of SimPops applications

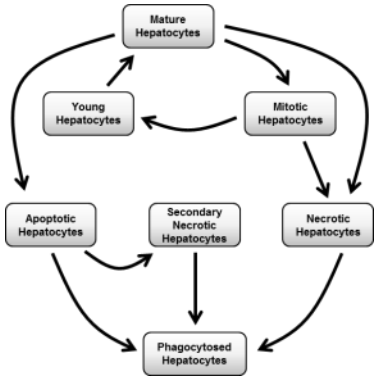
DILIsym: Quantitative Systems Toxicology



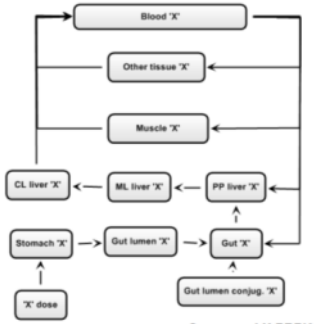
Mitochondrial dysfunction



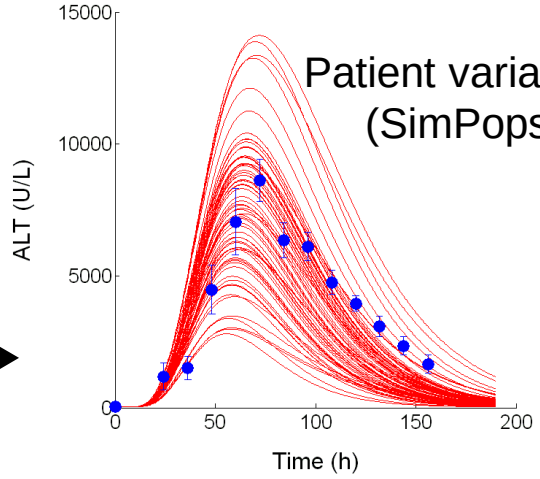
Hepatocyte life-cycle



Drug distribution & metabolism



Patient variability (SimPops)



Kuepfer 2010, Molecular Systems Biology

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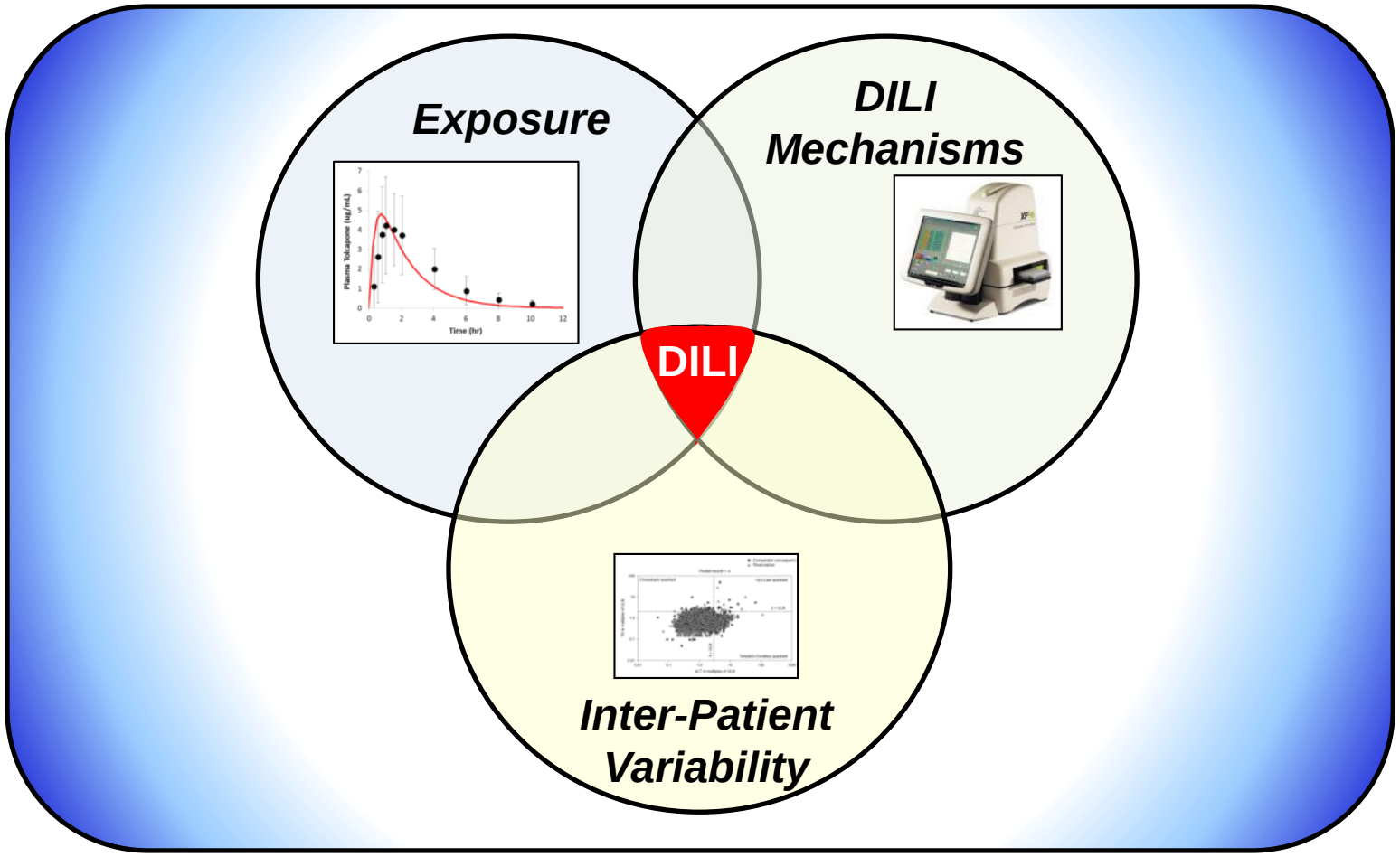
DILIsym Predicts DILI via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



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Population Variability is Important for the Prediction of Low-Frequency Events Such as Drug-Induced Liver Injury (DILI)

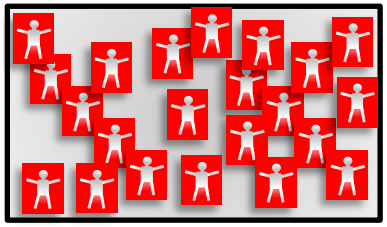
- Most drugs with DILI liabilities do not cause DILI for every individual
  - Would not be predicted by simulations of an “average” or “median” human or animal
- Including variability in individual characteristics that lead to DILI susceptibility is crucial
  - Can also isolate potential individual risk factors

