WebEx Tools will be Used to Record this Training Session

 Recording meeting for future reference and members not able to attend

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DILIsym [®] Training Agenda –	
September 26, 2013	
• 8:30 AM – Introduction and goals	
-DILIsym [®] overview and highlights	
-Model architecture notes • 8:45 AM – Biomarker analysis example	
• 8:45 AM – Biomarker analysis example • 9:45 AM – Break	
• 10:00 AM – Biomarker analysis example	
• 11:00 AM – MITOsym [™] overview and introduction	() Raise Hand Audio
• 11:30 AM – Lunch	
• 12:30 PM – Bile acid transport inhibitor example DILIsym [®]	🖵 Chat
• 1:30 PM – Break	
• 1:45 PM – Bile acid transport inhibitor example	
• 2:45 PM – Discussion and questions	
• 3:00 PM – Training concludes	
 DILI-sim modeling team is available for questions 	
	Send to: Brett H (Host & Presenter)
Institute for Drug Safety Sciences 🔟 CONFIDENTIAL 3	Select a participant in the Send to menu first, type
	chat message, and send
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THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

DILIsym® v2B In-depth User Training

September 26, 2013

Lisl Shoda, Yuching Yang, Kyunghee Yang Brett Howell, Scott Siler, Jeff Woodhead

*DILIsym[®] is a registered trademark, and MITOsym[™] a trademark, of The Hamner Institutes for Health Sciences for computer modeling software and for consulting services.



Please note: this presentation is being recorded

DILIsym[®] Training Agenda – September 26, 2013

8:30 AM – Introduction and goals

- -DILIsym[®] overview and highlights
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Goals for the DILIsym[®] v2B In-depth User Training Session

Participants should understand the following general concepts:

- The conceptual model architecture of DILIsym[®] v2B
- The concept of "translatability" as it applies to DILIsym®
- Use of DILIsym[®] for the retrospective interpretation of liver injury associated with clinical ALT signals
- Parameter selection for the non-mechanistic representation of hepatocyte necrosis
- Intended applications for MITOsym[™] v1A, a model of mitochondrial function
- Using in vitro transporter inhibition data to parameterize DILIsym[®] and make predictions about the potential hepatotoxic effects of inhibitors on humans and animals

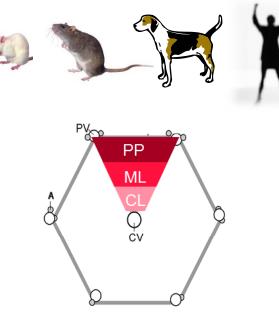


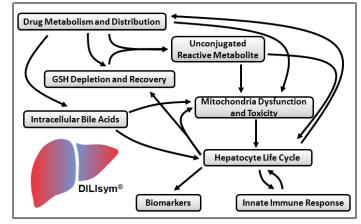


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DILIsym[®] v2B Overview

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





Hepatotoxicity exemplars

- Reactive metabolite mediated
 - Acetaminophen
 - Methapyrilene
 - Furosemide
 - Aflatoxin B1
- Mitochondrial dysfunction
 - Etomoxir
 - Buprenorphine
- Bile acid transporter inhibition
 - Glibenclamide
 - CP-724714
- Single, multiple dose protocols
- Single, combination drug protocols

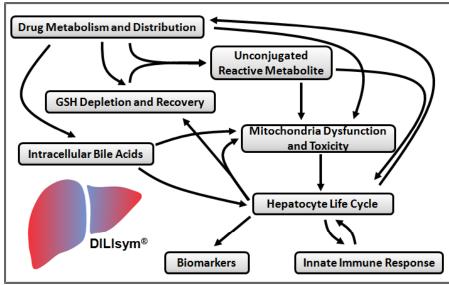
Compartment-based modeling

- >480 state variables
- 'Form to function' connection
- Ordinary differential equations
- Alternative mathematical approaches are possible
- Simulations can be run using code or GUI developed in house





Highlights of DILIsym[®] v2A



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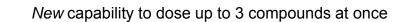
- Added direct mitochondria toxicitymediated hepatocellular necrosis
- Added bile acid-mediated toxicity hepatocellular necrosis
- Expanded representation of innate immune contributions to injury and recovery
- Expanded number of represented biomarkers of hepatocellular injury
 - Circulating (e.g., mir-122)
 - Hepatocellular (e.g., triglyceride)



- Introduced additional exemplar compounds for exposure-related toxicity
 - Etomoxir
 - Buprenorphine
 - CP-724714
- Additional SimPops[™], capturing impact of variability in key pathways
- Expanded capabilities of GUI interface



Expanded Capabilities and Features of DILIsym[®] v2A



- W, X, and Y; v1A included APAP, X, and NAC
- NAC representation still available
- New Compound Y option includes a simple, two compartment PK model representation
- Drug and Species parameters are now split into two separate value sets
 - Easier cross-species predictions
 - Improved clarity on what parameters apply to the biology versus the intersection of the drug and the biology
- New Output Table feature allows for easy calculation of Max, Min, AUC, Mean, and other metrics
- New Parameter Sweep option allows GUI users to sweep across a range of values for a given model parameter
 - Includes all model parameters; dose sweeps and sensitivity analyses possible
- *New* 2-Parameter Sweep option (MATLAB code version only)
- New Load/Save options for GUI results
- *New* Override protection for standard drug and species parameter sets (GUI version only)
- Data Comparisons include many more data sets and new plot options
- Caloric intake is now included for mitochondria toxicity and bile acid homeostasis; the role of caloric intake will continue to expand
- *New* 'events' feature avoids skipping discrete events, regardless of maximum step size
 - Compound W, X, and Y doses, caloric intake (meals), and mechanistic interventions included
- Added dog optimizations and capabilities
- Streamlined code base
 - No separate algebraics file
 - ODE file and many Excel and GUI files are now automatically called
- Expanded Zotero reference database (contact us for real-time access)



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of NORTH CAROLINA

DILIsym[®] Updates for version 2B

- Newly added functional model of bile acid homeostasis for the rat
- Additional SimPops[™] population samples
 - Relevant to mitochondrial dysfunction and bile acid homeostasis
- Faster, more efficient simulations
- Various bug fixes and GUI improvements
 - Semi-log plotting capability
 - Log sweep capability for parameter sweeps
 - Many others







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DILIsym[®] v2C Includes Changes for Multiple, Simultaneous Mechanisms of DILI

- Newest version of model released in September 2013
- Primary update:
 - Testing of v2B with multiple mitochondrial dysfunction mechanisms alerted DILI-sim team to changes that needed to be made for multi-hit simulations
- DILI-sim team recommends that members download and use v2C for future work, to the extent possible
- Changes do not affect simulations where any single mechanism for DILI were selected, or where one mitochondrial dysfunction mechanism or less was selected

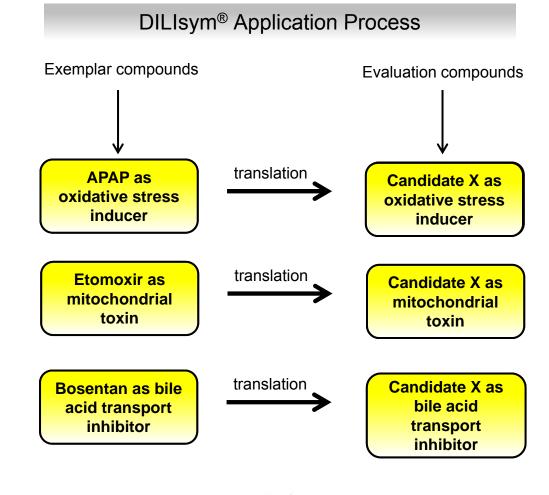






DILIsym[®] Architecture – Translation from Exemplar Compounds to Compounds of Interest

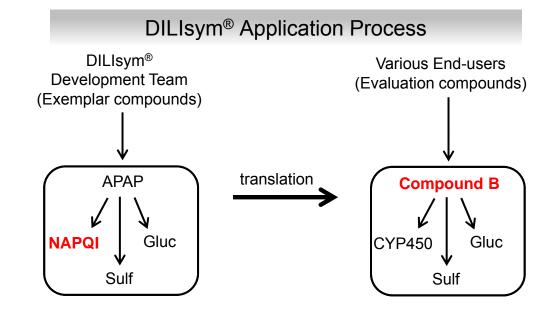
- The value proposition of DILIsym[®] lies in its ability to translate to compounds NOT used to build it
- This requires end-users with evaluation compounds to either have an idea of what mechanisms of hepatotoxicity might be in play or conduct hypothesisbased modeling
- Multiple, concurrent mechanisms of hepatotoxicity can be used and are being explored





DILIsym[®] Architecture – Using the Mechanism Selection Tool

- The mechanism selection tool allows the end-user to select an existing mechanism in the DILIsym[®] model
- Importantly, the tool also allows the mechanism to be applied anywhere in the metabolism tree
- The user can also apply multiple mechanisms to the same chemical species and different mechanisms to different levels of the tree
 - Parent and metabolite with same mechanism
 - Parent and metabolite with different mechanisms







DILIsym[®] Architecture – Using the Mechanism Selection Tool in the GUI

- The mechanism selection tool allows the end-user to select an existing mechanism in the DILIsym[®] model
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 - Parent and metabolite with same mechanism
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Species	RNS-ROS productio	n ATP utilization	Direct necrosis	BSEP/NTCP inhib	Pyruvate ox inhib	Fatty acid ox inhib	ETC inhib	Mito ATP synth inh	ib Mito uncoupler	1 Mito uncoupler	2 MPT initiator
Compound W											
Compound W metabolite A											
Compound W metabolite B											
Compound W reactive metabolite 1											
Compound W RM 1 protein adducts											
Compound W reactive metabolite 2											
Compound W RM 2 protein adducts											
Compound X											
Compound X metabolite A											
Compound X metabolite B											
Compound X reactive metabolite 1											
Compound X RM 1 protein adducts											
Compound X reactive metabolite 2											
Compound X RM 2 protein adducts											
Compound Y											

Species	RNS-ROS production	ATP utilization	Dir
Compound W			
Compound W metabolite A			
Compound W metabolite B			
Compound W reactive metabolite 1	\checkmark	✓	
Compound W RM 1 protein adducts			
Compound W reactive metabolite 2			
Compound W RM 2 protein adducts			
Compound X			
Compound X metabolite A			
Compound X metabolite B			
Compound X reactive metabolite 1			
Compound X RM 1 protein adducts			
Compound X reactive metabolite 2			
Compound X RM 2 protein adducts			
Compound Y			





DILIsym[®] Training Agenda – September 26, 2013

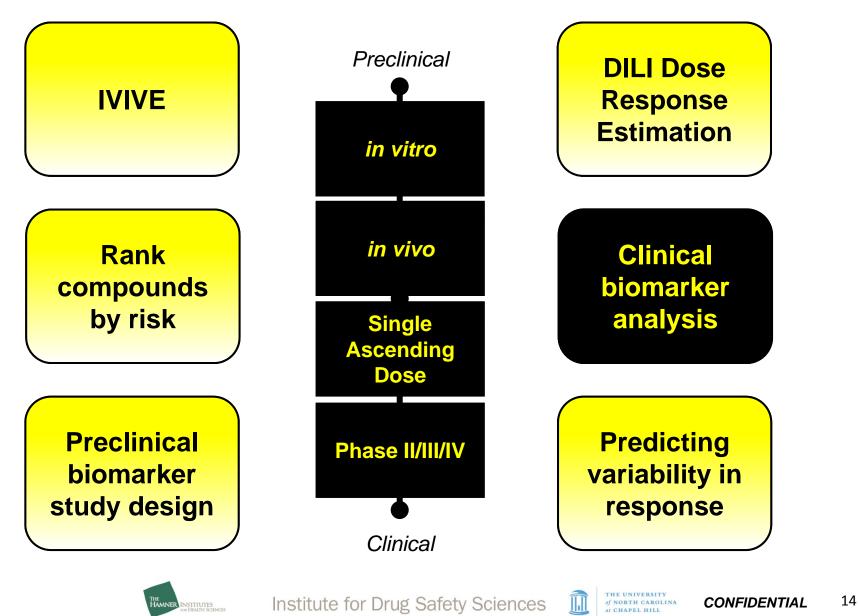
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Examples of DILIsym[®] Applications



Cleveland BioLabs Project Objectives

- Primary Objectives
 - Use simulations to infer hepatocellular dynamics associated with observed changes in liver biomarkers during CBLB502 clinical trials in normal, healthy volunteers (NHV)
 - Support Cleveland BioLabs in communications with regulatory agencies regarding CBLB502
- Secondary Objectives
 - Simulate protocols of past CBLB502 clinical trials
 - Determine impact of variability in key areas of hepatocellular dynamics (i.e., necrosis, proliferation) on generation of liver biomarkers using SimPops™, individual simulated patients with variability in key areas of hepatocellular dynamics
 - Present and/or publish findings at scientific conferences or in scientific journals



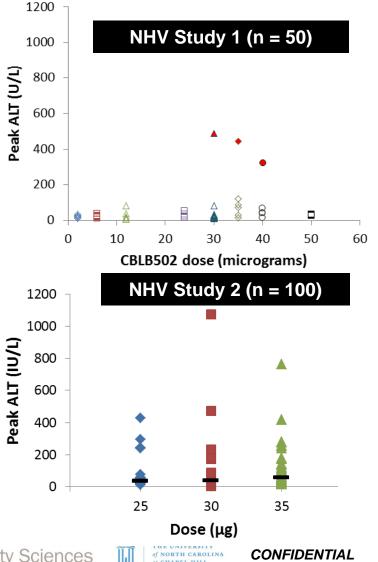


Observations of CBLB502 Clinical Data Applicable to Simulations

- Initial dose-ranging trial showed that some individuals had clinically relevant ALT increases at doses ≥30 micrograms
- Second trial included more narrow dosing range (25-35 micrograms)
- Preponderance of NHV exhibited only minor increases in liver signals
 - 70% < 1.5x ULN for ALT</p>
 - 65% < 1.5x ULN for AST</p>
- Increased ALT and AST in several NHV
 - 20% > 3x ULN for ALT
 - 26% > 3x ULN for AST
- Time to peak ALT is quite rapid (8-16 h)
 - More rapid than following APAP overdose
- AST and ALT increases are coincident
 - Implies hepatic vis a vis peripheral injury
- Slight increase in bilirubin
 - No correlation with ALT or AST

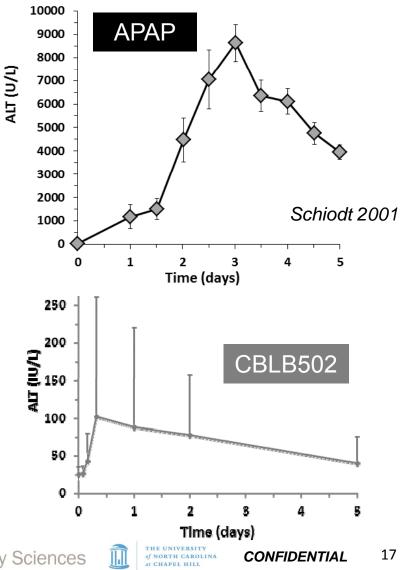
Clinical Data





Rapid Peak ALT with CBLB502 Compared with Acetaminophen Overdose

- Peak ALT after acetaminophen (APAP) overdose reported to be 48-84 h
- Peak ALT observed after CBLB502 8-24 h after dosing
 - Mean Tmax = 14.3 h
 - Median Tmax = 8 h
- Accelerated ALT Tmax with CBLB502 treatment required adjusting existing ALT sub-model



Clinical Data



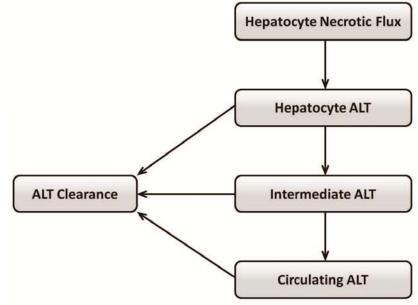
Approach for Using Simulations to Analyze **Entolimod Clinical Data**

- Approach: use ALT dynamics to infer hepatocyte loss
 - ALT content per cell based on cellular measurements
 - Boyd 1983, Remien 2012, Lindblom 2007
 - ALT release occurs upon hepatocyte necrosis
 - ALT elimination half-life based on clinical data
 - Nicoll 1997
- Initial simulations in DILIsym[®] ٠ baseline normal healthy volunteer (NHV)



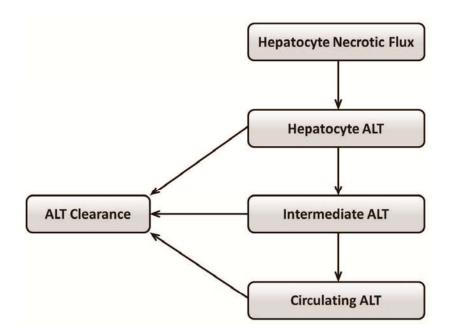






ALT Sub-Model Includes Hepatocellular Release and Clearance Dynamics

- Modeled ALT release is driven by rate of hepatocyte necrosis
- Liver, intermediate ALT pools included to provide timing of release consistent with reported clinical data
 - Primarily acute acetaminophen overdose
 - Transfer rate can be adjusted if necessary
- Model includes clearance from liver, intermediate, and plasma pools
 - Kupffer cells largely responsible for clearance from liver and intermediate ALT pools
 - Kidney largely responsible for clearance from plasma ALT pool
- AST sub-model is similarly designed

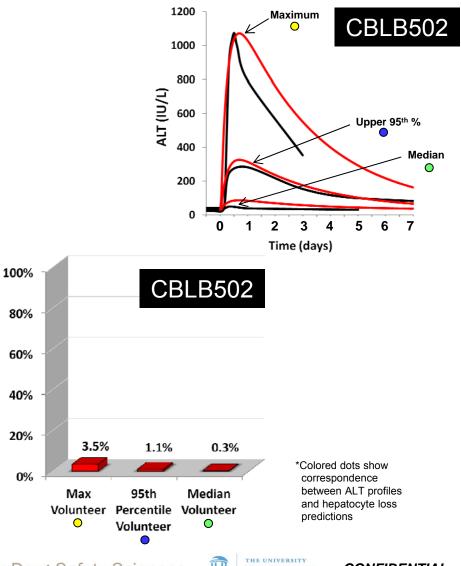






Baseline Human Simulations Indicate Minimal Hepatocyte Loss with CBLB502

- ALT time course data indicates consistent, early peaks
 - Variations in peak height observed
- Simulations performed in baseline NHV
 - Focused comparison of simulation results with Max, 95th percentile, and median volunteer ALT levels
- Simulations agree with ALT clinical data
 - By design via optimization
- Minimal hepatocyte loss associated with observed ALT profiles
 - Volunteer with greatest peak ALT predicted to have lost <5% hepatocytes



Clinical Data and Simulation Results

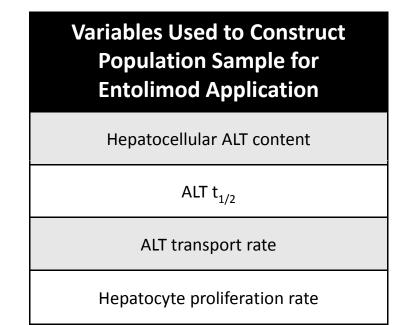


Percent of Hepatocytes Lost

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Approach for Introducing Population Variability into Simulations

- Varying parameters associated with ALT dynamics in accordance with variance described in literature
 - Remien 2012, Nicoll 1997, Portmann 1975, Prescott 1979
- Compared simulated humans (N ≈ 300) with clinical data from Prescott 1979 and Portmann 1975
 - Indirect link between ALT and necrosis
- Simulated humans used to simulate Entolimod trial protocol





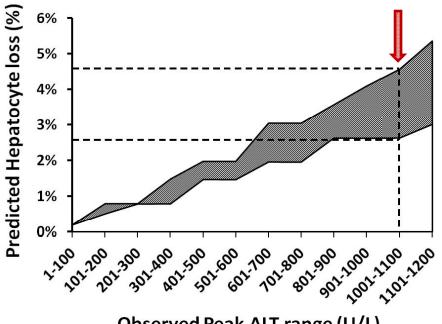




Variability in SimPops[™] Predicts Minimal Range of Hepatocyte Loss for CBLB502 Peak ALT

- SimPops[™] generated with variability in ٠ key aspects of ALT release
- ALT 1001-1100 U/L corresponds with 2.6-4.6% hepatocyte loss
- Did not simulate hepatocyte loss-ALT ٠ variability at ALT 201-300 due to systematic simulation approach

Peak ALT	Hepatocyte loss		
RANGE	LOWER BOUND	HIGHER BOUND	
1-100	0%	0.5%	
101-200	0.5%	0.8%	
201-300	0.8%	0.8%	
301-400	0.8%	1.5%	
401-500	1.5%	2.0%	
501-600	1.5%	2.0%	
601-700	2.0%	3.0%	
701-800	2.0%	3.0%	
801-900	2.6%	3.6%	
901-1000	2.6%	4.1%	
1001-1100	2.6%	4.6%	
1101-1200	3.0%	5.4%	



Observed Peak ALT range (U/L)

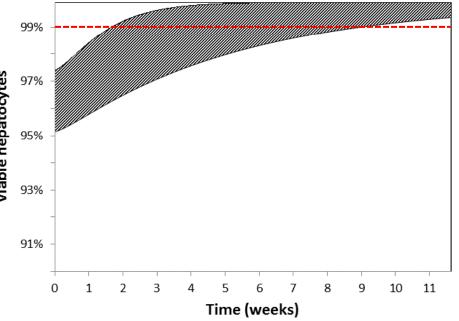
Simulation Results





Regenerative Hepatocyte Proliferation Predicted to be Complete 2-9 Weeks after CBLB502 Dosing

- SimPops[™] generated with variability in hepatocyte proliferation
- Hepatocyte restoration complete within ~2-9 stored weeks after onset of injury (median human prediction 3 weeks)
 Shaded region reflects variation in degree of injury and hepatocyte proliferative response from the SimPops™ ٠
 - from the SimPops™
 - Viable hepatocyte restoration considered complete at 99% (dashed red line)
 - Simulation results shown for maximal ALT response to CBLB502
- Hepatocyte proliferation begins with onset of injury and persists until complete regeneration
 - Simulation results plotted from nadir of viable hepatocytes until complete restoration









Evidence from Literature to Support Safety of Minimal Hepatocyte Loss with CBLB502

- Excision of 20% of liver volume in living donors is generally considered safe (Florman 2006)
 - Living donors routinely recover fully after even greater portions (40-60%) of liver are excised for adult-to-adult donations (Florman 2006, Lee 2010)
- Heparins are widely considered to be safe despite associated increases in ALT
 - Reported ALT increases after heparins comparable to observed ALT after CBLB502
 - DILIsym[®] modeling team performed comparable ALT-hepatocyte loss on published clinical data (Harrill 2012, analysis on following slides)
 - Comparable, minimal hepatocyte loss predicted for heparins and CBLB502
- Clinical correlative data from literature indicate that minimal loss of hepatocytes due to injury has little to no effect on bilirubin levels and prothrombin clotting time (Portmann 1975)





Project Summary

- Analyses based on clinical data and simulation results indicate that volunteers with ALT elevations following CBLB502 administration likely incurred hepatocyte losses of ≤5%
- The vast majority of necrotic hepatocyte loss was predicted to have occurred within the first 24 h following dosing, and recovery (restoration of 99% viable hepatocytes) times ranged from 2-9 weeks
- Based on literature review, ~15% of hepatocytes can be lost to a necrotic event without an increase in bilirubin or symptoms associated with liver injury





Application Example 1: Retrospective Analysis of Observed Liver Safety Signals

Issue

- ALT (and AST) elevations were reported in a single (few) individuals from early clinical trials
- No indications of liver dysfunction were observed in the early trials
- No mechanistic data for hepatotoxicity have been identified

Pending Decision

- Does the Company continue to advance this program?
 - Assume multiple inputs and data sets, potentially including modeling and simulation

Questions to Individual(s) Responsible for Liver Safety Assessment

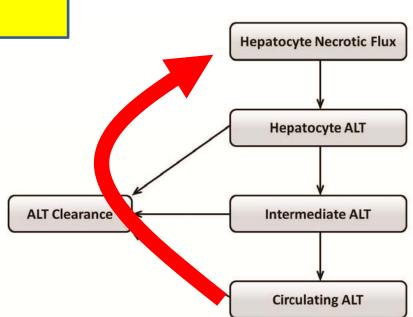
- Can DILIsym[®] be used to retrospectively interpret the observed ALT elevations?
 - What level of injury might be inferred from the reported ALT profile?
 - How much uncertainty is associated with the estimated level of liver injury?
 - What time frame of recovery would be expected for the simulated injury?





Approach for Using Simulations to Analyze Entolimod Clinical Data

- Approach: use ALT dynamics to infer hepatocyte loss
 - ALT content per cell based on cellular measurements
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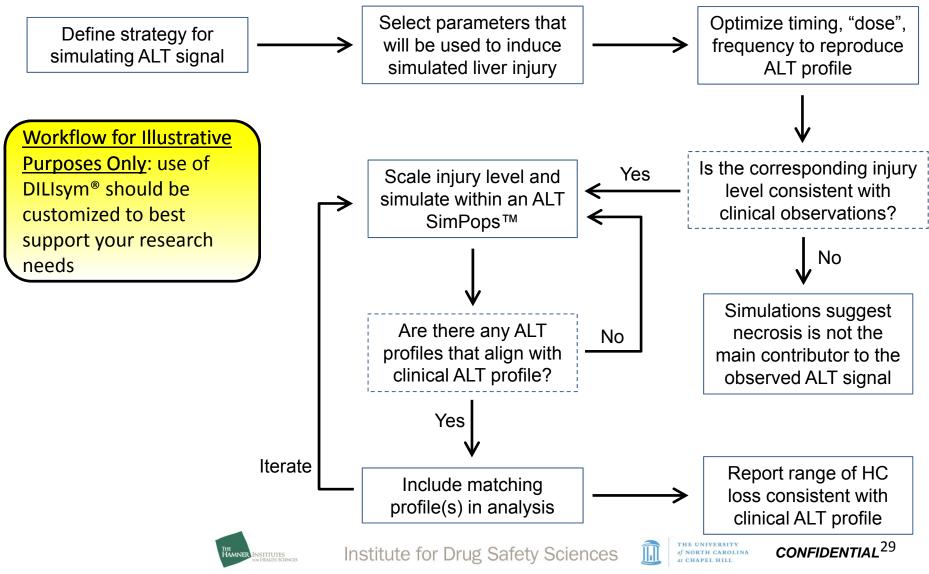
Methodological Approach for Using DILIsym[®] in Retrospective Analysis

Prospective analysis: use what is known about a compound to better understand potential hepatotoxicity (e.g., degree of necrosis, ALT, bilirubin)

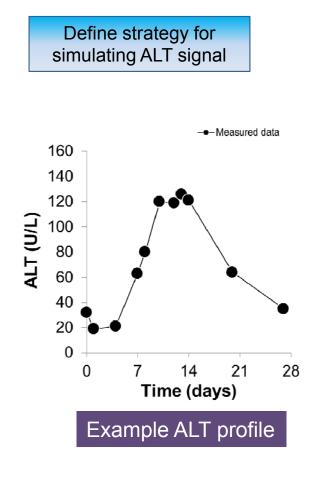
Model inputs Compound Necrosis **Mechanisms** characteristics Model outputs A simple retrospective analysis can be conducted without a detailed compound or Necrosis ALT mechanistic representation Including compound and mechanisms will result in a more Retrospective analysis: use ALT to robust analysis confirm when appropriate degree of necrosis has been simulated THE UNIVERSITY Institute for Drug Safety Sciences 28 of NORTH CAROLINA CONFIDENTIAL

CHAPEL HILL

Workflow for Retrospective Analysis of Clinical ALT Signals Using DILIsym[®]



How Can DILIsym[®] Be Used to Reproduce the Clinical ALT Signal?



- Clinical data
 - ALT elevations observed in a single individual
 - No liver dysfunction reported
 - No mechanistic data for liver signal available
- Using the DILIsym[®] baseline simulated human,
 - Assume observed ALT elevations are a result of hepatocyte necrosis
 - Apply parent compound W induces direct necrosis, to "hit" the hepatocytes and generate an ALT profile similar to the experimental data

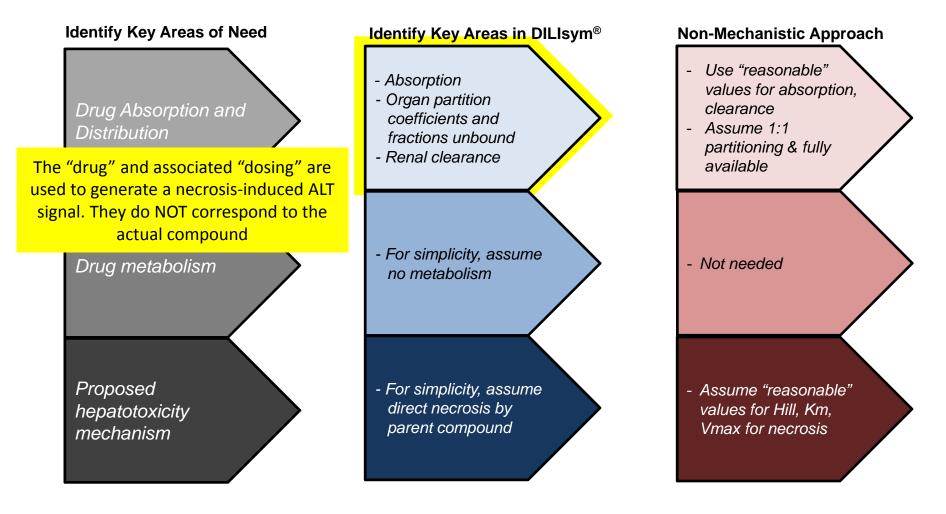






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Identifying the Inputs Needed to Reproduce the ALT Profile in DILIsym[®] (v2B)



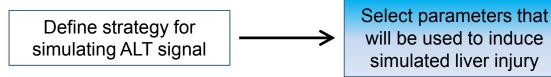


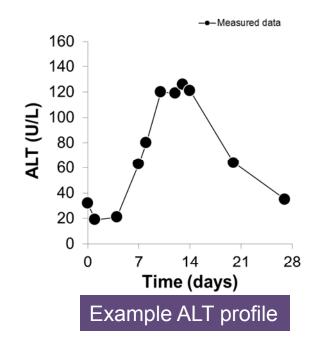
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Identification and Selection of Injury-Inducing Parameters





- Define simple Compound W PBPK
- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

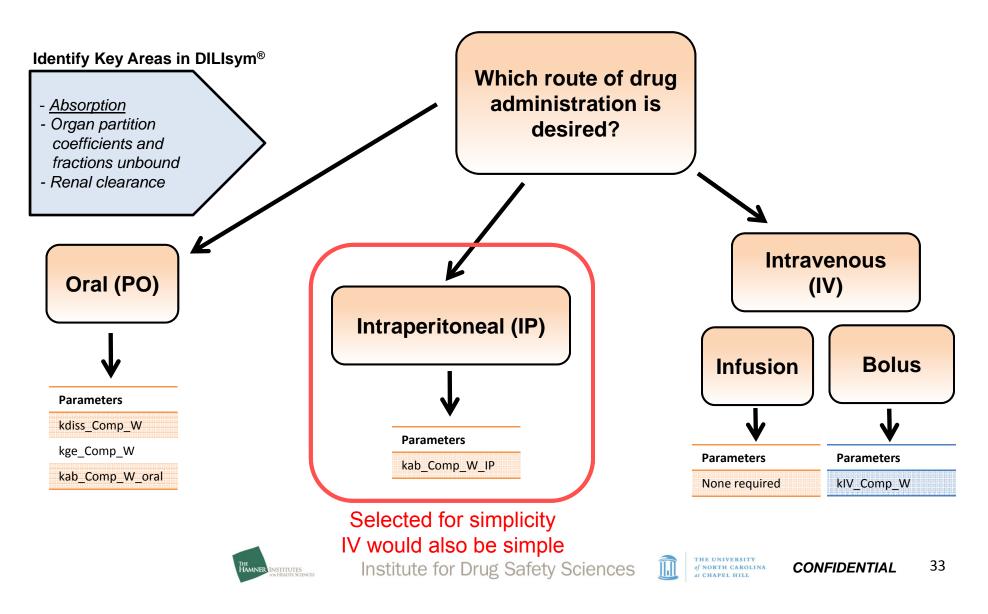
Note: Because "Compound W induces direct necrosis" is a simple stimulus & <u>its effects will be constrained to align with the observed ALT profile</u>, alternate parameter solutions are possible but not expected to impact the estimated liver injury

Clinical Data

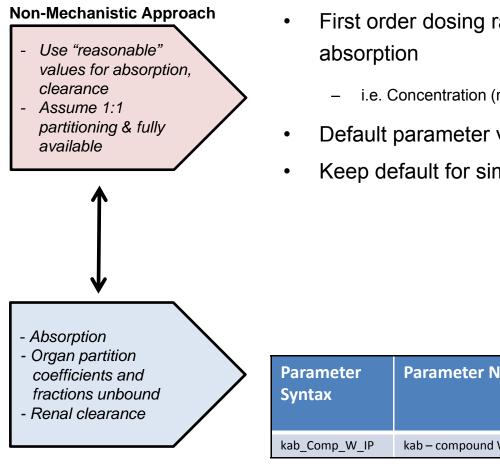


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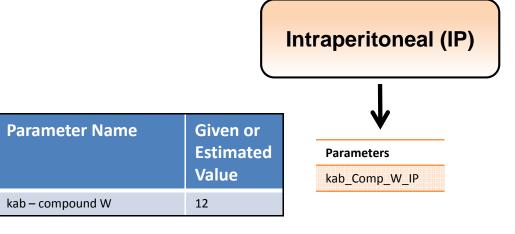
Selecting the DILIsym[®] Parameters to Use for Drug Delivery and Absorption



Determining Parameter Values for Absorption



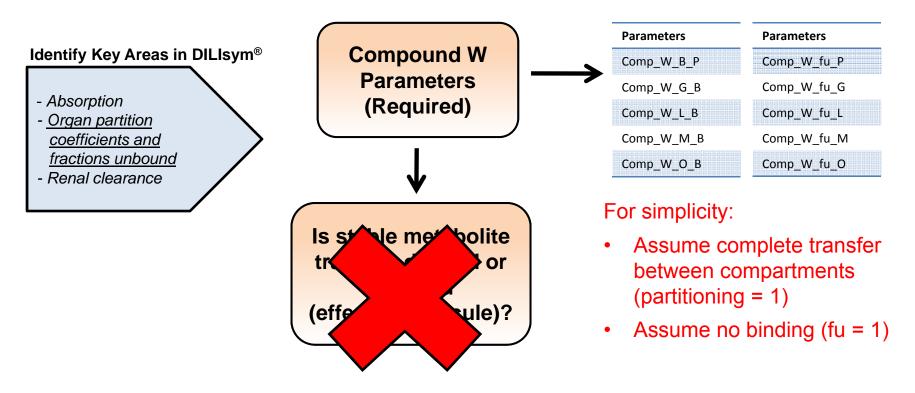
- First order dosing rate constants determine the rate of
 - i.e. Concentration (mass/volume) * Rate Constant (1/hour) = Rate
- Default parameter value is **12** (1/hour)
- Keep default for simplicity







Selecting the DILIsym[®] Parameters to Use for Drug/Tissue Partitioning and Binding



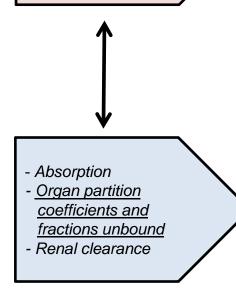




Determining Parameter Values for Tissue Distribution and Protein Binding

Non-Mechanistic Approach

- Use "reasonable" values for absorption, clearance
 Assume 1:1
- partitioning & fully available