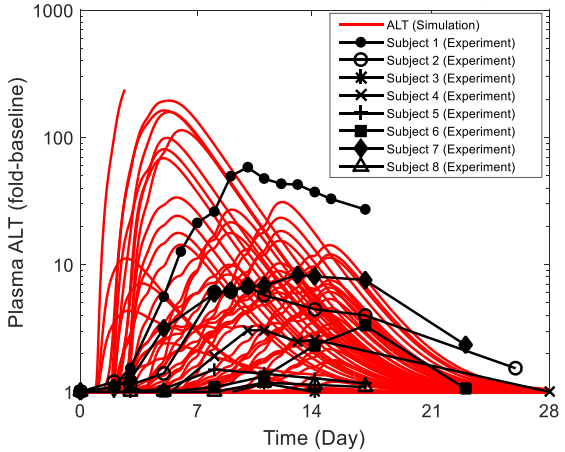
Drug	Clinical ALT Elevation Frequency
Troglitazone	2%
Bosentan	8-18%
Tolcapone	3%
APAP (Therapeutic Dose)	31-44%

Range of Hepatotoxic Responses in SimPops Due to Variability in Underlying Biochemistry

- SimPops are population samples with variability in hepatotoxic drug responses
- Multiple parameters are varied to produce diverse simulated patients
- Numerous simulated patients are generated, consistent with range of observed response data and known parameter distributions
- SimPops compared with reported clinical data where available
- SimPops are subsequently used to predict responses to novel compounds

Example of Variables Used to Construct SimPops
Body weight
Glutathione levels and synthesis
RNS-ROS clearance
Mitochondria function
Bile acid transporter function
Adaptive responses to bile acid levels
Apoptotic sensitivity to RNS-ROS
Necrotic sensitivity to ATP reductions
Hepatocyte regeneration

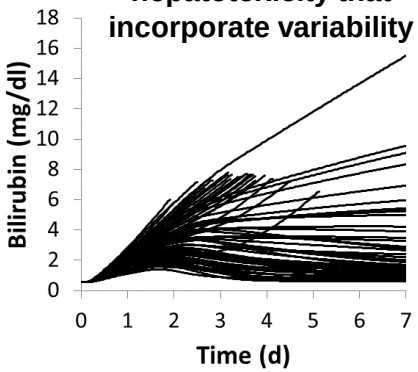




Clinical Data and Simulation Results

Treat SimPops with Compound X

Novel predictions of hepatotoxicity that incorporate variability



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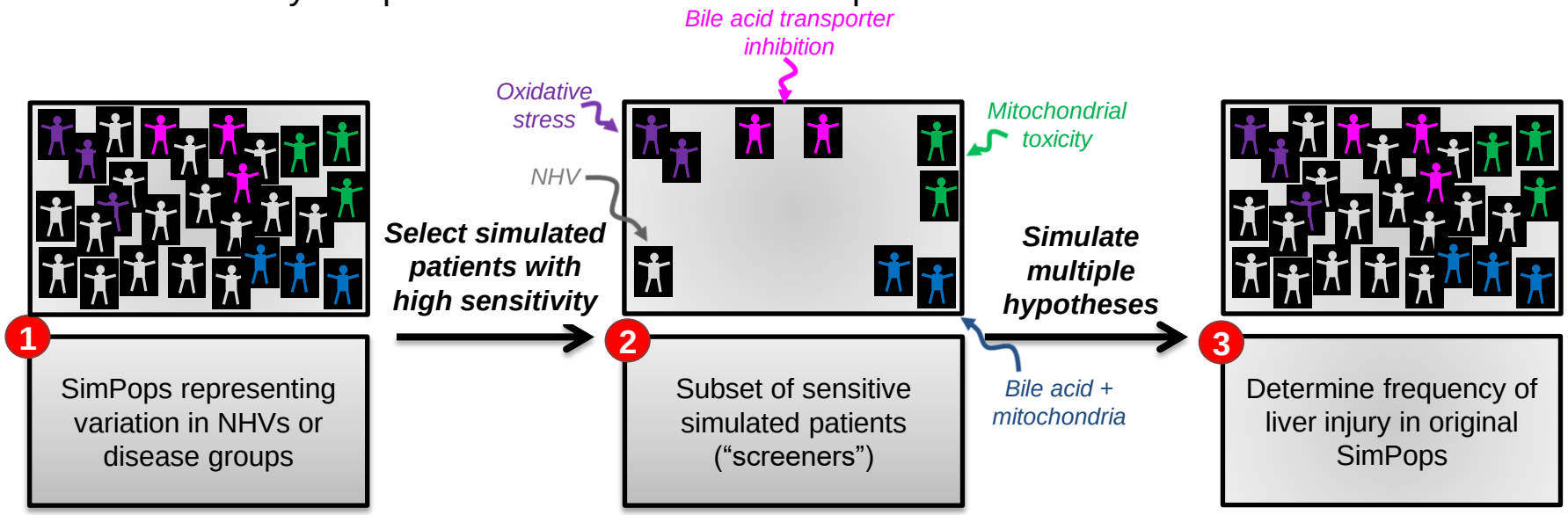
Generation and Use of Subsets of Simulated Humans (SimCohorts) in DILIsym

- SimCohorts consisting of a subset of individuals from existing SimPops are used for screening and preliminary simulations and are adequate for many analyses
  - Computationally less-expensive for testing multiple hypotheses prior to full SimPops
  - ***Provided with DILIsym for many of the SimPops – DILIsym documentation explains purpose of each***
- Multiple approaches for selecting individuals from larger SimPops
  - Select simulated individuals with high sensitivity
  - Select individuals based on parameter values (i.e. body weight)
  - Randomly sample individuals from full SimPops



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Overview of SimPops Training Session

- General introduction to SimPops concepts
- Processes for constructing SimPops
- SimPops included in DILIsym v7A
- Snapshots of real world examples of SimPops applications

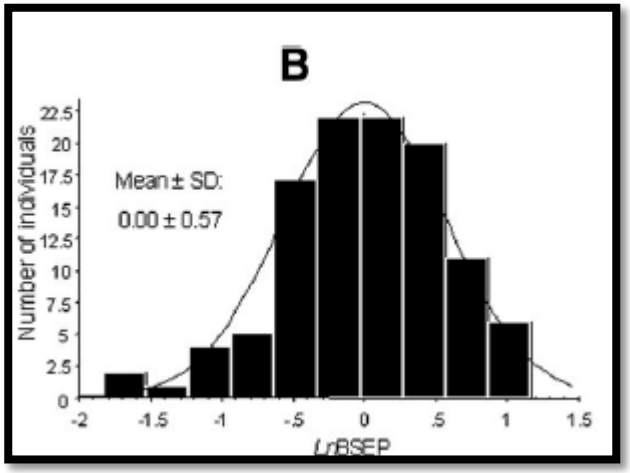
The Scope of a SimPops Was Determined As a First Step