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Parameter Name	Given or Estimated Value
Compound W blood to plasma	1
Compound W gut to blood	1
Compound W liver to blood	1
Compound W muscle to blood	1
Compound W other to blood	1
Compound W fraction unbound plasma	1
Compound W fraction unbound gut tissue	1
Compound W fraction unbound liver	1
Compound W fraction unbound muscle tissue	1
Compound W fraction unbound other tissue	1
	Compound W blood to plasmaCompound W gut to bloodCompound W liver to bloodCompound W muscle to bloodCompound W other to bloodCompound W other to bloodCompound W fraction unbound plasmaCompound W fraction unbound gut tissueCompound W fraction unbound liverCompound W fraction unbound muscle tissue

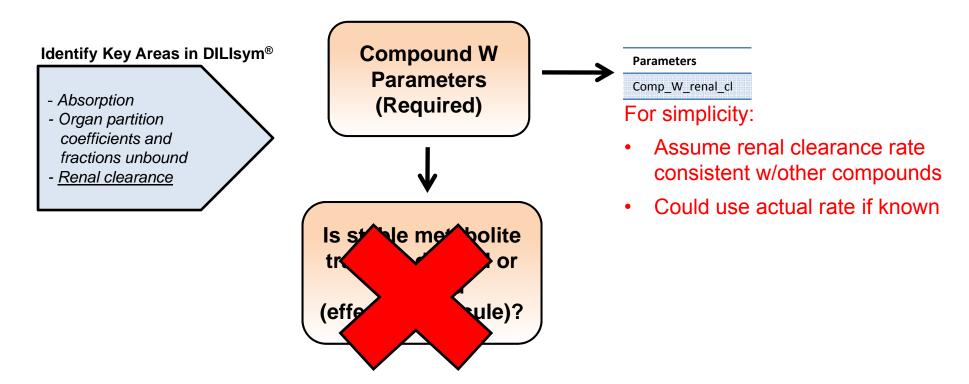
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Selecting the DILIsym[®] Parameters to Use for Renal Clearance







Determining Parameter Values for Renal Clearance

Non-Mechanistic Approach

- Use "reasonable" values for absorption, clearance
 Assume 1:1
- partitioning & fully available

- Absorption

- Organ partition coefficients and fractions unbound

- <u>Renal clearance</u>

Parameter Syntax	Parameter Name	Given or Estimated Value
Comp_W_renal_cl	Compound W renal clearance	25





Implementing Parameter Values for Compound W PBPK (1 of 2)

DILIsym v2C - 2013 Q3 Training		DILIsym v2C - 2013 Q3 Training		🛃 Drug Parameter Values-Parame 🗖 🗖 🗮 🌌
File View Results About		File View Results About		
😡 😡 🗘		🖸 🛃 🖸		۲ ک
SimSingle Setup File		SimSingle Setup File		
SimSingle Input Options	Se	SimSingle Input Options	Select 💌	Mechanism selection Drug toxicity parameters Mechanistic interventions
Simulation Time	Se	Simulation Time	Select 💌	Compound W PBPK
Species Parameters	Se	Species Parameters	Select 🔻	Compound W RM 2 PBPK
Drug Parameters	Se	Drug Parameters	Parameters_Blank_v2C	Compound X PBPK Comp X Metabolite A PBPK Comp X Metabolite B PBPK
Caloric Intake	Se	Caloric Intake	Select 💌	Compound X RM 1 PBPK Compound X RM 2 PBPK
Compound W Dosing	Se	Compound W Dosing	Select 💌	Compound Y PK Bile acid transporter inhibition constant
Compound X Dosing	Se	Compound X Dosing	Select 💌	CDCA transporter inhibition constants
Compound Y Dosing	Se	Compound Y Dosing	Select 💌	Noncompetitive inhibition constants Species identification
Solver Options	Se	Solver Options	Select 💌	
Simulate		Simulate		
Run	R	Run Output	Run in Parallel Data	Comparise
Export to Excel		Export to Exc	el Plot Ou	tput Table
		RIGHTINGEN TO THE REPORT OF TH		

Implementing Parameter Values for Compound W PBPK (2 of 2)

Parameter	Value	Units
Comp W bil cl		mL/hour/kg^0.75
Comp_W_B_P		dimensionless
Comp_W_fr_recir		dimensionless
Comp_W_fu_G		dimensionless
Comp_W_fu_L		dimensionless
Comp_W_fu_M	1	dimensionless
Comp W fu O	1	dimensionless
Comp_W_fu_P		dimensionless
u_correlation_Comp_W		dimensionless
Comp_W_fu_corr_2		dimensionless
Comp_W_fu_corr_1		dimensionless
Comp_W_fu_corr_0		dimensionless
Comp_W_G_B		dimensionless
Comp_W_L_B		dimensionless
Comp_W_mg_mol	1	mol/mg
Comp_W_mol_mg		mg/mol
Comp_W_M_B		dimensionless
Comp_W_O_B	1	dimensionless
Comp_W_renal_cl	0	mL/hour/kg^0.75
ab_Comp_W_oral		1/hour
ab_conj_Comp_W	0	1/hour
ab_Comp_W_IP	12	1/hour
diss_Comp_W		1/hour
ge_Comp_W		1/hour
IV_Comp_W		1/hour
/max_Comp_W_ab	0	1/hour
(m_Comp_W_ab	1.0000e+10	mg
_out_gut_Comp_W		1/hour
Comp_W_Km_L_B	1.0000e+10	mg/mL
Comp_W_perm	0	1/hour
Comp_W_Vmax_L_B Comp_W_Km_L_B Comp_W_perm	0 1.0000e+10	1/hour mg/mL

- Retain most default parameter values
 - Tissue distribution values set to 1
 - Fraction unbound set to 1
- Update renal clearance to selected value = 25
- Save parameter file by a new name, e.g.,
 - Parameters_Human_CompW_direct_necrosis

Parameter	Value	Units	_
Comp_W_mg_mol	1	mol/mg	1
Comp_W_mol_mg	1	mg/mol	
Comp_W_M_B	1	dimensionless	
Comp_W_O_B	1	dimensionless	
Comp_W_renal_cl	0	mL/hour/kg^0.7	E,
kab_Comp_W_oral	5	1/hour	-
kab_conj_Comp_W	0	1/hour	=
kab_Comp_W_IP	12	1/hour	-
kdiss_Comp_W	12	1/hour	
kge_Comp_W	12	1/hour	
•		•	3
	Ammha		
_	Apply		

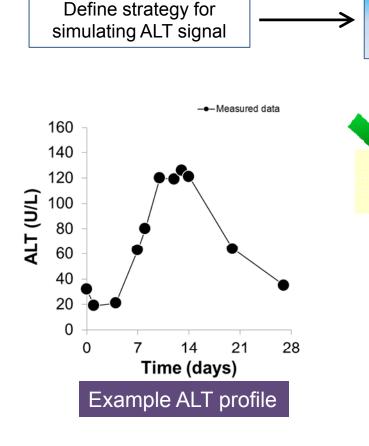
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Select "Reasonable" Parameters for Compound Induction of Direct Necrosis

Select parameters that

will be used to induce

simulated liver injury



Define simple Compound W PBPK

- Define parameters (K_m, Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & <u>its effects will be constrained to align with the observed ALT profile</u>, alternate parameter solutions are possible but not expected to impact the estimated liver injury

Clinical Data





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Implementing Drug Toxicity Parameters for Compound W

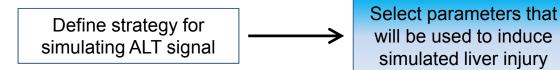
- Recall this is a dummy "drug" intended simply to induce necrosis (not simulate real drug)
- Insert "reasonable" values, e.g., as used in Cleveland BioLabs Project
 - Hill_direct_necrosis = 1
 - Vmax_direct_necrosis = 1
 - Km_direct_necrosis = 0.003
- Alternate values can be used at the researcher's discretion

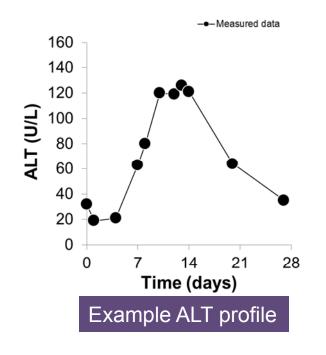
led ate used		Ag Parameter Valu Mechanism select Drug toxicity para Mechanistic inter Compound W PB Comp W Metabol Comp W Metabol Comp W Metabol Compound W RM Compound W RM Compound X PBF	tion wentions PK lite A PBPK lite B PBPK 1 1 PBPK 1 2 PBPK		r r
Drug toxicity parameters-	Parame	ters_Blank_v2C		• <mark>×</mark>	
Parameter		Value	Units		
RNS_ROS_prod_const		0	mL/hour/mol	<u> </u>	
Hill_direct_necrosis		0	dimensionless	s 👔	
Vmax_direct_necrosis		0	dimensionless	;	
Km_direct_necrosis		1	dimensionless	3	
ATP_util_Vmax		0	1/hour		
ATP_util_Km		1	mol/mL		
ATP_util_Hill		0	dimensionless	5	
	4	Apply			
		•		4	





Specify Compound W Induces Direct Necrosis





Define simple Compound W PBPK

- Define parameters (K_m , Hill, V_{max}) for induction of direct necrosis by Compound W
- Select mechanism Compound W (parent) induces direct necrosis
- Verify species selector set to human

Note: Because "Compound W induces direct necrosis" is a simple stimulus & <u>its effects will be constrained to align with the observed ALT profile</u>, alternate parameter solutions are possible but not expected to impact the estimated liver injury

Clinical Data





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DILIsym[®] Hepatotoxicity Mechanism Selection for Simply Reproducing an ALT Profile

Identify Key Areas in DILIsym®

 Absorption Organ partition coefficients and fractions unbound 	Parameter Syntax	Parameter Name	Given or Estimated Value	Units	Method of Estimation
- Renal clearance	Compound W	Mechanism for Compound W	Direct necrosis	dimensionless	Not applicable
- For simplicity, assume no metabolism					
- For simplicity, assume direct necrosis by parent compound					





Implementing Compound W Direct Necrosis

J Drug Parameter Values-Parame				x
				ъ
Mechanism selection			*	
Drug toxicity parameters				
Mechanistic interventions				
Compound W PBPK				
Comp W Metabolite A PBPK				
Comp W Metabolite B PBPK				
Compound W RM 1 PBPK				
Compound W RM 2 PBPK				
Mechanism selection-Parameters_Blank_v2	с	-		

1

- Select "direct necrosis" for the parent • compound W
- Leave all other mechanisms unchecked •

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Species	RNS-ROS production	ATP utilization	Direct necrosis	BSEP/NTCP inhib	Pyruvate ox inhib	Fatty acid ox inhib	ETC inhib	Mito ATP s
ompound W								·
ompound W metabolite A								
ompound W metabolite B								
ompound W reactive metabolite 1								
ompound W RM 1 protein adducts								
ompound W reactive metabolite 2								
ompound W RM 2 protein adducts								
ompound X								
ompound X metabolite A								E
ompound X metabolite B			100	(m)	E			E
ompound X reactive metabolite 1			F					E.
	III	Di Pilen						•

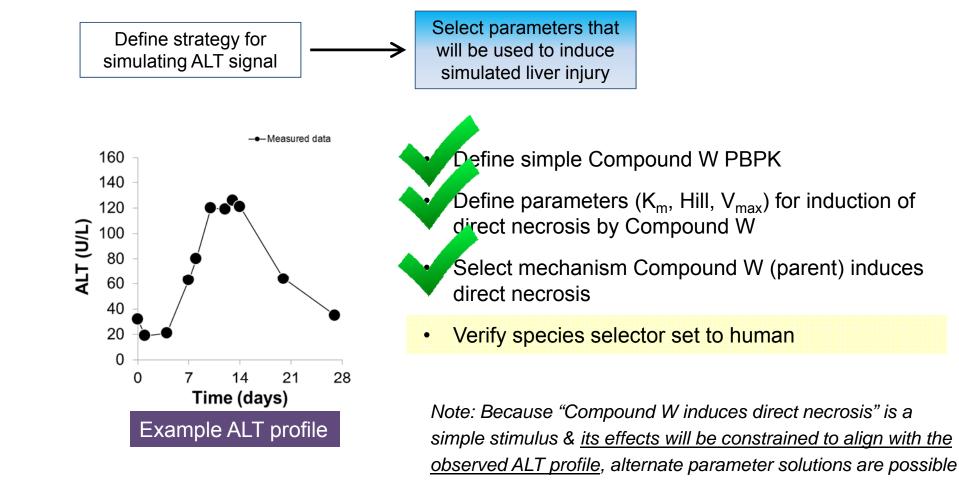
Appropriate Species Selection

but not expected to impact the estimated liver injury

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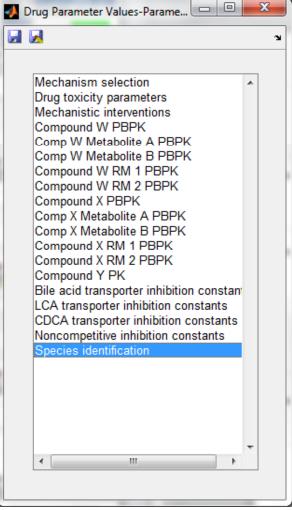
Clinical Data

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Set Species Selection for Human Simulations

- DILI simulations may be run for mice, rats, dogs, or humans
- Species is specified by number
 - **1** mice
 - **2** rats
 - **3** dogs
 - **4** humans

Param	eter	Value	Units	
pecies		4	↓ n/a	
		Apply		

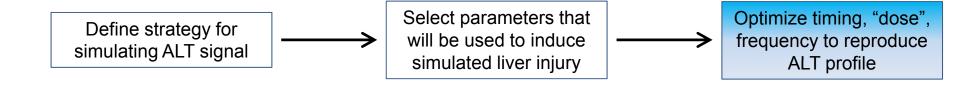


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Optimization to Reproduce the ALT Profile



Workflow for Illustrative Purposes Only: use of DILIsym[®] should be customized to best support your research needs

- Optimize for a Compound W "protocol" that reproduces the ALT profile of interest
 - Time of 1st "dose" §
 - "Dose" magnitude
 - "Dose" frequency

[§] Compound W and associated "dosing" are used to induce injury. They do NOT correspond to the real compound and therefore need not reflect the real compound protocol.



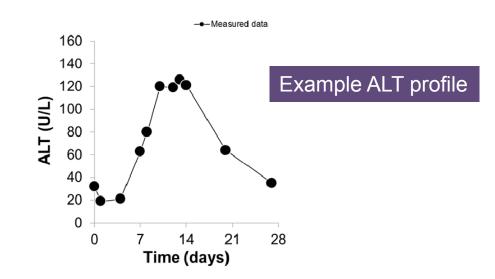
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Optimization Goal: Simulate Injury that Results in the Measured ALT Profile

- Objective is to characterize:
 - Timing of ALT elevation
 - Relationship between "dose" and ALT
- Initial set-up design
 - Short duration
 - Single "dose"
- Save Compound W dose scheme & SimSingle[™] under appropriate names



Time (days)	Time (hours)	ALT (U/L)
0	0	32
1	24	19
4	96	21
7	168	63
8	192	80
10	240	120
12	288	119
13	312	126
14	336	121
20	480	64
27	648	35

Clinical Data



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Set up an Initial SimSingle™

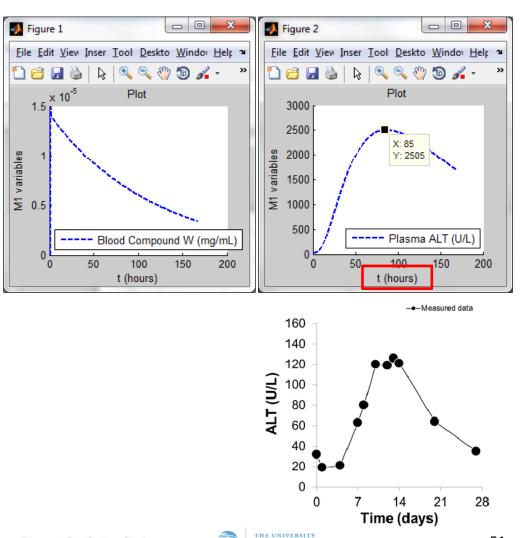
- Simulation Time
 - 1_week_Default
- Species Parameters
 - Parameters_human_specific_v2C
- Drug Parameters
 - As specified in previous section
 - Parameters_human_CompW_direct_necrosis
- Caloric Intake
 - Caloric_intake_parameters_blank_v2C
- Compound W Dosing
 - Create a new test set
 - Specify 1 mg dose, 1 total dose
- Compound X Dosing
 - Compound_X_dosing_blank_v2C
- Compound Y Dosing
 - Compound_Y_dosing_blank_v2C
- Solver Options
 - Select_Human_Sims_Solver_Options

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	JILIsym v2C - 2013 Q3 Training	Cature an Initial (
	File View Results About		۲.
	🖬 🛃 🛈	ave SimSingle™ file	
	SimSingle Setup File		
		Human Ex1 0h 1mg Training	
c v2C		Human_Ex1_0h_1mg_Training	
0_720	SimSingle Input Options	Select a short default tin	ne
tion	Simulation Time	Select human species p	arameters
<i>W_direct_necrosis</i>		Ocicet numari species p	arameters
	Species Parameters	Coloct Comp/A/ direct po	orogio
		Select CompW direct ne	CIOSIS
blank v2C	Drug Parameters	Select Calorie Intake de	fault narameter
		Select Calolle Illiake de	iault parameter
	Caloric Intake	Customize Compound V	V docina
		Customize Compound V	vuosing
	Compound W Dosing	Calact Correspond V daf	
ose		Select Compound X defa	auit parameters
	Compound X Dosing		14 4
		Select Compound Y defa	ault parameters
k_v2C	Compound Y Dosing		0.5700 dimensionless
		Select Human Solver	0.0500 hours
	Solver Options	Select_Human start_IP_Comp_W_bolus_dose_	0 hours
k_v2C		period_iP_Comp_VV_bolus_dose	40 hours
		IP_Comp_W_bolus_dose_1	1 mg
	Simulate	total_IP_Comp_W_bolus_dose_ start_IP_Comp_W_bolus_dose_	1 dimensionless
er_Options	Run	Period IP Comp W bolus dose	24 hours
		IP_Comp_W_bolus_dose_2	0 mg
Run SimSingl	е™	total IP Comp W bolus dose	0 dimensionless
	Export to Exc	start_IP_Comp_W_bolus_dose_	96 hours
		neriod IP Comp W bolus dose	24 hours
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Use Initial Results to Guide Next Steps in Optimization