Select Key Features of the SimPops

1. Species
2. DILI mechanisms to include
3. Population characteristics
   - Healthy volunteers or disease patients?
   - Any demographic focus (i.e. ethnicity, age subgroup, etc.)?
4. Size of the SimPops
   - Target size is usually ~200-300 individuals
   - Tradeoff between including high number of individuals and computation time to run the SimPops
5. Define endpoints for successful sample population generation
   - Identify available outcome data

Understanding Available Data Was Necessary for Constructing the SimPops

- Creation of a SimPops requires an understanding of *which characteristics are likely to be variable among a population and how those relate to parameters and groups of parameters in DILIsym*
  - e.g., protein expression is likely to vary while protein affinity for substrate is not; this means a transporter V_{max} should be in a SimPops while a transporter K_m should not
  - Some parameters can represent the variability in a larger system



Meier *Hepatology* 2006

	Before fenofibrate			
Bile acid	Mean	SEM	10th[a]	90th[a]
CDCA	256.9	29.5	16.4	610.9
TCDCA	92.9	8.8	12.7	268.5
GCDCA	745.9	54.2	169.8	1,694.2
CA	176.2	27.1	2.6	403.2
TCA	113.7	15.8	8.1	269.5
GCA	198.4	16.9	29.8	461.5
UDCA	106.2	7.9	13.6	269.1
TUDCA	6.1	1.2	0.00	13.3
LCA	17.6	1.3	4.4	34.3
TLCA	19.1	1.1	BLQ	32.3
GLCA	18.4	1.7	4.6	35.0
LCA-S	7.0	0.6	BLQ	14.4
DCA	393.1	24.6	102.9	782.4
TDCA	35.1	3.2	4.5	81.6

Trottier *Clin Pharm Ther* 2011

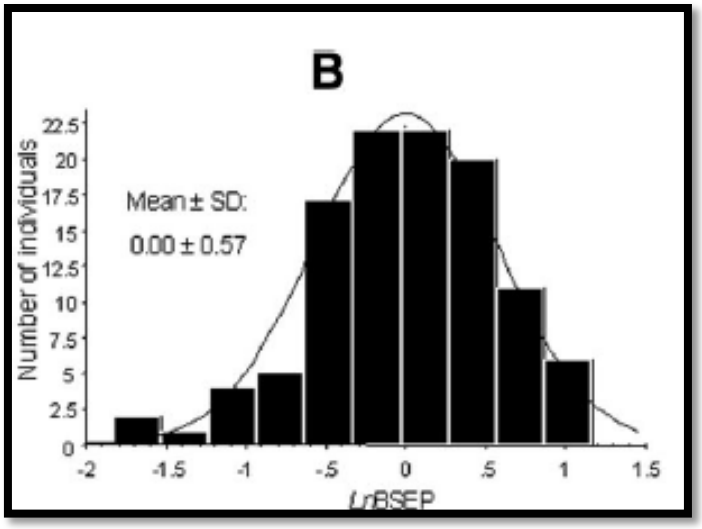
- In order to construct a SimPops, two kinds of data can be used
  - Data representing the range of the variable parameter
  - Data representing the potential range of outcomes for a population (toxicity, bile acid profiles, etc.)

Parameters to Vary in the SimPops Were Selected Based on Literature Review

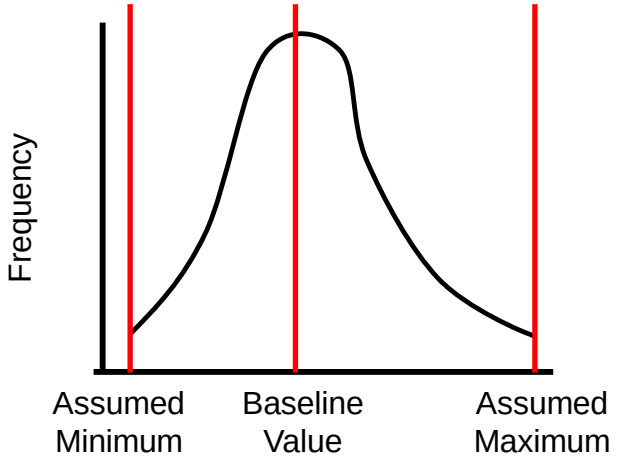
- Determine parameters to be varied based on the scope of the SimPops and available data
  - If the size of your SimPops is limited by computational power, you will need to compromise on the number of parameters included
    - Focus on the primary purpose of the SimPops
  - Gather available information on the distribution for each parameter
    - Parameter distributions defined based on reported ranges or distributions when available
    - Parameter distributions assumed when no reported information on the variation is available
      - Use reasonable assumptions for CV or standard deviation
- To make predictions with parameter values that are extremely unlikely (at the far tails of the distribution), weighting of simulated patients and very precise data may be needed
  - **This is different than predicting an unlikely event based on a combination of parameter values**

Methods Used for Determining Parameter Distributions for SimPops

- Published or internal data can be used to determine range and distribution of parameters
  - Example: BSEP protein assay can be used to determine likely distribution for canalicular V_{max} parameters
  - Care should be taken when extrapolating mRNA/gene data; DILIsym parameters depend on protein activity, which may or may not correlate
- If no data exist, parameter ranges can be assumed
  - Can choose a maximum and minimum value based on the baseline parameter value in DILIsym
  - Can choose a standard deviation based on desired amount of variability
  - 50% +/- as max/min representing 2.5 SDs from the mean tends to be a good starting point (SD of 20% assumed)
  - ***All the distributions for DILIsym SimPops can be found in the SimPops documentation, including parameters where distribution was assumed***



Meier *Hepatology* 2006



Some Basic Methods Used for Creating SimPops

1. Genetic algorithm method
   - Focuses parameter distribution on known outcomes (e.g. ALT elevation distribution, PK profile, BA plasma concentrations)
2. Known distribution method
   - Focuses on creating individuals based on adherence to a known population distribution of the parameters
3. Combination of both methods
   - Includes generation of initial, starting simulated population with known distribution bias, followed by the genetic algorithm fitness function
   - Allows parameters to adhere to defined distributions (to some degree) while also ensuring outcomes are reasonable for simulated individuals
4. Method depends on data available and confidence in those data

Generating SimPops by Sampling from Distribution for Each Parameter

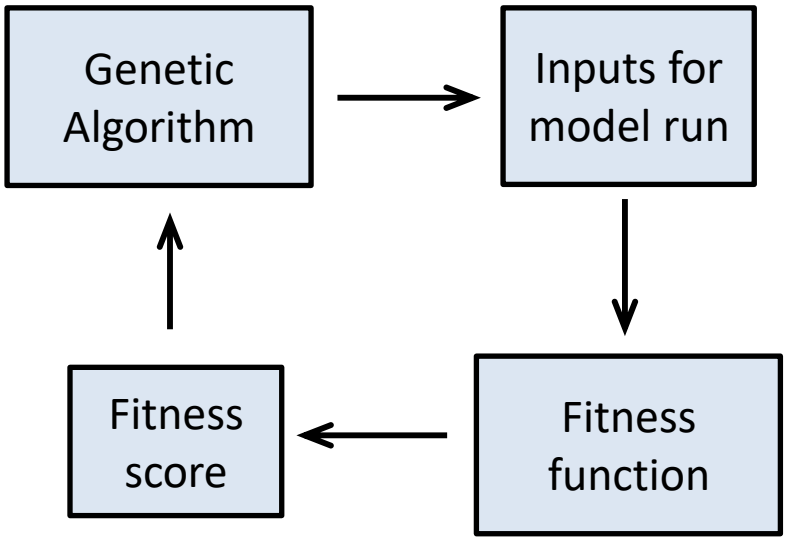
- Create individuals based on adherence to a known or assumed population distribution for each of the parameters
- Validate by comparing simulated outcomes with clinical data
- Curate, if necessary, by removing unlikely simulated individuals
- Example:
  - Assume creating a SimPops of n=300 individuals with variation in 5 parameters relevant to a key DILI mechanism

Parameter 1	Data available describing distribution
Parameter 2	Data available describing distribution
Parameter 3	Data available describing distribution
Parameter 4	No data available on distribution, assume parameter distribution
Parameter 5	No data available on distribution, assume parameter distribution

  - Generate n=300 individuals for each parameter by sampling from the known or assumed distribution for each parameter
  - Validate with outcome data, curate if necessary

Generating SimPops with Genetic Algorithm Method

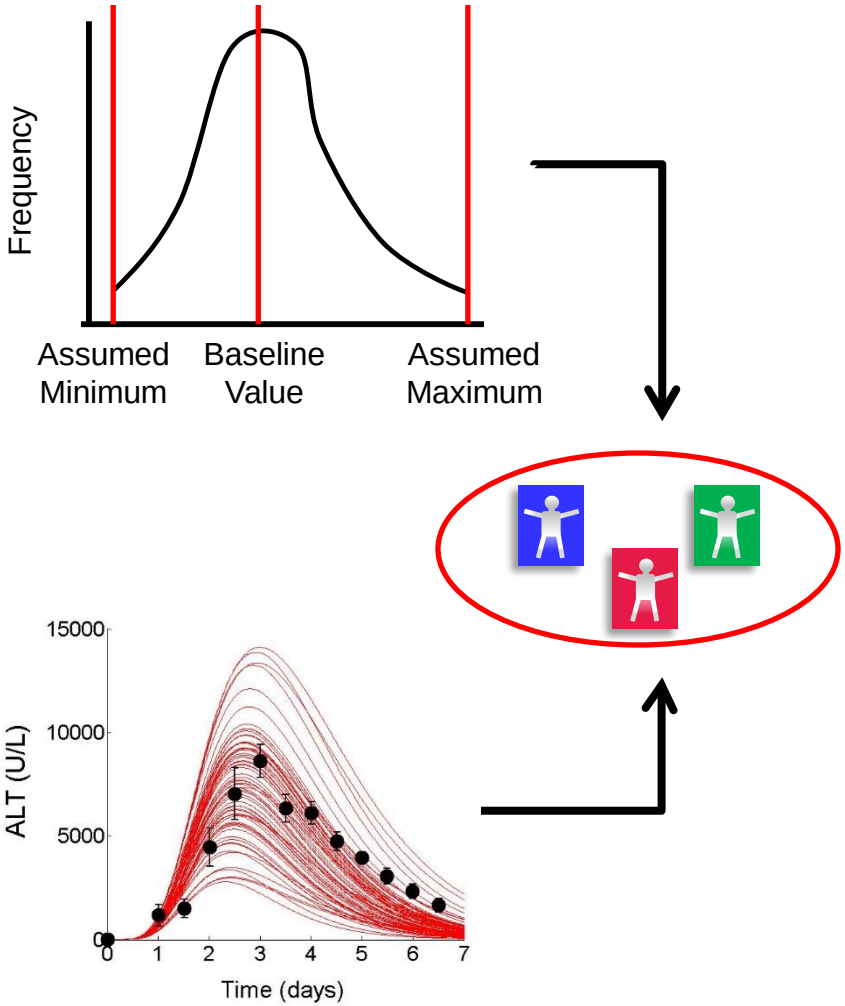
- Genetic algorithm loops through the following components:
  - Initial population generation (GA)
  - Fitness score generation (fitness function)
  - Next generation production (GA)



- Fitness function compares model inputs and model outputs with data
- Focus parameter distribution on known outcomes (ex. serum bile acid profiles)
- May be limited by computation time required
- May lead to odd parameter distributions

Combining Outcomes Data with Parameter Distribution Information for Selection

- The likelihood of occurrence of a particular simulated individual may be as important as their simulated outcomes
- Incorporate both data and distribution into a fitness function
  - Score for data can be produced by least-squares calculation
  - Score for probability can be produced by product of z-scores for each parameter
  - Sum of these scores would be overall fitness score