- Use **Plot** button on GUI to visualize simulation results
- Plotting blood compound W verifies that a single dose was simulated
- Plotting ALT reveals 1 mg elicits too much injury, too fast
- Use parameter sweep
 feature to test
 - Lower "doses"
 - Alternate start times

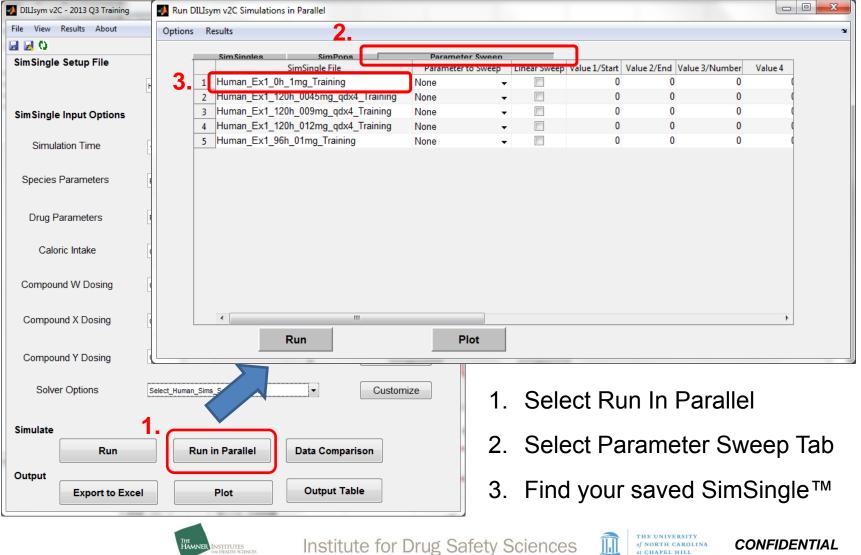


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Parameter Sweep Functionality is Available Through Run In Parallel



52

Use Log Sweep to Identify a "Dose" Range Better Aligned with Target ALT Profile

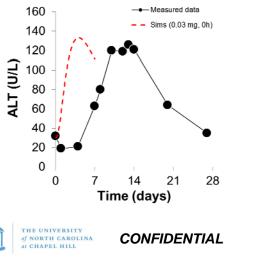
	e DILI and R1 (fo		imPons			ter Sween							
	thmic Sweep (ba		Sweep Lin	near Sweep	Value 1/Start	Value 2/End	Value 3/Nu	imber V	alue 4	Value	5 V	alue 6	Value 7
	Human_E		•		0	0		0	0		0	0	
2	Human_E	None	•		0	0		0	0		0	0	
DILIS	sym v2C Simulat	ions in Paral	llel										
		SimSi	ngle File		Pa	arameter to Sv	veep	Log Swee	p Value	1/Start	Value 2/E	nd Value 3	3/Number
2		_0h_1mg_T _120h_004	5mg_qdx4_		IP_Com None	arameter to Sv p_W_bolus_q	- 1_eaob			00e-03 0	Value 2/E	nd Value 3 1 0	3/Number 7 0
2	Human_Ex1 Human_Ex1	_0h_1mg_T _120h_004 _120h_009i	Training 5mg_qdx4_ mg_qdx4_T	raining	IP_Com None None		dose_1 - - -			00e-03	Value 2/E	1	3/Number 7 0 0 0
2 3 4	Human_Ex1 Human_Ex1	_0h_1mg_T _120h_004 _120h_009 _120h_012	Training 5mg_qdx4_ mg_qdx4_T mg_qdx4_T	raining	IP_Com None		- 1_eaob			00e-03 0 0	Value 2/E	1 0 0	8/Number 7 0 0 0 0





Use Parameter Sweep Results to Guide Further Optimization

- 🚺 Figure 1 Insert Tools Desktop File Edit View Window Help 🐌 🗜 🔏 🕤 Ð ् 🥎 리 R Human Ex1 0h 1mg;IP Comp W bolus dose 1 =0.001 Human Ex1 0h 1mg;IP Comp W bolus dose 1 =0.0031623 Human Ex1 0h 1mg;IP Comp W bolus dose 1 =0.01 Human Ex1 0h 1mg; IP Comp W bolus dose 1 =0.031623 Human Ex1 0h 1mg;IP Comp W bolus dose 1 =0.1 Human Ex1 0h 1mg;IP Comp W bolus dose 1 =0.31623 Human Ex1 0h 1mg;IP Comp W bolus dose 1 =1 Plasma ALT (U/L) vs t (hours) 3000 2500 Plasma ALT (U/L) vs t (hours) Plasma ALT (U/L) 2000 140 **Delete ALT profiles** X: 98 120 1500 Y: 133.2 that are too high Plasma ALT (U/L) 100 1000 80 500 60 40 50 100 150 t (hours) 20 L 50 100 150 200 t (hours) Clinical Data and THE HAMNER INSTITUTES Institute for Drug Safety Sciences Simulation Results
 - Lowering injury-inducing "dose" range by ~2 orders of magnitude puts ALT into a range similar to data
 - Dynamics of single "dose" are a poor match and suggest delayed start time and multiple "doses" should be evaluated
 - <u>Note</u>: multiple "dose" scenario will necessitate further "dose" lowering



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Create a Derivative SimSingle[™] with Closer "Dosing" to Continue Optimization

DILIsym v2C - 2013 Q3 Training	Canada Contraction of Canada			×			
File View Results About						of Walasa 2	,
SimSingle Setup File				 Delay start ti 	me for 1	st "dose"	•
SimSingle Input Options	Human_Ex1_96h_01mg_Training		Customize	Lower "dose" reported ALT	0	nto the	
Simulation nine	4_weeks_Training		Gustomize				
Species Parameter: Ac	djust to target tin	ne frame	Customize	IP Bolus	Dosing		
Drug Parameters	Parameters_human_CompW_direct_necros	sis_T 🔻	Customize	Parameter	Value	Units	
Caloric Intake	Caloric_intake_parameters_blank_v2C	•	Customize	IP_ratio_gut_Comp_W_IP		dimensionless	
Commental Williams				duration_IP_Comp_W_bolus	0.0500		
Compound W Dosing	CompW_96h_01mg_Training		Customize	start_IP_Comp_W_bolus_dose_ period_IP_Comp_W_bolus_dose		hours hours	=
Compound X Dosir FL	urther customize	•	Customize	IP_Comp_W_bolus_dose_1	0.0100	ng	
Compound Y Dosing	Compound_Y_dosing_blank_v2C	•	Customize	total_IP_Comp_W_bolus_dose_		dimensionless	
g				start_IP_Comp_W_bolus_dose_		hours	- 1
Solver Options	Select_Human_Sims_Solver_Options	•	Customize	period_IP_Comp_W_bolus_dose		hours	- 1
				IP_Comp_W_bolus_dose_2		mg	- 1
Simulate				total_IP_Comp_W_bolus_dose_		dimensionless	- 1
Run	Run in Parallel	Data Comparis	on	start_IP_Comp_W_bolus_dose_ period_IP_Comp_W_bolus_dose		hours hours	-
Output					/4	nours •	
Export to Exe	Plot	Output Table	•				





Use Linear Sweep to Identify a Frequency Range Better Aligned with Target ALT Profile

	SimSingles SimPops	Parameter Sween	_				
	SimSingle File	Parameter to Sweep	1	Linear Sweep	Value 1/Start	Value 2/End	Value 3/Number
1	Human_Ex1_0h_1mg_Training	None	-		0	0	0
2	Human_Ex1_120h_0045mg_qdx4_Training	None	•		0	0	0
3	Human_Ex1_120h_009mg_qdx4_Training	None	•		0	0	0
4	Human_Ex1_120h_012mg_qdx4_Training	None	-		0	0	0
5	Human_Ex1_96h_01mg_Training	total_IP_Comp_W_bolus_dose_1	•	V	1	7	8
	Swee	p number of "dose	es"	(necr	osis-in	ducing	j hits)
	Swee	p number of "dose	s"	(necr	osis-in	ducing	g hits)





Use Linear Sweep to Identify Better Timing of 1st Dose

	SimSingles SimPons	Parameter Sweep					
	SimSingle File	Parameter to Sweep		Linear Sweep	Value 1/Start	Value 2/End	Value 3/Number
1	Human_Ex1_0h_1mg_Training	None	•		0	0	0
2	Human_Ex1_120h_0045mg_qdx4_Training	None	•		0	0	0
3	Human_Ex1_120h_009mg_qdx4_Training	None	•		0	0	0
4	Human_Ex1_120h_012mg_qdx4_Training	None	•		0	0	0
5	Human_Ex1_96h_01mg_Training	start_IP_Comp_W_bolus_dose_1	•	V	24	192	8

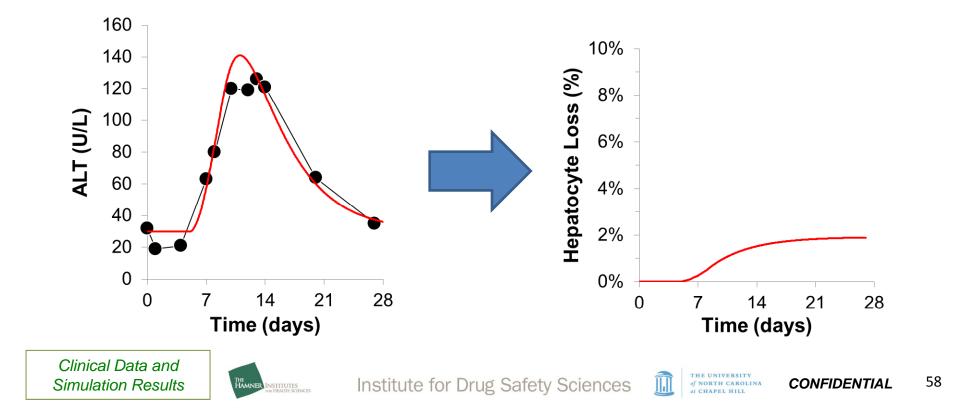




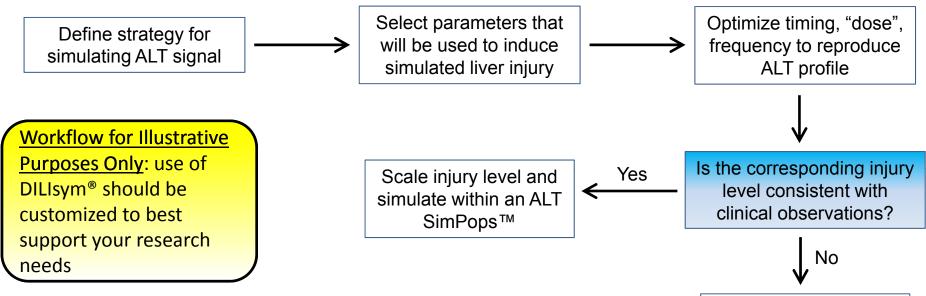
Optimization Provides an ALT Profile Similar to the Clinical Data

Time of 1st dose = 120 h Dose number = 4 Dose frequency = daily Dose magnitude = 0.009 mg

Reproducing the observed ALT profile in DILIsym[®] corresponds to ~2% hepatocyte loss



Injury Level Associated with Optimized ALT Profile is Evaluated



- Clinical data indicated "no evidence of liver dysfunction"
- It seems reasonable that ~2% hepatocyte loss would not result in measurable liver dysfunction
- Select "yes" direction on flow chart & continue DILIsym® retrospective analysis



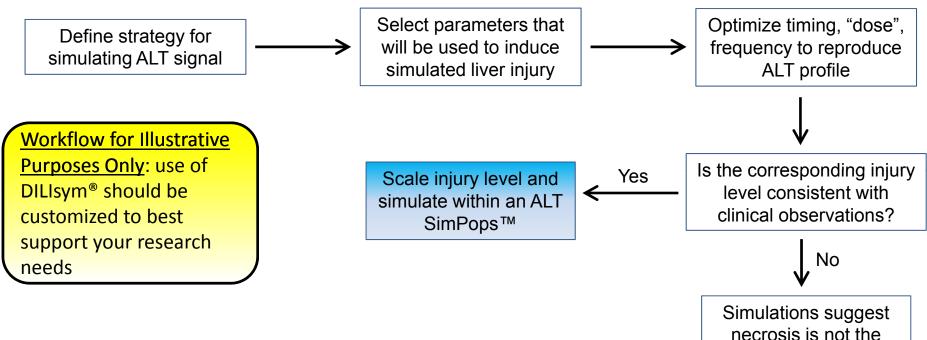


Simulations suggest

necrosis is not the main contributor to the

observed ALT signal

Variability in Predicted Injury Can Be Assessed Using SimPops™



Simulate the optimized compound W scheme in all simulated humans within the SimPops[™]



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main contributor to the

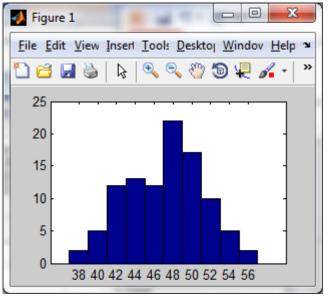
observed ALT signal





ALT SimPops[™] Include Variation in Biomarker Appearance and HC Regeneration

- SimPops[™] Variables
 - HC ALT content
 - HC ALT release rate
 - ALT half-life
 - HGF production rate
 - HGF effect on regeneration
- Use normal distribution function to generate parameter combinations (alternate simulated individuals)
 - 1000 for full SimPops™
 - 100 for training SimPops™
- Screen simulated individuals against available data on liver function vs. biomarker



Distribution of ALT half-life across 100 simulated individuals[§]

 $\$ Plasma ALT half-live of 47 \pm 10h. Normal distribution function used to fill in range from 37-57h.



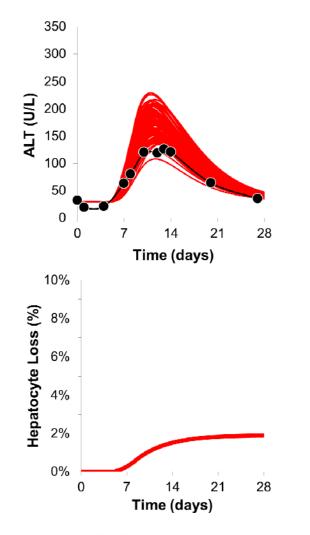


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Use the Optimized Injury Profile in Training SimPops[™] (n=100) to Guide Scaling

- Optimized injury profile in training SimPops[™] shows peak ALT varying from 108-229 U/L
 - Max injury is 2% loss for all these profiles (lower figure)
- Evaluate max and min ALT profiles to guide "dose" scaling
 - Optimized to peak 141 U/L
 - Max 229 U/L, suggests "dose" reduction ~0.5x
 - Min 108 U/L, suggests "dose" escalation ~1.3x



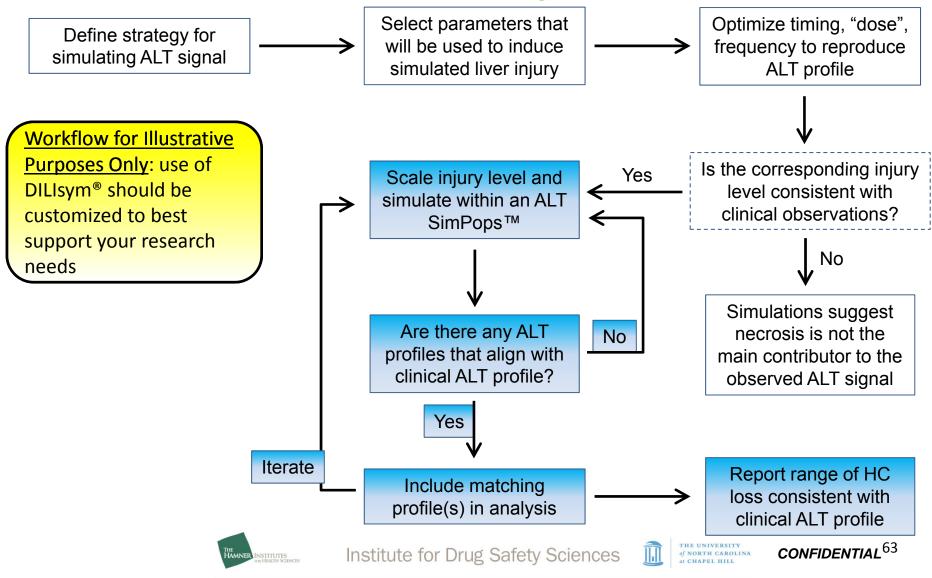
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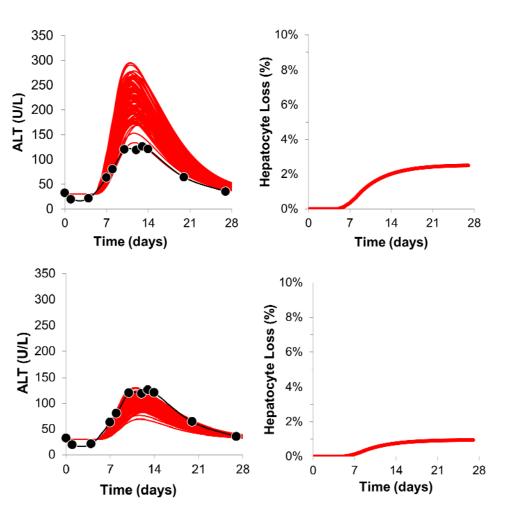


Test Different Injury Levels for Consistency with ALT Data Using SimPops™



Scale Injury to Identify the Limits of Injury that are Still Consistent with ALT Data

- Injury scaled up and down in the SimPops[™]
 - Injury limits identified by the ability to match the ALT profile within the SimPops[™]
 - Injury inducing ~3% hepatocyte loss remains consistent with the measured ALT data
 - Injury inducing ~1% hepatocyte loss remains consistent with the measured ALT data
- Analysis suggests the clinical ALT profile is consistent with 1-3% hepatocyte loss



Clinical Data and Simulation Results



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Retrospective Analysis of Observed Liver Safety Signals

Issue

- ALT (and AST) elevations were reported in a single (few) individuals from three early clinical trials
- No indications of liver dysfunction were observed in the early trials
- No mechanistic data for hepatotoxicity have been identified

Pending Decision

- Does the Company continue to advance this program?
 - Assume multiple inputs and data sets, potentially including modeling and simulation

Questions to Individual(s) Responsible for Liver Safety Assessment

- Can DILIsym[®] be used to retrospectively interpret the observed ALT elevations?
 - What level of injury might be inferred from the reported ALT profile?
 - How much uncertainty is associated with the estimated level of liver injury?
 - What time frame of recovery would be expected for the simulated injury?