Clinical Data and Simulation Results

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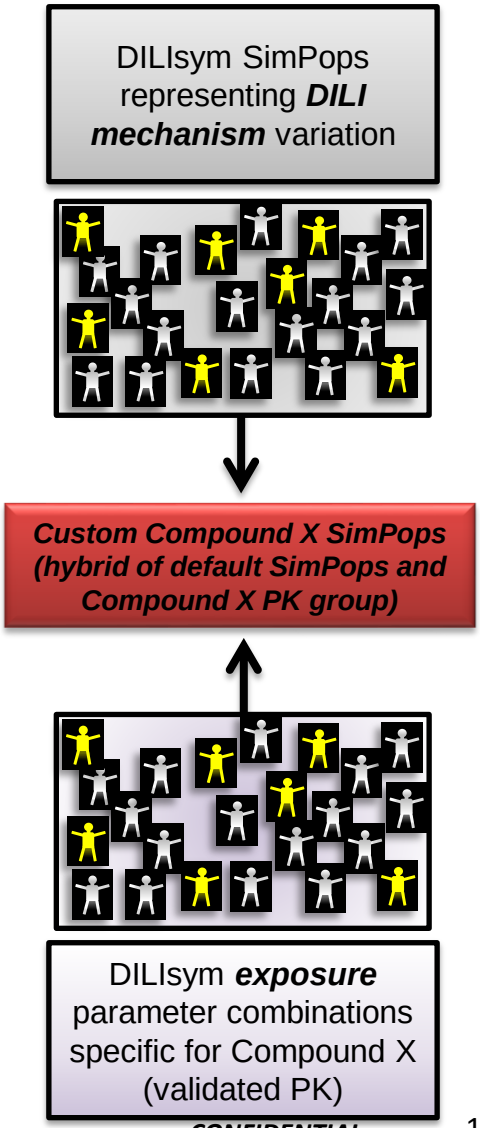
Customized SimPops Can Be Constructed to Recapitulate Compound X PK Variability

- Variability in parameters specific to exposure of a given compound can be superimposed on the existing SimPops
- Data used to optimize and validate the Compound X PK SimPops
- Parameter sets combined to create new SimPops
- PBPK parameters and associated distributions must be picked for each compound, if using DILIsym for this
  - GastroPlus PBPK outputs can also be exported and then imported into DILIsym using Specified Data to accomplish same goal more quickly
  - GastroPlus includes several special populations with respect to PK variability



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Approach Used to Generate Compound-Specific SimPops with PK Variability

- Existing SimPops in DILIsym used as a starting point
- Variability in parameters specific to compound of interest superimposed upon the existing SimPops
- Example:
  - Add PK variability for compound of interest to n= 285 Human v4A_1 SimPops
  - Select parameters to reflect clinical PK variability
    - For this example, assume variation in 3 PK parameters for compound of interest:
      - Compound X metabolism Vmax
      - Compound X renal clearance
      - Compound X biliary clearance
  - Generate n=285 individuals for the 3 PK parameters
    - Distributions based on data where available (i.e. reported variation in activity of relevant metabolic enzymes, etc.)
    - Confirm that variation in PK parameters recapitulates the range of exposure for the drug of interest
  - Combine the 3 PK parameters with the 34 v4A_1 parameters to generate the 37 parameter, n=285 compound-specific SimPops

Human v4A_1 SimPops:
34 parameters, 285 individuals

Compound PK variability:
3 parameters, 285 individuals

Compound-specific SimPops:
37 parameters, 285 individuals

Overview of SimPops Training Session

- General introduction to SimPops concepts
- Processes for constructing SimPops
- SimPops included in DILIsym v7A
- Snapshots of real world examples of SimPops applications

Select SimPops Included in DILIsym version 7A

SimPops	Abbreviated Reference Number	Species and Type	Important Notes
Human_ROS_apop_mito_BA_v4A_1	v4A_1	Human, healthy volunteers	
Human_T2D_ROS_apop_mito_BA_v6A_2	v6A_2	Human, Type 2 Diabetics	Includes disease-related variability in body mass, plasma glucose, plasma FFA, liver GSH, mitochondria function, lipogenesis, and lipotoxicity
Human_NAFLD_ROS_apop_mito_BA_v5A_1	v5A_1	Human, NAFLD (non-alcoholic fatty liver disease)	Includes disease-related variability in body mass, plasma glucose, plasma FFA, liver GSH, mitochondria function, lipogenesis, liver TG synthesis, plasma TG, liver bile acid uptake transporters, and lipotoxicity
Human_ROS_apop_mito_BA_ALT_v7A_1	v7A_1	Human, healthy volunteers	Combines DILI mechanisms with biomarker (ALT) parameters
Human_ROS_apop_mito_BA_Biogenesis_v7A_2	v7A_2	Human, healthy volunteers	Includes mitochondrial biogenesis (adaptation) parameters added to the v4A_1 SimPops
Dog_ROS_apop_mito_v3B_3	v3B_3	Dog, healthy	Beagle
Mouse_ROS_apop_mito_v3B_4	v3B_4	Mouse, healthy	Data from various strains used but most like C57Bl6
Rat_ROS_apop_mito_BA_v4A_2	v4A_2	Rat, healthy	Data from various strains used, but most like Sprague Dawley

All SimPops and SimCohorts are described at www.DILIsymHelp.com and within the Help Menu of the SimPops Feature

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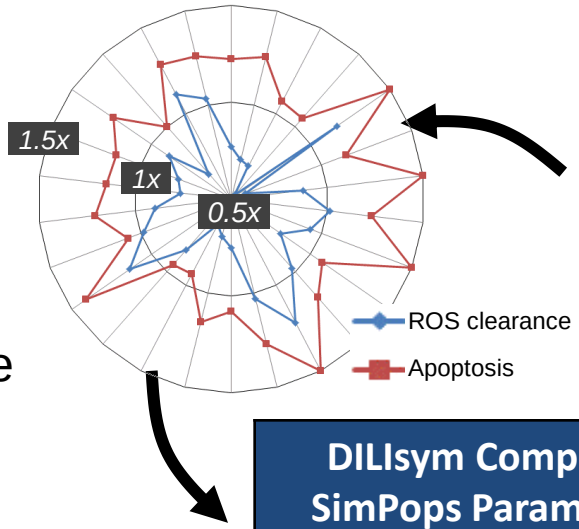
SimPops Results Used to Explore Multi-Factorial Contributions to Predicted Injury

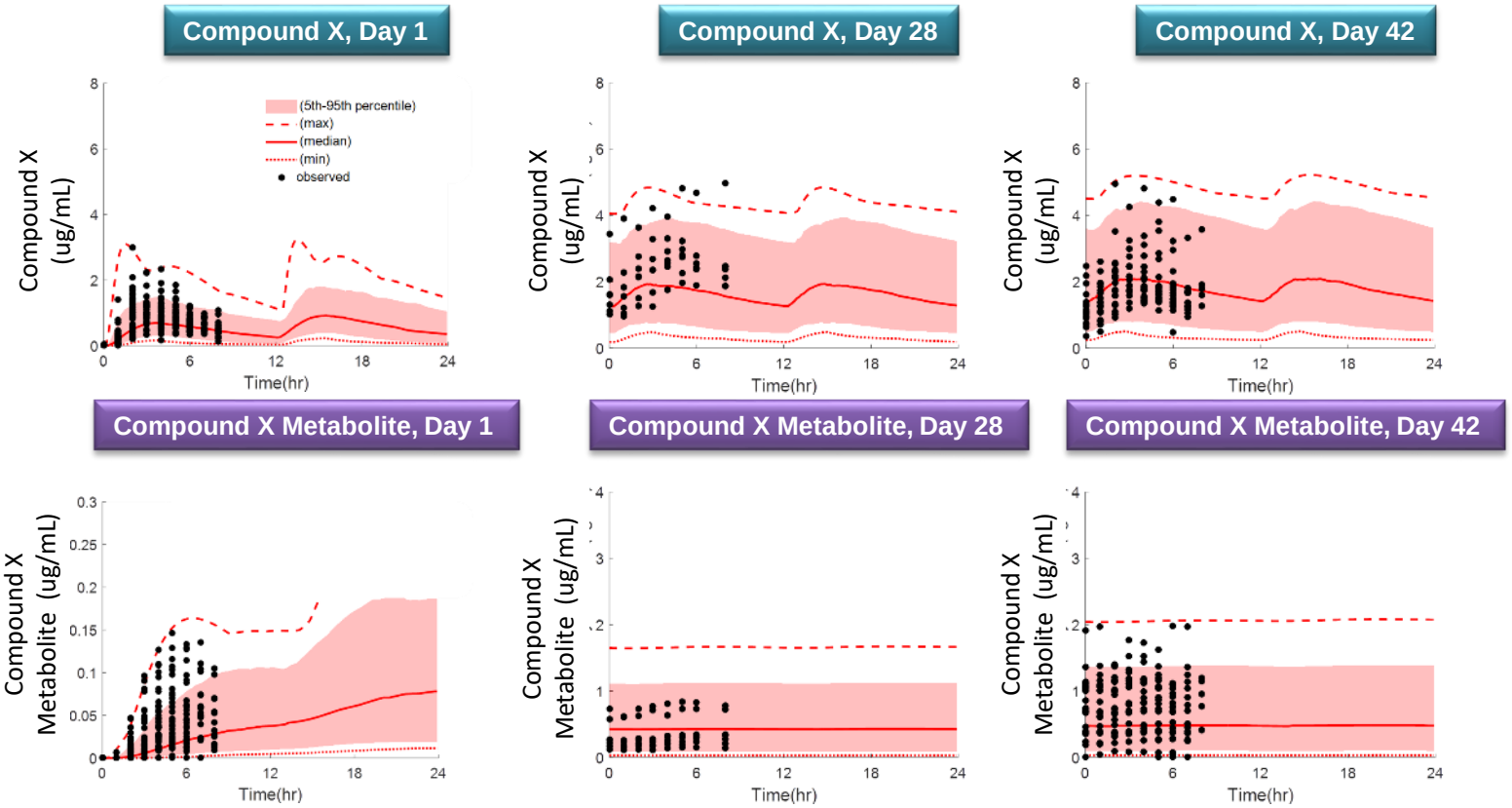
- Performed analyses with SimPops simulation results to identify factors contributing to susceptibility to injury with APAP and Compound E
  - Multifactorial contributions assessed
- Sensitive simulated healthy patients have <u>combination</u> of higher apoptosis + lower ROS clearance capacity
  - These findings can be used to guide patient selection and clinical trial protocols
- Consistent with previous efforts to identify susceptibility factors
  - Previously indicated that individuals who are nutritionally compromised are at risk



DILIsym Compound E Project SimPops Parameter Categories
Body weight
ROS clearance and production
Caspase-mediated apoptosis
Glutathione concentrations
Glutathione synthesis
Mitochondria function
ATP-dependent necrosis
Hepatic regeneration
Acetaminophen metabolism

Custom Compound X PK SimPops Covers Observed Plasma Concentration Ranges

- Custom SimPops compared to data from studies several Compound X clinical studies
- Observed concentration ranges for Compound X and Compound X metabolite recapitulated by PK SimPops; some profiles extend beyond max and min values measured (by design)



Simulation Results and Clinical Data

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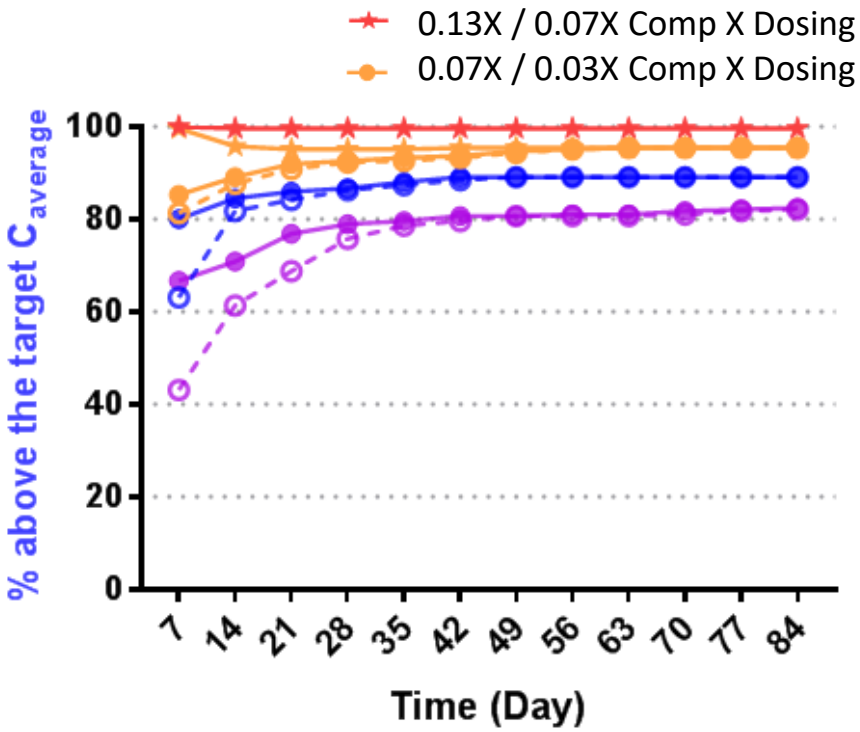
Prospective Doses Identified Showing Good Compound X Exposure