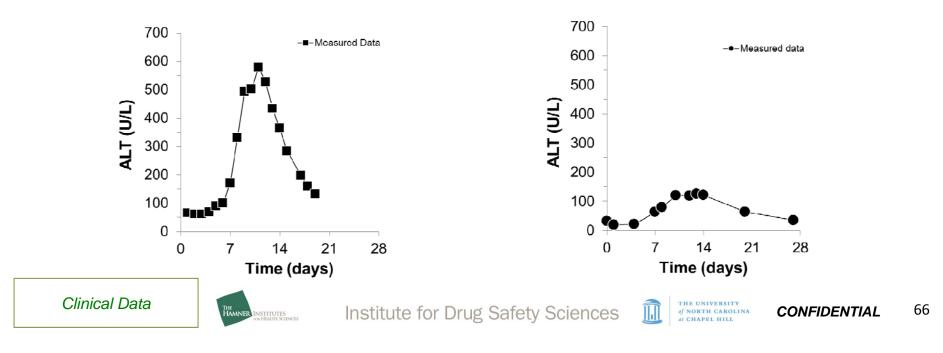
Compare & Contrast Two ALT Profiles from Compounds Intended for Different Indications

Compound 1 – Indication A

- ALT measurements shown for a single NHV
- Increase first noted at d5
- Increase >3x ULN by d8
- Max ALT ~ 600 U/L

Compound 2 – Indication B

- ALT measurements shown for a single NHV
- Increase first noted at d7
- Increase >3x ULN by d10
- Max ALT ~ 125 U/L



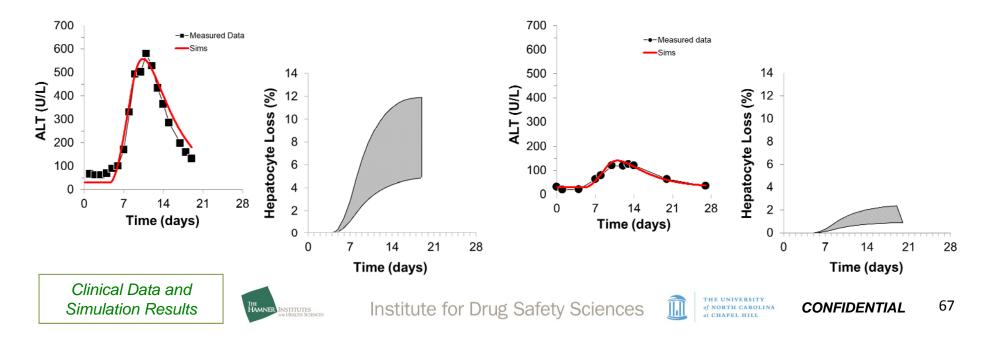
Reproducing ALT Curves Provides Estimates for Associated Hepatocyte Necrosis

Compound 1 – Indication A

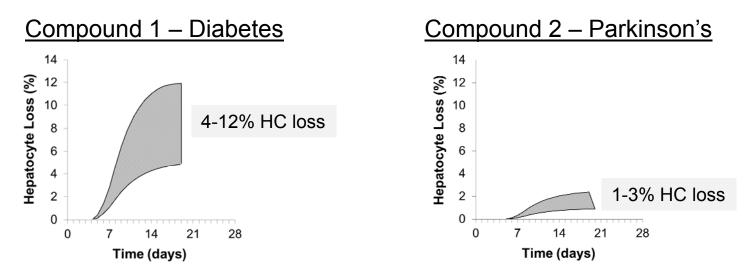
- ALT match in baseline simulated person
- Varying injury level & matching ALT profile in SimPops[™] yields an estimated 4-12% range of hepatocyte loss

Compound 2 – Indication B

- ALT match in baseline simulated person
- Varying injury level & matching ALT profile in SimPops[™] yields an estimated 1-3% range of hepatocyte loss



Estimated Hepatocyte Necrosis in the Context of Disease Indication



- Estimated range for hepatocyte loss may directly figure into the risk assessment
- Speed of recovery (simulation results not shown) may directly figure into the risk assessment
- Hepatocyte loss may be considered in the context of the intended indication (e.g., disease morbidity and mortality, availability and efficacy of currently approved drugs; market size)
 - Would Compound 1 estimated HC loss be considered too risky for a diabetes drug?
 - Would Compound 2 estimated HC loss be considered acceptable for a Parkinson's drug?
 - Interpretation is user and company specific open for general discussion





Evidence from Literature to Support Interpretation of Hepatocyte Loss Simulations

- Excision of 20% of liver volume in living donors is generally considered safe (Florman 2006)
 - Living donors routinely recover fully after even greater portions (40-60%) of liver are excised for adult-to-adult donations (Florman 2006, Lee 2010)
- Heparins are widely considered to be safe despite associated increases in ALT
 - Reported ALT increases after heparins were moderate (>700 U/L peak, 1-2 week time frame)
 - DILIsym[®] modeling team performed comparable ALT-hepatocyte loss on published clinical data (Harrill 2012)
 - Maximal hepatocyte loss predicted for heparins of around 5% of viable hepatocytes
- Clinical correlative data from literature indicate that minimal loss of hepatocytes due to injury has little to no effect on bilirubin levels and prothrombin clotting time (Portmann 1975)





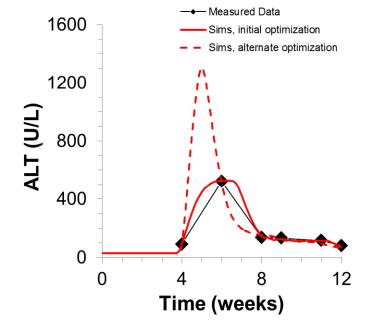
Additional Insights: Fitting Long-Term ALT **Elevations Reveals Critical Uncertainties**

- Long time courses (weeks to months) often include infrequent sampling
 - 1-2 week sampling intervals allow for missing the true peak
- Simulations can still provide an estimate of ٠ liver injury but with room for alternate solutions
 - Note that this can require a necrotic "event" to last for weeks to months
- Additional sources of uncertainty can have ٠ greater impact with long-term ALT elevation
 - ALT clearance rates
 - Mechanisms of injury
 - Adaptation
 - Regeneration

Clinical Data and Simulation Results

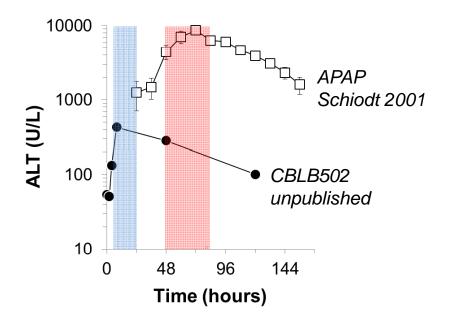






Additional Insights: CBLB502 Data Highlighted Additional Variables Affecting Measured ALT

- CBLB502 induced ALT elevations much more rapidly that the more prototypical APAP profile
 - CBLB502 peak 8-24h (shaded blue)
 - APAP peak 48-84h (shaded red)
- Dramatically different dynamics highlighted additional areas of potential variability
 - Speed of injury onset
 - Speed of ALT release from necrotic cells
- 4 possible combinations identified



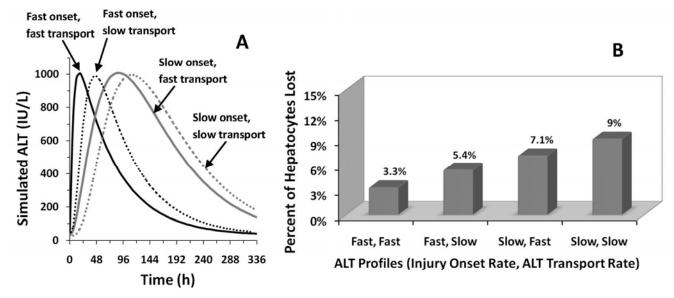
	Injury onset rate	ALT transport rate
Injury onset rate	Fast, fast	Fast, slow
ALT transport rate	Slow, fast	Slow, slow

Clinical Data





Simulating the Combinations Demonstrates the Most Conservative Assumption



- "Dose" adjusted such that all combinations achieve similar peak ALT levels
- Corresponding hepatocyte loss illustrates that slow injury onset and slow ALT release from necrotic hepatocytes is associated with the greatest level of necrosis, i.e., most conservative
- The examples described thus far have used the slow, slow parameter settings (i.e., most conservative) in the optimization of ALT profiles
 - Re-optimizing to fast, fast can be conducted and is expected to provide lower estimates of hepatocyte loss







Assumptions and Limitations

- Simulations are based on induced hepatocyte necrosis (i.e., apoptosis and extra-hepatic ALT release are not accounted for)
- Optimization examples assume the experimental data illustrate the shape of the ALT curve
 - Wide sampling intervals can potentially miss the "true" ALT peak
 - More frequent sampling increases confidence that the optimized ALT profile accurately reflects the human experience
 - Prolonged ALT elevation (weeks to months) may not be simultaneously compatible with hepatocyte necrosis and absence of clinical signs
- Alternate optimization solutions that result in the same ALT profile will give the same level
 of HC loss
 - Solutions resulting in a different ALT peak or AUC are expected to alter the estimated level of injury
- Optimization examples assume slow onset of injury and slow ALT release, leading to conservative estimates of hepatocyte loss
 - Speeding up either parameter and re-optimizing to the ALT data is expected to result in less simulated hepatocyte injury
- Larger SimPops[™] will provide a more complete distribution of injury vs. ALT





Retrospective Analysis of Observed Liver Safety Signals - Summary

Participants should understand the following general concepts:

- Use of DILIsym[®] for the retrospective interpretation of liver injury associated with clinical ALT signals
- Parameter selection for the non-mechanistic representation of hepatocyte necrosis
- Set-up, simulation, and visualization for parameter sweeps
- Use of SimPops[™] to identify a range of injury consistent with a particular ALT profile
- Key uncertainties associated with large time interval sampling
- Impact of speed of injury onset and ALT release on estimated hepatocyte injury

And for compound comparisons:

- Simulations suggest the ranges of liver injury associated with ALT signals from compound 1 and compound 2 were both less than 15%
- While no clinical measures of liver dysfunction (e.g., bilirubin) were observed, the estimated level of injury could inform the compound safety assessment





DILIsym[®] Training Agenda – September 26, 2013

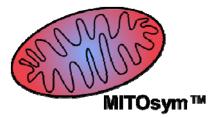
- 8:30 AM Introduction and goals
 - -DILIsym[®] overview and highlights
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MITOsym[™] Training Agenda



Introduction

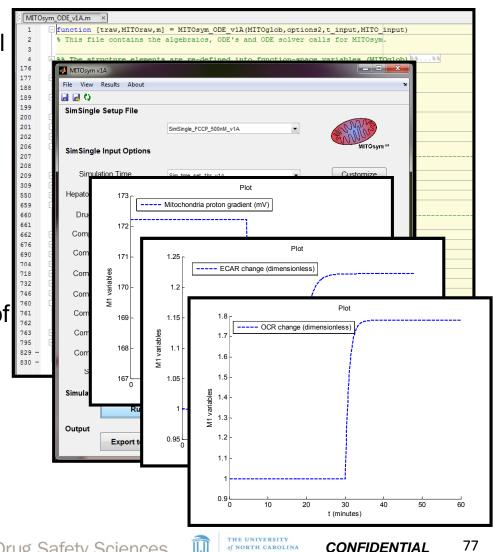
- ✤ Components of MITOsym[™] model
- ♦ Optimization of MITOsym[™] model
- Tolcapone as example of translation of *in vitro* data to predictions of *in vivo* toxicity





MITOsym[™] Is Designed to Support IVIVE DILI Predictions and Mechanistic Data Interpretation

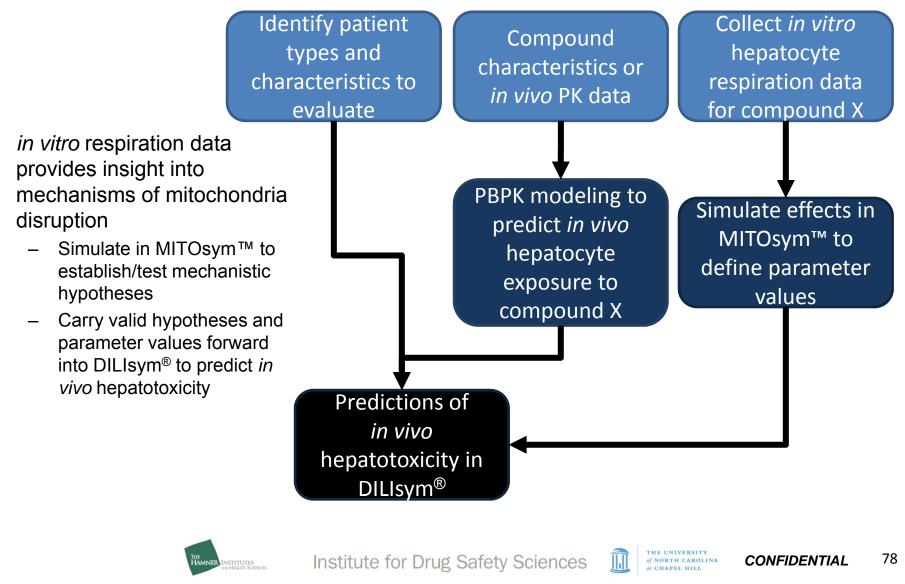
- MITOsym[™] is a standalone model of hepatocyte bioenergetics
- MITOsym[™] can be used to facilitate predictions of hepatotoxicity based on *in vitro* cellular respiration data
 - Combine with DILIsym[®] model
- MITOsym[™] can be used to develop and explore hypotheses of the mechanisms underlying observed changes in respiration and glycolysis in hepatocytes



at CHAPEL HILL

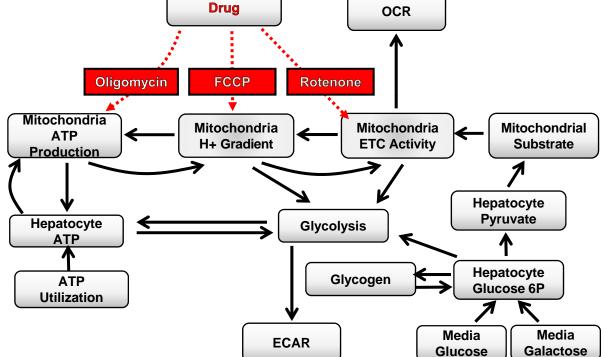


Workflow for Predicting *in vivo* Risk Based on *in vitro* Mitochondria Function Data



MITOsym[™] Model Includes Essential Components of Hepatocyte Bioenergetics

- Includes mitochondria ETC activity, proton gradient and ATP production
 - Also includes glucose uptake, glycolysis, and ATP utilization
- Includes respiration (OCR) as a primary model output
 - Also includes ATP, $\Delta \Psi m$, ECAR
- Includes effects of exemplar drugs
- Includes adaptive, compensatory glycolysis increases with declining mitochondria function
 - Provides ATP unless galactose is primary media substrate





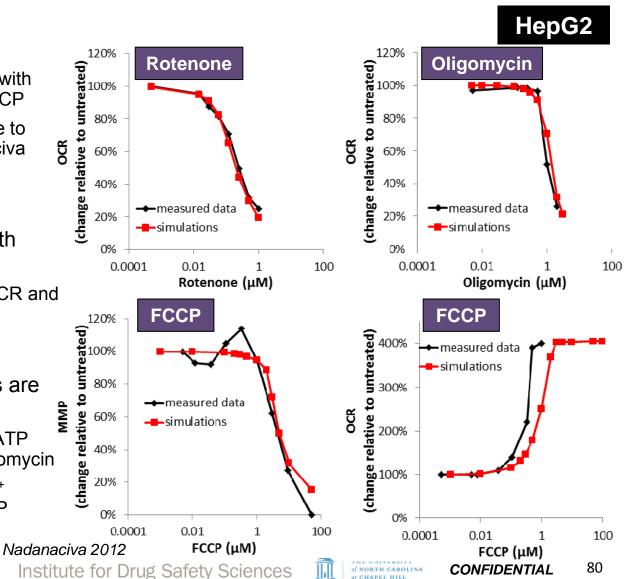


OCR Simulations Optimized to Align with **Response to Individual Mitochondrial Effectors**

- Relative changes to oxygen consumption rate (OCR)
 - Dose response vs. treatment with rotenone, oligomycin, and FCCP
 - Simulation results comparable to measured data from Nadanaciva 2012
- Reduction in mitochondria membrane potential (MMP) with increasing doses of FCCP
 - Also simulating changes in OCR and MMP with time
- Simulation results provide confidence that ETC dynamics are captured
 - Decreased respiration when ATP _ synthase is inhibited with oligomycin
 - Increased respiration when H⁺ gradient is reduced with FCCP



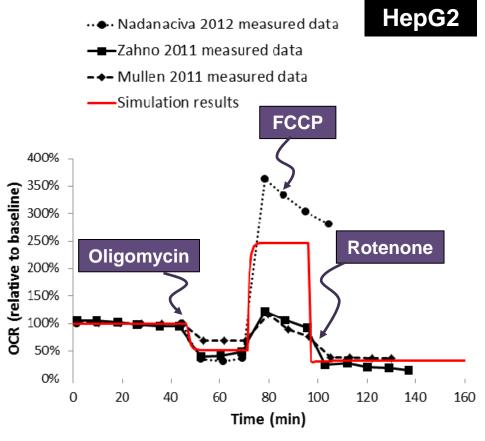




I.