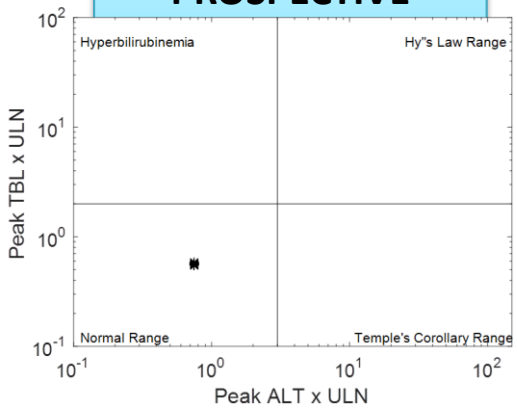
- The percentage of the simulated population who achieved the target Compound X $C_{average}$ at the steady-state increases with increasing daily maintenance dose
  - Two protocols taken forward for safety simulations
- The higher the loading dose, the greater percentage of the simulated population achieving the target during the first few weeks



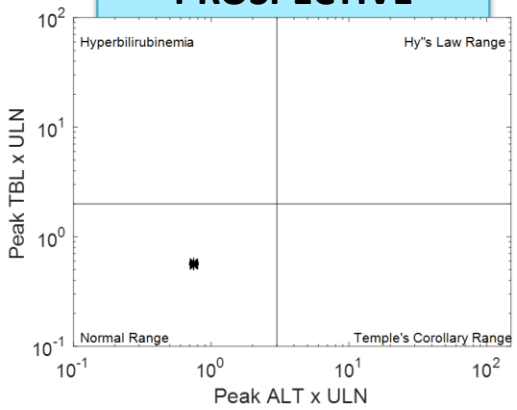
No Hepatotoxicity Predicted for Additional Prospective Clinical Protocols

- Dose dependent DILI frequency and severity predicted for Compound X – prospective dose levels clean
- Severity of response not appropriate to consider
  - Clinical stop protocol not included in simulations
  - DILIsym does not yet represent some likely key adaptation mechanisms like mitochondria biogenesis

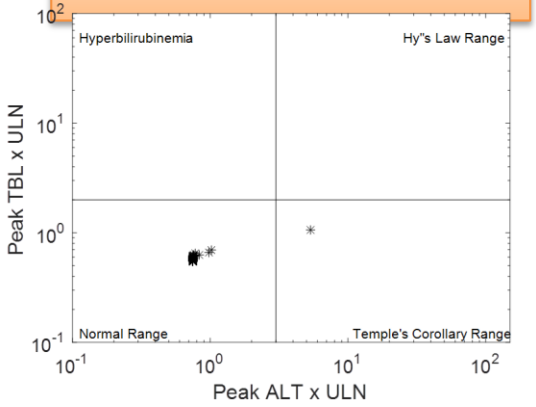
0.07X/0.03X Compound X Dosing **PROSPECTIVE**



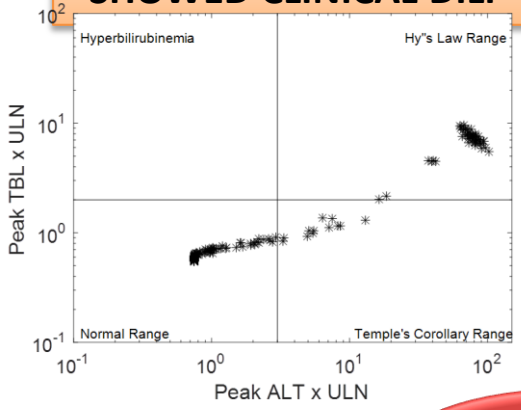
0.13X/0.07 Compound X Dosing **PROSPECTIVE**



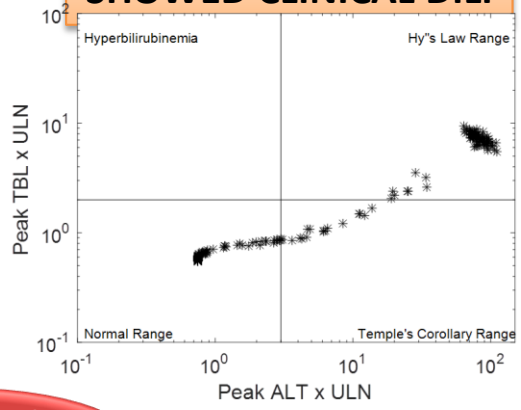
0.3X Compound X Dosing **SHOWED CLINICAL DILI**



0.5X Compound X Dosing **SHOWED CLINICAL DILI**



1X Compound X Dosing **SHOWED CLINICAL DILI**



Simulation Results

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No clinical stop protocol

Frequency of Simulated Compound G Hepatotoxicity Generally Consistent with Clinical Data

- Compound G effects simulated in SimPops
- ALT > 3X ULN predicted in 0 – 12.6 % of population administered 1X – 2X doses over multiple weeks
  - Covers clinically observed ALT > 3X ULN frequencies of around 2-3%
  - Simulated time to reach ALT > 3X ULN was similar to observed timing
- Low frequency of ALT elevation predicted with shorter term 2X dosing
- No ALT elevations predicted with alternative treatment protocol, consistent with clinical data

Compound G

Protocol	Peak ALT > 3X ULN*	
	Observed	Simulated**
2X BID 12 weeks	2.5-3.5%	12.6% (36/285)
1.5X BID 12 weeks	No Data	2.1% (6/285)
1.25X BID 12 weeks	No Data	0.7% (2/285)
1X BID 12 weeks	1-2%	0/285

*Upper limit of normal (ULN) in DILIsym is 40 U/L
**SimPops Human_ROS_apop_mito_BA_v4A_1 (n=285) used
***18% of subjects experienced ALT elevation > 2X ULN
†ALT elevation > 2X ULN predicted in 3.2% of simulated individuals

- Simulations reasonably captured frequency of Compound G hepatotoxicity across multiple clinical protocols with different doses and duration and validate DILIsym representation of Compound G

Simulations of Compound H (New Drug Candidate) in DILIsym SimPops Up to 0.64X BID Showed No Liver Injury Responses

- Compound H effects simulated in SimPops
  - Incorporates inter-individual variability in parameters related to toxicity mechanisms
- ALT > 3X ULN predicted in 0% of population administered **clinically proposed dose of 0.43X BID**
  - 0.64X BID simulation also showed no responders
- Low frequency of ALT elevation predicted with 0.86X and 1.26X BID doses
  - ALT > 3X ULN predicted in 0.7% and 7.4% of the population, respectively

Compound H

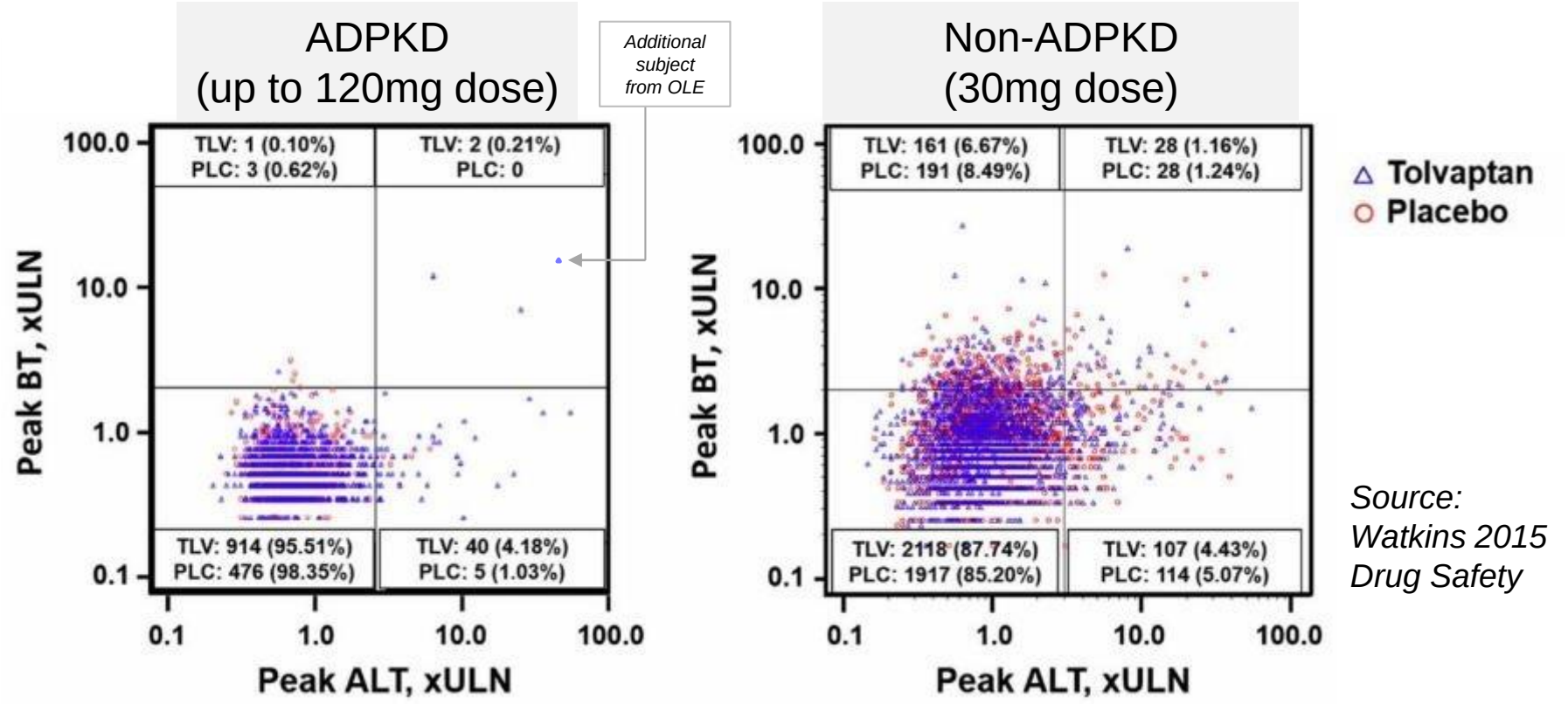
Protocol	Simulated* ALT > 3X ULN**
0.43X BID 12 weeks	0/285
0.64X BID 12 weeks	0/285
0.86X BID 12 weeks	0.7% (2/285)
1.26X BID 12 weeks	7.4% (21/285)

*SimPops Human_ROS_apop_mito_BA_v4A_1 (n=285) used for simulations

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

Lixivaptan Project Example - Comparator

- Tolvaptan has shown DILI in clinical trials for ADPKD



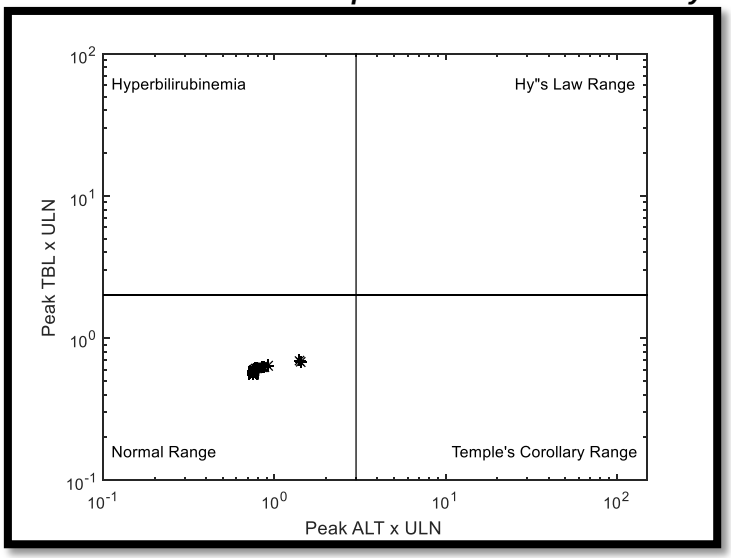
Source: Watkins 2015 Drug Safety

- The long-lived metabolite of tolvaptan DM-4103 may contribute to DILI

Lixivaptan Project Executive Summary

- Simulations of lixivaptan dosing in custom SimPops of 285 simulated individuals with exposure variability showed no ALT elevations (0/285 >2X ULN) at 200/100 mg BID dosing
- The DILIsym results suggest that lixivaptan is likely safer than tolvaptan
  - Tolvaptan had significant ALT elevations at its clinical dose (simulated and clinically observed); lixivaptan simulations predict none

Simulated 200/100 mg dosing over 12 weeks in Custom SimPops of 285 with PK variability



Simulation Results

DILIsym Services

A SIMULATIONS PLUS COMPANY

Contribution to Predicted ALT Elevations in Simulated Human Population

DILI Mechanism	Solithromycin	Telithromycin	Erythromycin	Clarithromycin
Mitochondrial Respiration Inhibition	**Predominant**	None	None	**Predominant**
Oxidative Stress	None	None	Minor	None
Bile Acid Transporter Inhibition	**Minor**	**Predominant**	**Predominant**	Minor

Data presented at Nov 4 2017 anti-infective Ad com