OCR Simulations Optimized to Align with Response to Multiple Mitochondrial Effectors

- Oxygen consumption rate (OCR) predicted to change as reported by Mullen 2011, Zahno 2011, and Nadanaciva 2012
 - Classic mitochondria disruptors used to characterize mitochondria function
 - 1 µM oligomycin, 1 µM FCCP,
 1 µM rotenone added sequentially
- Simulation results provide confidence that integrated dynamics are appropriately represented
 - Decreased maximum in respiration when FCCP follows oligomycin
 - Fully suppressed respiration when rotenone follows FCCP



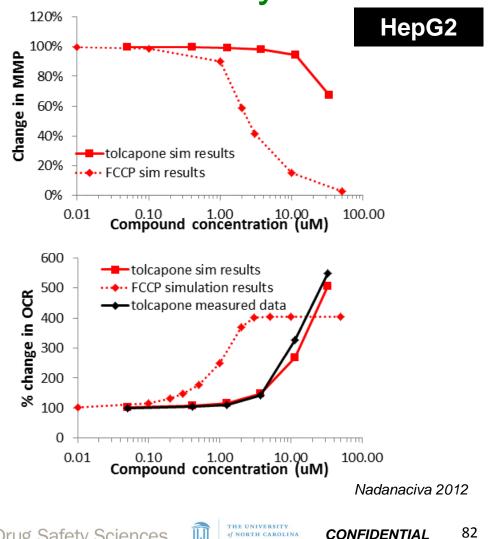
Mullen 2011, Zahno 2011, Nadanaciva 2012





in vitro Respiration Data Used to Determine Tolcapone Mitochondria Uncoupler Parameter Values with MITOsym[™]

- Used MITOsym[™] model to simulate OCR, ECAR, and MMP response to tolcapone
 - Confirmed mechanism is uncoupling
 - Used FCCP measured data and simulations to infer tolcapone parameter values
 - Good agreement with measured OCR data (by design)
- MitoK_UC1_Km parameter value is 10x greater for tolcapone than FCCP
 - MitoK_UC1_Vmax and MitoK_UC1_Hill parameter values unchanged



CHAPEL HILL



DILIsym[®] and MITOsym[™] Have Minor Differences in Mito Drug Parameter Values

		MITO	sym™			DILIS	sym®	
Parameter	Rote- none	Oligo- mycin	FCCP	Tolca- pone	Rote- none	Oligo- mycin	FCCP	Tolca- pone
MitoS_ETC_Inhib_1 (mM)	1.2e-05				1.2e-04			
MitoS_ATP_Inhib_1 (mM)		1.0e-03			-	1.0e-03	-	
MitoK_UC1_Vmax (unitless)			40	40			190	190
MitoK_UC1_Km (mM)			2.0e-02	2.0e-01			2.0e-03	2.0e-02
MitoK_UC1_Hill (unitless)			1	1			1	1

- Parameter values relative to the mitochondria exemplar drugs in MITOsym[™] are what should be used in DILIsym[®]
 - e.g., MitoK UC1 Km for tolcapone
- Minor differences between MITOsym[™] and mitochondria sub-model of DILIsym[®]

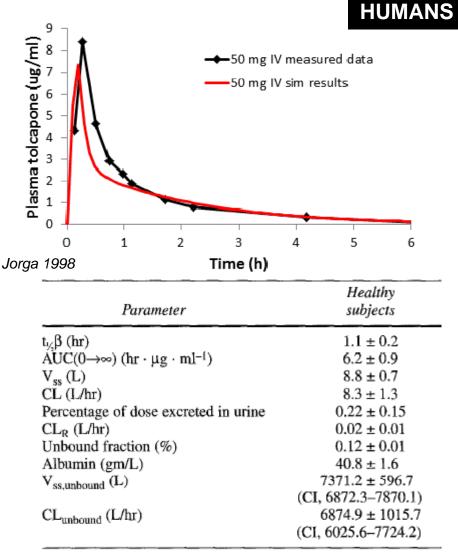
- Account for differences in mitochondria drug-related parameters





Good Agreement for PBPK Modeling of Tolcapone

- Used Compound Y PBPK structure in DILIsym[®]
 - Simpler than Compounds W and X
 - No explicit hepatic metabolism of parent compound with this paradigm
- Used data from series of tolcapone PK studies (Jorga 1998)
 - Used for parameter values and optimization
- Reasonable agreement between simulation results and measured data
 - Maintained 5-15% liver to plasma tolcapone ratio





Parameters Varied in SimPops[™] Used for Tolcapone Simulations

HUMANS

Parameter	Pathway	Baseline	Minimum	Maximum	Mitochondria compromised patients
Basal_Stdzd_MitoETC_Flux	ETC flux	25	19.26	30.77	5-25
Resp_Reserve_Scalar	Respiratory reserve	1	0.39	1.53	0.3-1.0
Basal_Glycogen_Conc	Glycogen	284	250.54	316.94	284
ATP_dec_necrosis_Vmax	ATP-dependent necrosis	0.370	0.19	0.55	0.370
Body_mass	Drug distribution	70	50.05	108.07	70
HGF_regen_Vmax	Hepatocyte regeneration	1.650	0.84	2.43	1.650

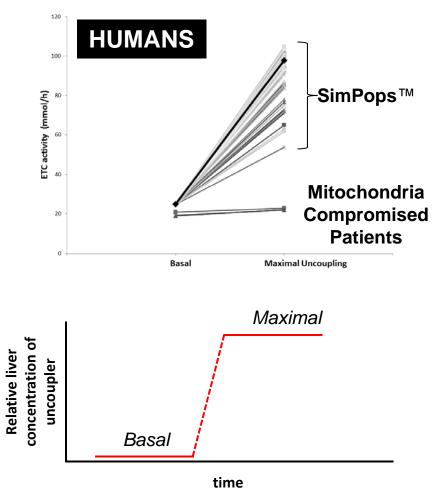
- Used SimPops[™] 'Human_mito_v2B_1_exploration' in DILIsym[®]
 - Parameters varied based on ranges extracted from literature
 - Used a Gaussian distribution pattern, n=176
 - Currently, there aren't population response data with which to validate
- Mitochondria compromised patients included changes to mitochondriarelated parameters exclusively
 - Substantial reduction in basal and adaptive ETC flux





SimPops[™] Mito Characteristics Variability Described by Simulated Max Uncoupler Protocol

- Used simulation protocol to understand how collection of parametric changes to individual simulated patients within SimPops[™] affected overall system
 - SimPops[™] based on control patient mitochondria function variability in Perez-Carrera 2003
 - Mitochondria compromised patients based on NASH patient variability in Perez-Carrera 2003
- Simulated administration of a potent uncoupling agent (i.e., FCCP) to each simulated patient
 - Determined predicted ETC activity at basal and maximal response



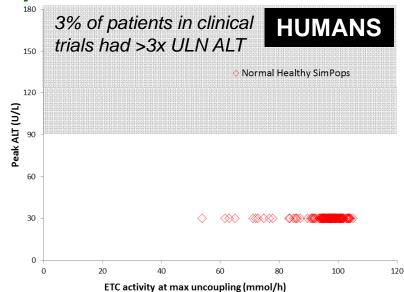
Simulation Results





No Injury Predicted in Normal Healthy SimPops[™] with Tolcapone Treatment

- Simulated 200 mg t.i.d. tolcapone treatment in NHV mitochondria SimPops™
- No liver injury was predicted in any of the simulated patients
 - 97% of clinical patients treated with tolcapone did not have increased ALT or AST
- ATP loss due to tolcapone uncoupling is prevented due to adaptive increase in ETC flux
- What are characteristics of patients who are sensitive to tolcapone-induced liver injury?



Variable	Pre- treatment	Post- treatment
ALT (U/L)	30±0	30±0
ATP decrease (%)	0.1±0.1	0.5±0.1
ETC flux increase (%)	0.7±0.2	18±2
simulated patients with liver signals (#)	0	0



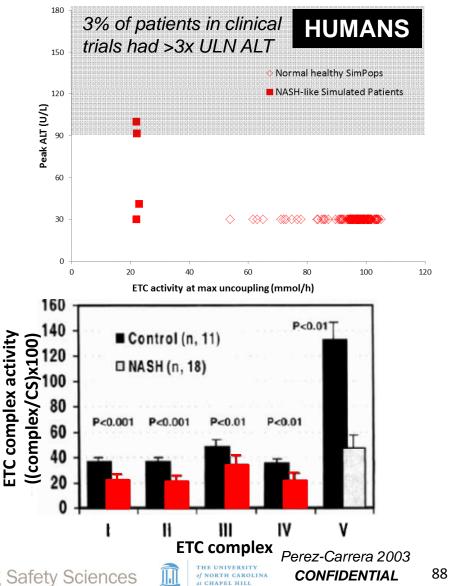
Liver Injury Predicted when Including Patients with Severely Compromised Mito Function

- Generated simulated patients with substantial reductions in mitochondria function
 - Within observed range for NASH patients¹
 - ETC activity at max uncoupling substantially lower in these simulated patients
- ALT increases predicted for several of the mitochondria-compromised simulated patients
 - More sensitive to effects of uncoupler
 - Unable to increase ETC flux to adequately compensate for uncoupling effect
- NASH incidence estimated to be 3-5%²
 - NAFLD incidence estimated to be 20%³
- Hypothesis: NASH-type mitochondria function patients were included in tolcapone clinical trials
 - Ability to screen and exclude these patients from treatment could reduce incidence of DILI

¹Perez-Carrera 2003, ²Ruhl 2004, ³Papandreou 2007

Clinical Data and Simulation Results





DILIsym[®] Training Agenda – September 26, 2013

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 - -Model architecture notes
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• 11:30 AM – Lunch

- 12:30 PM Bile acid transport inhibitor example
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Application Example 2: Potential for Bile Acid Transporter Inhibitors to Cause Clinical DILI

Issue

- Two drugs (bosentan and telmisartan) have been flagged by *in vitro* assays as BSEP inhibitors
- Clinical DILI is linked to BSEP inhibition
- Rat studies have shown no signs of liver injury

Pending Decision

• Should the Company take extra precautions for potential liver injury during clinical trials?

Questions to Individual(s) Responsible for Liver Safety Assessment

- Can DILIsym[®] be used to predict whether DILI might occur in humans?
 - Interpretation of lack of rat toxicity
 - Determination of maximum safe dose for drugs not expected to be toxic





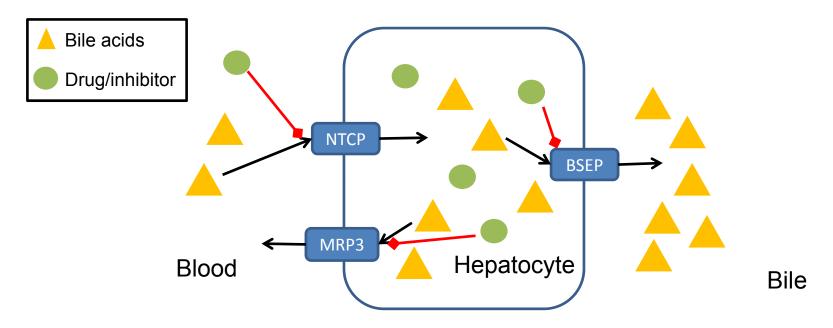
DILIsym[®] Prediction of Potential Bile Acid-Induced Hepatotoxicity

- Using the DILIsym[®] baseline simulated human and rat,
 - Build a model of bosentan and telmisartan including results from *in vitro* inhibition assays
 - Where mechanism of inhibition is unclear, perform simulations with both competitive and non-competitive inhibition
- Using human and rat SimPops[™],
 - Simulate bosentan within the rat and human SimPops[™]
 - Simulate telmisartan within the human SimPops™
 - Identify the presence of human individuals with ALT elevations
 - Explore hepatocyte necrosis, ATP, and bile acid accumulation data to better understand differences between drugs and between species
 - Identify potential risk factors that would make certain individuals more sensitive to toxicity from these drugs





Bile Acid Transport Inhibition

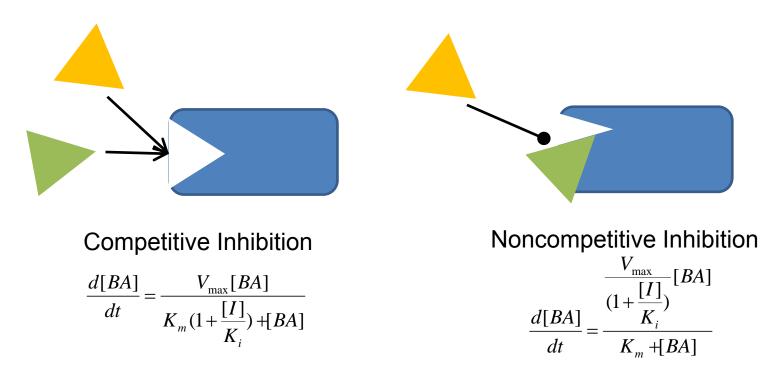


- Bile acids (^A) are taken up into hepatocytes by uptake transporters (NTCP) and transported out of cells by basolateral and canalicular efflux transporters (MRP3 and BSEP)
- Drugs () can inhibit any of these transport processes
- Bile acid buildup can cause toxicity in liver cells
- Our model represents bile acid transport and its inhibition by drugs mechanistically





Competitive and Noncompetitive Inhibition

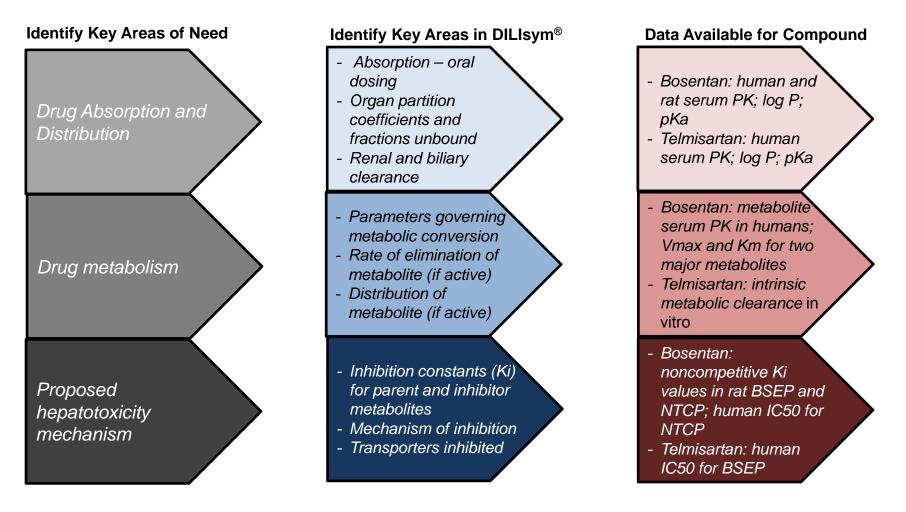


- Competitive inhibition involves drug and bile acids competing for same active site on an enzyme
 - Affects enzyme affinity for the bile acid, i.e. K_m
- Noncompetitive inhibition involves drug preventing bile acid from binding on the enzyme altogether
 - Affects enzyme activity with respect to bile acid, i.e. V_{max}





Identifying the Inputs Needed for Bile Acid Toxicity Prediction in DILIsym[®] v2B

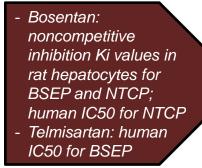






Determining Parameter Values for Transporter Inhibition: Bosentan

Data Available



- Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters
- Mechanism of inhibition
- Transporters inhibited

- Value of Ki and mechanism known in rat hepatocytes for BSEP
 - Both parent and metabolite
 - Will use this value for humans too; literature has shown that bosentan has similar potency for rat and human BSEP
- Value of Ki for NTCP known in rat hepatocytes

Ki for NTCP known in human hepatocytes

Parameter Syntax	Parameter Name	Experimental Value
Ki_noncomp_BSEP_CompX	Noncompetitive Ki for BSEP; parent Compound X	12 uM (Fattinger 2001)
Ki_noncomp_BSEP_CompX _MetA	Noncompetitive Ki for BSEP; Compound X metabolite A	8.5 uM (Fattinger 2001)
Ki_NTCP_CompX	Competitive Ki for bulk bile acid uptake; parent Compound X (human)	18 uM* (Leslie 2007)
Ki_noncomp_NTCP_Comp X	Noncompetitive Ki for bile acid uptake; parent Compound X (rat)	0.28 uM (Leslie 2007)





Selecting the Appropriate Mechanism

Data Available

- Bosentan: noncompetitive inhibition Ki values in rat hepatocytes for BSEP and NTCP; human IC50 for NTCP
 Telmisartan: human IC50 for BSEP
- Competitive vs. noncompetitive inhibition can be important to outcome of model
 - Can be difficult to discern from experimental data; often a blend of the two is responsible
 - Assay to differentiate competitive from noncompetitive inhibition is often not performed
- Can set the model up to run both mechanisms if mechanism is unknown/in doubt
 - In our case, that applies to telmisartan BSEP

Competitive	Noncompetitive
Ki_BSEP_CompX	
LCA_canal_Ki_CompX	
LCAamide_canal_Ki_CompX	Ki nancomn BCED ComnY
LCAsulfate_canal_Ki_CompX	Ki_noncomp_BSEP_CompX
CDCA_canal_Ki_CompX	
CDCAamide_canal_Ki_CompX	

- Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters
- Mechanism of inhibition
- Transporters inhibited





Selecting the Appropriate Mechanism

.

Data Available

 Bosentan: noncompetitive inhibition Ki values in rat hepatocytes for BSEP and NTCP; human IC50 for NTCP
 Telmisartan: human IC50 for BSEP

- In DILIsym[®], competitive inhibition is governed by individual Ki values for each bile acid species, while noncompetitive inhibition is governed by a single constant for each inhibitor
 - Constants can often be different for different bile acid species (e.g. glibenclamide)
 - However, assays are often done using only one substrate (generally TCA)
 - To represent competitive inhibition accurately, all six Ki values must be defined in the parameter set

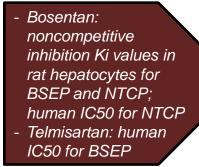
Competitive	Noncompetitive
Ki_BSEP_CompX	
LCA_canal_Ki_CompX	
LCAamide_canal_Ki_CompX	Ki noncomp BSEP CompX
LCAsulfate_canal_Ki_CompX	KI_HORCOMP_BSEP_COMPX
CDCA_canal_Ki_CompX	
CDCAamide_canal_Ki_CompX	

- Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters
- Mechanism of inhibition
- Transporters inhibited

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Determining Parameter Values for Transporter Inhibition: Telmisartan

Data Available



- Inhibition constants (Ki) for parent compound and any metabolite that also inhibits transporters
- Mechanism of inhibition
- Transporters inhibited

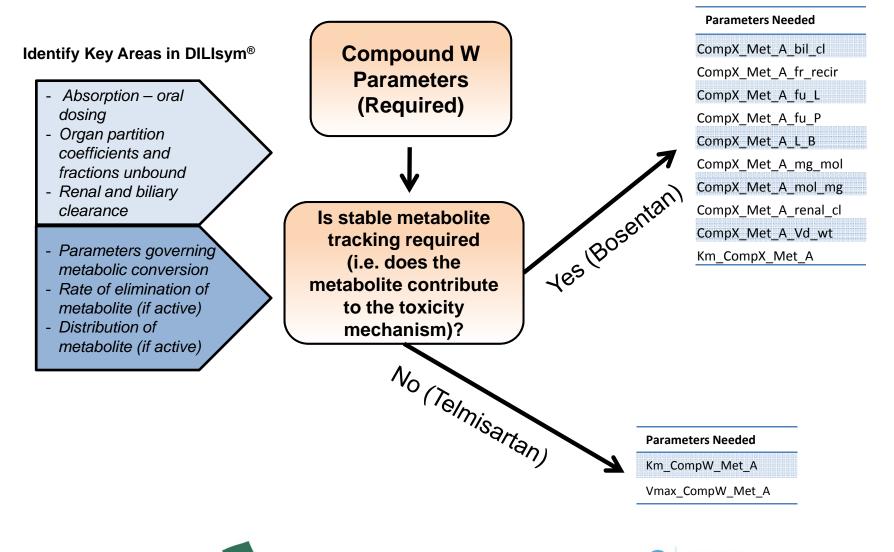
- Value of Ki unknown
- Value of IC50 for human BSEP known from *in vitro* experiment
 - Can use this value as a crude approximation of Ki
 - Will set up parameter sets representing both competitive and noncompetitive inhibition

Parameter Syntax	Parameter Name	Experimental Value
Ki_BSEP_CompW	Competitive Ki for bulk bile acids for Compound W	
LCA_canal_Ki_CompW	Competitive Ki for lithocholic acid for Compound W	
LCAamide_canal_Ki_CompW	Competitive Ki for lithocholic acid amide conjugates for Compound W	
LCAsulfate_canal_Ki_CompW	Competitive Ki for lithocholic acid sulfate conjugates for Compound W	16.2 uM
CDCA_canal_Ki_CompW	Competitive Ki for chenodeoxycholic acid for Compound W	
CDCAamide_canal_Ki_CompW	Competitive Ki for chenodeoxycholic acid amide conjugates for Compound W	





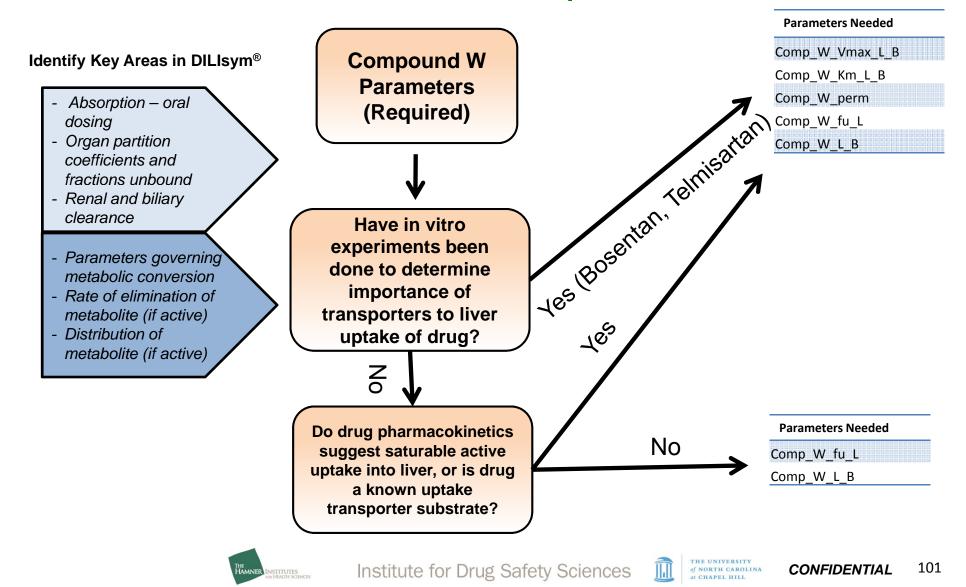
Selecting the DILIsym[®] Parameters to Use for Metabolite PBPK



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Selecting the DILIsym[®] Parameters to Use for Active Liver Uptake



Implementation of Bile Acid Toxicity – Creating Parameter Sets to Account for Unknown Inhibition Mechanisms

- For some transporters, the mechanism of inhibition is unknown, i.e. the drug could be either a competitive or a noncompetitive inhibitor
 - Telmisartan and bosentan for human uptake
 - We will treat bosentan as a competitive inhibitor of human uptake for this exercise
- We will need to build alternate drug parameter sets for competitive and noncompetitive inhibition and run both
- We will make four parameter sets in total today
 - Human telmisartan competitive
 - Human telmisartan noncompetitive
 - Human bosentan
 - Rat bosentan
- The PBPK portion of the input has been filled in already; we will concentrate on inputs that are special to the bile acid model



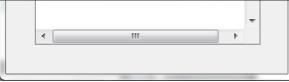


Implementing Hepatotoxicity Mechanism for Bile Acid Toxicity

🛃 Drug Parameter Values-Parame 💷 😐	-	x
		3
Mechanism selection		
Drug toxicity parameters		
Mechanistic interventions		
Compound W PBPK		
Comp W Metabolite A PBPK		
Comp W Metabolite B PBPK		
Compound W RM 1 PBPK		
Compound W RM 2 PBPK		
Compound X PBPK		
Comp X Metabolite A PBPK		
Comp X Metabolite B PBPK		
Mechanism selection-Parameters Blank v2B		

- Telmisartan: check "BSEP/NTCP inhib" for Compound W
- Bosentan: check "BSEP/NTCP inhib" for Compound X and Compound X metabolite A
- Leave all other mechanisms unchecked

Species	RNS-ROS production	ATP utilization	Direct necrolis	BSEP/NTCP inhib	ruvate ox inhib	Fatty acid ox inhib	ETC inhib	Mito ATP synth inhib	Mito uncoupler 1	Mito uncoupler 2	MPT initiator	
Compound W												
Compound W metabolite A												
Compound W metabolite B												Ξ
Compound W reactive metabolite 1												
Compound W RM 1 protein adducts												
Compound W reactive metabolite 2												
Compound W RM 2 protein adducts												
Compound X									100	(11)		÷



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Activating the Bile Acid Model using Mechanistic Interventions

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	<u>d</u>	۲
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Mechanism selection Drug toxicity parameters Mechanistic interventions Compound W PBPK Comp W Metabolite A PBPK Comp W Metabolite B PBPK Compound W RM 1 PBPK Compound W RM 2 PBPK Compound X PBPK Compound X PBPK Comp X Metabolite A PBPK Comp X Metabolite B PBPK Compound X RM 1 PBPK Compound X RM 2 PBPK Compound X RM 2 PBPK Compound X RM 2 PBPK Compound Y PK Bile acid transporter inhibition constants CDCA transporter inhibition constants Species identification	
Ī	<u>د المحمد الم</u>	•
	Mechanistic interventions Compound W PBPK Comp W Metabolite A PBPK Comp W Metabolite B PBPK Compound W RM 1 PBPK Compound W RM 2 PBPK Compound X PBPK Comp X Metabolite A PBPK Comp X Metabolite B PBPK Compound X RM 1 PBPK Compound X RM 1 PBPK Compound X RM 2 PBPK Compound Y PK Bile acid transporter inhibition constants CDCA transporter inhibition constants Noncompetitive inhibition constants	•

Parameter	Value	Units
Anti_TNF_time_stop	0	hour
Anti_TNF_effect_level	0	dimensionles
Anti_HGF_switch	0	dimensionles
Anti_HGF_time_start	0	hour
Anti_HGF_time_stop	0	hour
Anti_HGF_effect_level	0	dimensionles
start_time_HGF_infusion	0	hour
stop_time_HGF_infusion	0	hour
HGF_infusion_rate	0	ng/hour
BA_model_switch	(1	dimensionles
•		

- The bile acid portion of the model is normally switched off in order to preserve computational time
- The bile acid model **must** be turned on in order to properly represent transporter inhibitors





Implementation of Bile Acid Toxicity – Inhibition Constants (1 of 3)

nug Parameter Values-Parameters_B	- 🗆 🗵
	ъ.
Mechanism selection Drug toxicity parameters Mechanistic interventions Compound W PBPK Comp W Metabolite A PBPK Comp W Metabolite B PBPK Compound W RM 1 PBPK Compound W RM 2 PBPK Compound X PBPK Comp X Metabolite A PBPK Comp X Metabolite B PBPK Compound X RM 1 PBPK Compound X RM 1 PBPK Compound X RM 2 PBPK Compound X RM 2 PBPK Compound Y PK Bile acid transporter inhibition constants CDCA transporter inhibition constants Species identification	
	•

Parameter	Value	Units
(i_NTCP_CompX_MetB	1.0000e+10	mg/mL 🔄
ki_NTCP_CompX_RM1adducts	1.0000e+10	mol/mL
<pre>Ki_NTCP_CompX_RM2adducts</pre>	1.0000e+10	mol/mL
Ki_NTCP_CompY	1.0000e+10	
Ki_BSEP_CompW	0.0033	mg/mL
Ki_BSEP_CompW_MetA	1.0000e+10	n ighti L
Ki_BSEP_CompW_MetB	1.0000e+10	mg/mL
Ki_BSEP_CompW_RM1	1.0000e+10	mol/mL
Ki_BSEP_CompW_RM1adducts	1.0000e+10	mol/mL
Ki_BSEP_CompW_RM2	1.0000e+10	mol/mL
Ki_BSEP_CompW_RM2adducts	1.0000e+10	moVmL
VI POED COMPY	1.0000-±10	malmi

- For competitive inhibitors (telmisartan canalicular, bosentan uptake in human)
- Note units in input column
 - For telmisartan, 16.2 μ M = 8.33x10⁻³ mg/mL
 - For human bosentan uptake, 19 μ M = 9.67x10⁻³ mg/mL





Implementation of Bile Acid Toxicity – Inhibition Constants (2 of 3)

	Parameter	Value Units
echanism selection 📃	CDCAamide_uptake_ki_Compx_ CDCAamide_uptake_Ki_CompY	1.0000e+10/movmL
ug toxicity parameters		
echanistic interventions	CDCA_canal_Ki_CompW	0.0083 mg/mL
mpound W PBPK mp W Metabolite A PBPK	CDCA_canal_Ki_CompW_MetA	1.0890e+10 mg/mL
mp W Metabolite B PBPK	CDCA_canal_Ki_CompW_MetB	1.0000e+10 mg/mL
mpound W RM 1 PBPK	CDCA_canal_Ki_CompW_RM1	1.0000e+10 mol/mL
mpound W RM 2 PBPK	CDCA_canal_Ki_CompW_RM1a	1.0000e+10 mol/mL
mpound X PBPK	CDCA canal Ki CompW RM2	1.0000e+10 mol/mL
mp X Metabolite A PBPK mp X Metabolite B PBPK	CDCA canal Ki CompW RM2a	1.0000e+10 mol/mL
npound X RM 1 PBPK	CDCA canal Ki CompX	1.0000e+10 ma/mL
npound X RM 2 PBPK	CDCA_canal_Ki_CompX_MetA	1.0000e+10 mg/mL
npound Y PK acid transporter inhibition constan	CDCA_canal_Ki_CompX_MetB	1.0000e+10 mg/mL
transporter inhibition constants	CDCA canal Ki CompX RM1	1.0000e+10 mol/mL
CA transporter inhibition constants'	CDCA_canal_Ki_CompX_RM1ac	1.0000e+10 mol/ml
competitive inhibition constants	CDCA canal Ki CompX RM2	1.0000e+10 mol/mL
cies identification	CDCA canal Ki CompX RM2ac	1.0000e+10 mol/mL
	CDCA_canal_Ki_CompY	1.0000e+10 m/mL
	CDCAamide_canal_Ki_CompW	0.0083 majmL
	CDCAamide_canal_Ki_ComnW	11089a (48 ma/m)
		ply

- For competitive inhibitors (telmisartan canalicular, bosentan uptake in human)
- Because we do not know the Ki values for each individual bile acid species, we must give each the same inhibition constant
- This must be done for the three LCA species as well as the two CDCA species





Implementation of Bile Acid Toxicity – Inhibition Constants (3 of 3)

Mechanism selection	Parameter	Value Units	
Drug toxicity parameters	KI_NONCOMP_BSEP_COMPVV_WERA	1.0000e+10/mg/mL	
Mechanistic interventions Compound W PBPK	Ki_noncomp_BSEP_CompW_MetB	1.0000e+10 <i>mg/mL</i>	
omp W Metabolite A PBPK	Ki_noncomp_BSEP_CompW_RM1	1.0000e+10 moVmL	
comp W Metabolite B PBPK	Ki noncomp_BSEP_CompW_RM1adducts	1.0000e+10 mol/mL	
ompound W RM 1 PBPK ompound W RM 2 PBPK		1.0000e+10 mol/mL	
ompound X PBPK	Ki_noncomp_BSEP_CompW_RM2		
omp X Metabolite A PBPK	Ki_noncomp_BSEP_CompW_RM2adducts	1.8000e+10 moVmL	
omp X Metabolite B PBPK ompound X RM 1 PBPK	Ki_noncomp_BSEP_CompX	0.0068 mg/mL	
ompound X RM 2 PBPK	Ki_noncomp_BSEP_CompX_MetA	0.0046 mg/m2	
ompound Y PK			
ile acid transporter inhibition constan CA transporter inhibition constants	Ki_noncomp_BSEP_CompX_MetB	1.0000e+10 mg/mL	
DCA transporter inhibition constants*	Ki_noncomp_BSEP_CompX_RM1	1.0000e+10 mol/mL	
oncompetitive inhibition constants	Ki_noncomp_BSEP_CompX_RM1adducts	1.0000e+10 mol/mL	
pecies identification	Ki_noncomp_BSEP_CompX_RM2	1.0000e+10 mol/mL	
	Ki noncomp BSEP CompX RM2adducts	1.0000e+10 mol/mL	
		1.0000e+10 mol/mL	

- For noncompetitive inhibitors (bosentan canalicular, bosentan uptake in rat)
 - Again, note unit conversion
 - For rat bosentan uptake, 0.28 μ M = 1.51x10⁻⁴ mg/mL



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Implementing Parameter Values for Compound W/X PBPK (1 of 2)

DILIsym v2B	DILIsym v2B	🛛 🛃 Drug Parameter Values-Parame 🗖 🖻 💻 🏹	
File View Results About	File View Results About		
O N		⊿ ⊿ °	
SimSingle Setup File	SimSingle Setup File		
Sei		Mechanism selection Drug toxicity parameters	
SimSingle Input Options	SimSingle Input Options	Mechanistic interventions Compound W PBPK	
Simulation Time	Simulation Time	Comp W Metabolite A PBPK Comp W Metabolite B PBPK	
Species Parameters	Species Parameters	Compound W RM 1 PBPK Compound W RM 2 PBPK Compound X PBPK	
Drug Parameters Se	Drug Parameters Parameters_Blank_v2B	Comp X Metabolite A PBPK Comp X Metabolite B PBPK	
Caloric Intakese	Caloric Intake	Compound X RM 1 PBPK Compound X RM 2 PBPK	
Compound W DosingSe	Compound W Dosing	Compound Y PK Bile acid transporter inhibition constant LCA transporter inhibition constants	
Compound X Dosing	Compound X Dosing	CDCA transporter inhibition constants Noncompetitive inhibition constants	
Compound Y DosingSe	Compound Y Dosing	Species identification	
Solver OptionsSe	Solver Options		
Simulate Run Ru	Simulate Run Run in Parallel Data Compa	ri +	
Output Export to Excel	Output Export to Excel Plot Output Ta		

Implementing Parameter Values for Compound W/X PBPK (2 of 2)

Parameter	Value Units
Comp_W_bil_cl	0 mL/hour/kg^0.75
Comp W B P	1 dimensionless
Comp_W_fr_recir	0 dimensionless
Comp W fu G	1 dimensionless
Comp_W_fu_L	1 dimensionless
Comp_W_fu_M	1 dimensionless
Comp_W_fu_O	1 dimensionless
Comp_W_fu_P	1 dimensionless
Fu_correlation_Comp_W	0 dimensionless
Comp_W_fu_corr_2	0 dimensionless
Comp_W_fu_corr_1	0 dimensionless
Comp_W_fu_corr_0	0 dimensionless
Comp_W_G_B	1 dimensionless
Comp_W_L_B	1 dimensionless
Comp_W_mg_mol	1 mol/mg
Comp_W_mol_mg	1 mg/mol
Comp_W_M_B	1 dimensionless
Comp_W_O_B	1 dimensionless
Comp_W_renal_cl	0 mL/hour/kg^0.75
kab_Comp_W_oral	5 1/hour
kab_conj_Comp_W	0 1/hour
kab_Comp_W_IP	12 1/hour
kdiss_Comp_W	12 1/hour
kge_Comp_W	12 1/hour
kIV_Comp_W	60 1/hour
Vmax_Comp_W_ab	0 1/hour
Km_Comp_W_ab	1.0000e+10 mg
k_out_gut_Comp_W	0 1/hour
Comp_W_Vmax_L_B	0 1/hour
Comp_W_Km_L_B	1.0000e+10 mg/mL
Comp_W_perm	0 1/hour

- For proprietary compounds, this would need to be filled out using data from earlier
- For bosentan and telmisartan, the values have been filled in for you

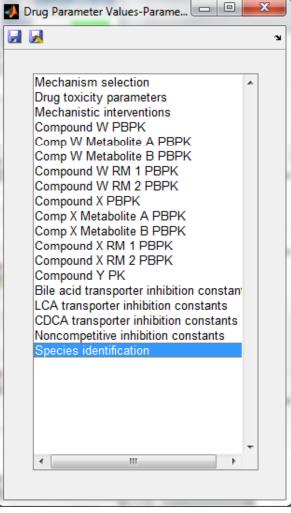
Parameter	Value	Units	
kdiss_Comp_W	12	1/hour	
kge_Comp_W	12	1/hour	
kIV_Comp_W	60	1/hour	
Vmax_Comp_W_ab	0	1/hour	
Km_Comp_W_ab	1.0000e+10	mg	
k_out_gut_Comp_W	0	1/hour	
Comp_W_Vmax_L_B	80	1/hour	
Comp_W_Km_L_B	0.0010	mg/mL	
Comp W_perm	168	1/hour	-

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Implement Appropriate Species Selection for Simulations

- DILI simulations may be run for mice, rats, dogs, or humans
- Species is specified by number
 - **1** mice
 - **2** rats
 - **3** dogs
 - **4** humans

Parameter	Value	Units	
pecies	4	n/a	



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Initial SimSingle[™] Set-Up

- Objective is to predict toxicity for:
 - Multiple dosing in humans
 - Standard rat protocol
- Set-up design
 - Long duration for multiple doses
- Create time files for both rat
 and human

File View Results About			لا	
SimSingle Setup File				-
		Select 💌		
SimSingle Input Options				
Simulation Time	1_week_Defa	ault 💌	Customize	
Species Parameters	Parameters_h	numan_specific_v2B_bili_v2	Customize	
Drug Parameters	Parameters_h	numan_CompW_direct_necrosis 💌	Customize	
Caloric Intake	Caloric_inta	💋 Simulation Time Parameter	rs-human_long_sim*	
Compound W Dosing	Compound_	Parameter	Value	Units
0 IVD :		Time Step	0.5000 hours	j
Compound X Dosing	Compound_	Simulation Time	900 hours)
Compound Y Dosing	Compound_			
Solver Options	Select_Hun			
Simulate				
Run				





Setting Up the Bile Acid Model to Equilibrate Properly

- Bile acid model must be run for a period of time without drug dosing so bile acids can reach their initial concentrations
- Human model reaches stable bile acid concentrations in 240 hours
- Rat model requires 480 hours to reach equilibrium

l 🛃 Oral Bolus	Dosing	
Parameter	Value	Units
duration_oral_Comp_W_bolus	0.0500	hours
start_oral_Comp_W_bolus_dose 🤇	240	hours
period_oral_Comp_W_bolus_dos	24	hours
oral_Comp_W_bolus_dose_1	50	mg
total_oral_Comp_W_bolus_dose	30	dimensionless
start_oral_Comp_W_bolus_dose	48	hours
period_oral_Comp_W_bolus_dos	24	hours
oral_Comp_W_bolus_dose_2	0	mg
total_oral_Comp_W_bolus_dose	0	dimensionless
start_oral_Comp_W_bolus_dose	96	hours
period_oral_Comp_W_bolus_dot	24	hours
oral_Comp_W_bolus_dose_3	0	mg
total oral Comp W bolus dose	0	dimensionless .





Drug Dosing for Bile Acid Simulations

Oral Bolus	Dosing	
Parameter	Value	Units
duration_oral_Comp_W_bolus	0.0500	hours 🔺
start_oral_Comp_W_bolus_dose	240	hours
period_oral_Comp_W_bolus_dos	24	hours
oral_Comp_W_bolus_dose_1	50	mg
total_oral_Comp_W_bolus_dose	30	dimensionless
start_oral_Comp_W_bolus_dose	48	hours
period_oral_Comp_W_bolus_dos	24	hours
oral_Comp_W_bolus_dose_2	0	mg
otal_oral_Comp_W_bolus_dose	0	dimensionless
start_oral_Comp_W_bolus_dose	96	hours
period_oral_Comp_W_bolus_dos	24	hours
oral_Comp_W_bolus_dose_3	0	mg
total_oral_Comp_W_bolus_dose	0	dimensionless 🖵

Compound X Dosing Parameter Values-human_bosentan_dosing*

🛃 🛃

Oral Bolus Dosing

Parameter	Value	Units
duration_oral_Comp_X_bolus	0.0500	hours
start_oral_Comp_X_bolus_dose_	240	hours
period_oral_Comp_X_bolus_dos	12	hours
oral_Comp_X_bolus_dose_1	500	mg
total_oral_Comp_X_bolus_dose_	60	dimensionless
start_oral_Comp_X_bolus_dose_	48	hours
period_oral_Comp_X_bolus_dos	24	hours
oral_Comp_X_bolus_dose_2	0	mg
total_oral_Comp_X_bolus_dose_	0	dimensionless
start_oral_Comp_X_bolus_dose_	96	hours
period_oral_Comp_X_bolus_dos	24	hours
oral_Comp_X_bolus_dose_3	0	mg
total_oral_Comp_X_bolus_dose_	0	dimensionless .
•		

- Set up simulations to run at maximum clinical dose
 - Telmisartan: 50 mg QD
 - Bosentan: 500 mg BID



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Caloric Intake for Bile Acid Simulations

		DILIsym v2C - 2013 Q3 Tra	ining	- 🗆 🗙
File View	Results About			3
🖂 🔀 Q				
	e Setup File e Input Options	Select	¥	
Simula	ation Time	Select	~	Customize
Species	Parameters	Select	¥	Customize
Drug P	arameters	Select	*	Customize
Calo	oric Intake	Caloric_intake_parameters_human_v2C	v	Customize
Compou	nd W Dosing	Select	¥	Customize
Compou	und X Dosing	Select	*	Customize
Compou	und Y Dosing	Select	~	Customize
Solve	er Options	Select	¥	Customize
Simulate				
	Run	Run in Parallel	Data Compar	ison
Output	Export to Exce	l Plot	Output Tak	ble

Parameter	Value	Units
aloric_intake	Default	kcal/day
racCHO	0.5500	dimensionless
racTG	0.3000	dimensionless
_meal_start	192	hovr
neal_duration	0.2500	hour
neal_1_on_off_switch	1	dimensionless
neal_1_start_time		hour
neal_2_on_off_switch	1	dimensionless
neal_2_start_time	6	hour
neal_3_on_off_switch	1	dimensionless
neal_3_start_time	12	hour
neal_4_on_off_switch	0	dimensionless
neal 4 start time	0	hour

 Set up caloric intake so that meals are taken at same time as drug





Exploring Simulation Results Using the Output Table

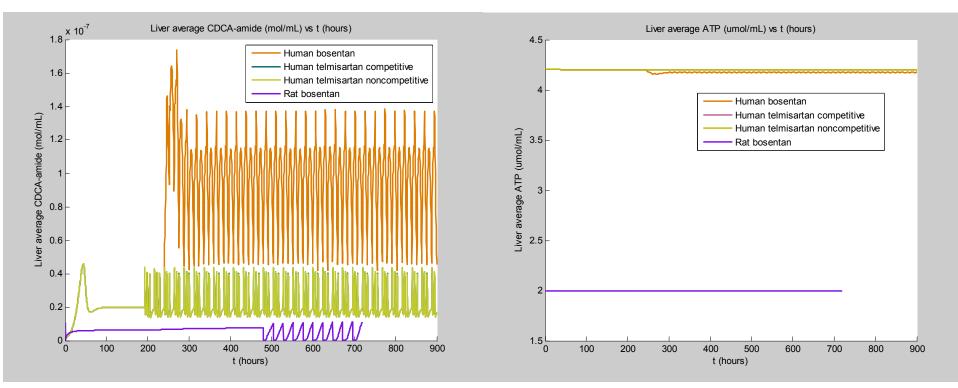
-		DILIsym v2C - 2013 Q3 Training			×					
File View	Results About					3 Output Table				
Ø 🛃 🖸				Outpu	t List Optio	ns				¥۲.
SimSingle	Setup File					Output Variable	Metric	Valu	e	Units
		Human_telmisartan_competitive	v /	1	PP ATP		Minimum	•	- 4.2000 umol/m	
				2			-	-		
SimSingle	Input Options			3			-	-		
Simula	tion Time	Level to a transfer to a	v Cu	4			•	<u> </u>		_
Sirridia	alon nine	human_long_sim_Training	v Cu	5	-		▼	▼ ▼		
Species I	Parameters	Parameters_human_specific_v2C	✓ Cu:	7	-		•			
opecies	urumeters	Parameters_numan_specific_v2c	• 00	8			•	•		
Drug Pr	arameters	Parameters_human_telmisartan_Training_Compl	. v Cu	9			-			
Diugia	arameters	Parameters_numan_termisartan_naming_compr.	. • Ou	10						
Caloric Intake Caloric_intake_parameters_human_v2C v Cu		11								
Compour	nd W Dosing	human_telmisartan_dosing_Training	✓ Cu	-						
	Ū.							,		
Compou	nd X Dosing	Compound_X_dosing_blank_v2C	✓ Cu:				Calculat	te		
Compou	nd Y Dosing	Compound_Y_dosing_blank_v2C	✓ Cus	tomize						
· ·	5				_	 Output 	t table	can be	used	to
Solver	r Options	Default_Solver_Options	✓ Cus	tomize		•				
					_	explor	e basic	: simula	ation	
Simulate						•				-
	Run	Run in Parallel D	ata Comparison			result	s for sin	igie sin	iuialio	11
	Kun		ata companson			_ Ma	x, min, a	vorade	ato	
Output						- 1018	л, пшт, а	verage,		
	Export to Exce	el Plot	Output Table)						
		THE HAMNER INSTITUTES FOR HEALTH SCIENCES	Institute	e for	Drug	Safety Scienc	es 🗊	HE UNIVERSITY North Carolina	CONFID	ENTIAL 115
		FOR HEALTH SCIENCES	Diug	Surety Selence		CHAPEL HILL				

Running Different SimSingles[™] in Parallel

3	DILIsym v2C - 2013 Q3 Training – 🗆 💌	Options Results
File View Results About	r.	SimSingles SimPops Parameter Sweep
0		SimSingle File Run
		1 Select All
SimSingle Setup File		2 Human_bosentan 3 Human telmisartan co ☑
	Human_telmisartan_competitive	3 Human_telmisartan_co
		5 Rat bosentan
SimSingle Input Options		
Simulation Time	human_long_sim_Training v Customize	
Species Parameters	Parameters_human_specific_v2C v Customize	
Drug Parameters	Parameters_human_telmisartan_Training_Compl	
Caloric Intake	Caloric_intake_parameters_human_v2C v Customize	
Compound W Dosing	human_telmisartan_dosing_Training v Customize	
Compound X Dosing	Compound_X_dosing_blank_v2C v Customize	
Compound Y Dosing	Compound_Y_dosing_blank_v2C v Customize	
Solver Options	Default_Solver_Options Customize	Run Plot
Simulate		 Can compare the results of
Run	Run in Parallel Data Comparison	each simulation by running
Output		
Export to Exc	el Plot Output Table	them in parallel together
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NTIAL 116

SimSingle[™] Results Summary



- SimSingle[™] results show no toxicity in average individual
 - Average individual does not generally show bile acid-induced toxicity
 - There are some slight elevations of bile acids in the human bosentan baseline
- Will need SimPops[™] to investigate if these elevations could cause problems in the general population

Simulation Results





Load SimPops[™] results

📣 R	un D	ILIsym	v2B Simul	ations in	Paralle	el 🛛				_ 🗆 🗵
Optic	ons [Results								R
		✓ Save	SimSingle R	esults						
	Sin	Load	SimSingle R	esults		Sweep				_
		🗸 Save	SimPops Re	sults		SimPops		- 4	; Size (n)	
	1	Load	SimPops Re	sults		•		·	0	
	2	✓ Save	Parameter	Sweep Res	sults	e		·	0	
	3	Load	Parameter :	5weep Res	sults	e	1	<u>.</u>	0	
	4	Rat_b	osentan		Non	9		r	0	
1										
							-		1	
		F	Run			Plot		Outpu	ut Table	

- SimPops[™] for human bosentan, rat bosentan, and human telmisartan have been run
 - Time to run would be prohibitive (~2 days)
 - Files are too large to fit on thumb drives; I will show results here
- Bosentan results include enzyme induction equations that are not in v2B
 - Will be included in v3A





Exploring SimPops[™] Using the Output Table

Sim Sir	ngles SimPop SimSingle File	SimPops File	SimPops Size (n)	-						
1 Hum	nan bosentan	None	▼ 0	📣 DIL	Isym v2B Output Table					
	nan telmisartan co		▼ 0	Output List Options						
and the second se	nan telmisartan co		– 0							
_	nan_telmisartan_no		• 0		Output Variable		Metric	Value	Units	
and the second se	nan telmisartan no		• 0	1	Number of deaths	•	*		0 Individuals	
6 Hum	nan_telmisartan_no	None	• 0	2	ALT over 3x baseline		-		2 Individuals	
	nan_telmisartan_no		▼ 0	3	Bilirubin over 2x baseline		•		1 Individuals	
8 Hum	nan_telmisartan_no	None	• 0	4	Hy"s Law cases				1 Individuals	
9 Hum	nan_telmisartan_no	None	• 0	5			× 1			
10 Hum	nan_telmisartan_no	None	• 0	6						
11 Hum	nan_telmisartan_no	None	- 0	7			•			
12 Rat_	_bosentan	None	▼ 0 ▼ 0	8		•				
13 Rat_	_bosentan_long	None	▼ 0	9		•	•			
				10						
				10	-	• •				
				11			Ma			
							Calcu	ulate		
							-	-		

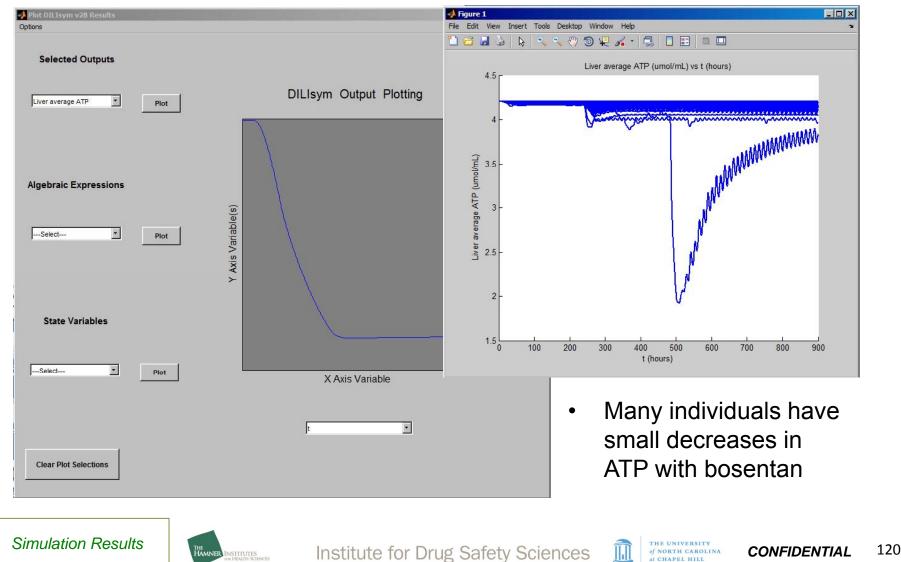
- Bosentan SimPops[™] shows 2 individuals with elevated ALT
- Potential cause for worry; deeper investigation of the results is needed



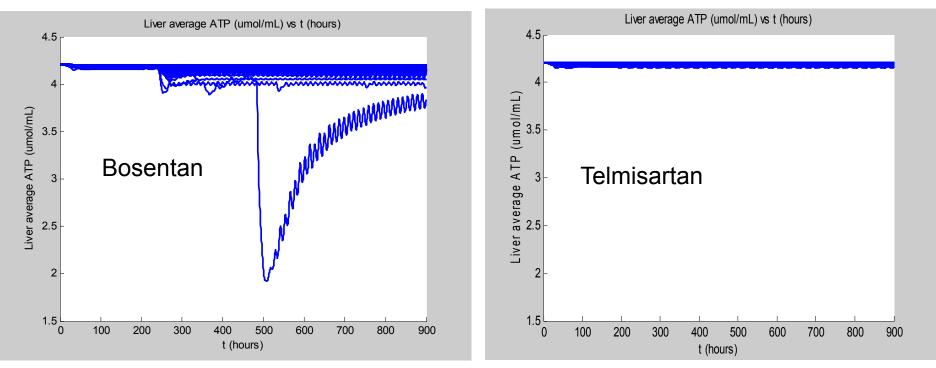
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Exploring SimPops[™] Using the Plotting Function (1/2)



Exploring SimPops[™] Using the Plotting Function (2/2)



 ATP does not decline at all in telmisartan SimPops[™]







Application Example 2: Analysis of Modeling Results

Issue

- Two drugs (bosentan and telmisartan) have been flagged by *in vitro* assays as BSEP inhibitors
- Clinical DILI is linked to BSEP inhibition
- Rat studies have shown no signs of liver injury

Pending Decision

• Should the Company take extra precautions for potential liver injury during clinical trials?

Conclusions from DILIsym[®] Modeling

- Bile-acid induced hepatotoxicity may be an issue with bosentan in certain individuals
 - Average individual will be fine; toxicity may appear rare
- Rat models are not predictive of the hepatotoxicity that may be seen with bosentan
 - Serum bile acid measurements can be misleading in this regard
- Telmisartan is likely clear of any bile-acid induced hepatotoxicity





DILIsym[®] Training Agenda – September 26, 2013

- 8:30 AM Introduction and goals
 - -DILIsym[®] overview and highlights
 - -Model architecture notes
- 8:45 AM Biomarker analysis example
- 9:45 AM Break
- 10:00 AM Biomarker analysis example
- 11:00 AM MITOsym[™] overview and introduction
- 11:30 AM Lunch
- 12:30 PM Bile acid transport inhibitor example
- 1:30 PM Break
- 1:45 PM Bile acid transport inhibitor example
- 2:45 PM Discussion and questions
- 3:00 PM Training concludes
 - -DILI-sim modeling team is available for questions





