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DILIsym User Training –

Importance of Integration of DILI Mechanisms within DILIsym

DILIsym Development Team

Goals for This Training Session

Participants should understand the following general concepts:

- How to approach mechanistic DILIsym projects from a workflow perspective
- How to analyze and present DILIsym results
  - Frequency, magnitude, timing of ALT elevations
  - Different mechanisms
  - Regulatory experience
- The importance of integrating all mechanisms in DILIsym together for accurate predictions

Overview of the DILIsym Workflow and Mechanism Integration Training Session

- How to approach mechanistic DILIsym projects from a workflow perspective
- The importance of integrating all mechanisms in DILIsym together for accurate predictions – background information
- The importance of integrating all mechanisms in DILIsym together for accurate predictions – hands-on example

Common Goals for Mechanistic DILIsym Projects

- To assess the risk of hepatotoxicity with drug candidate, including how the risks (or lack thereof) compare against marketed drug
- To help provide biological plausibility for any genetic or non-genetic biomarkers that emerge from the master research plan and to identify key hepatotoxicity risk factors that may be specific to the patient population
- To determine if the *in vitro* data and simulations support the hypothesis that an observed liver injury episode during clinical studies was unrelated to the Compound
- Determine the mechanisms responsible for liver enzyme elevations in serum of healthy volunteers and patients in Phase I and IIa clinical trials with Compound, focusing on the following possible mechanisms:
  - Mitochondria toxicity
  - Oxidative stress (potentially from a reactive metabolite)
  - Bile acid toxicity

For Mechanistic Projects, DILIsym Utilizes Various Data Types to Inform Decisions

Exposure Data

PBPK Modeling

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

Modeling & Simulation

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Simulations and Assays inform:

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

In vitro Mechanistic DILI Data

Assays performed to determine quantitative aspects of DILI mechanisms

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

Clinical Data

- **Dosing Protocols, meal times if necessary**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

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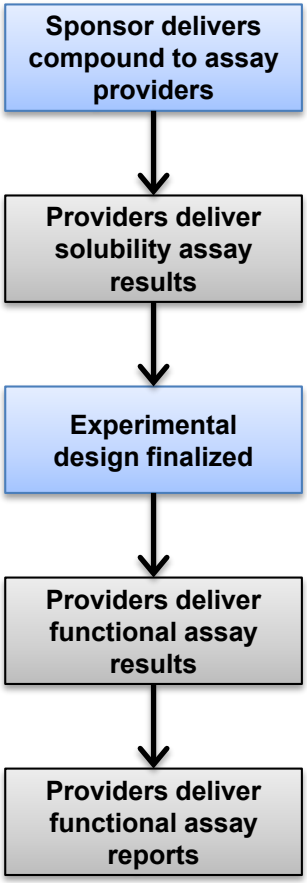
General Workflow for Collection of *In Vitro* Data



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- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

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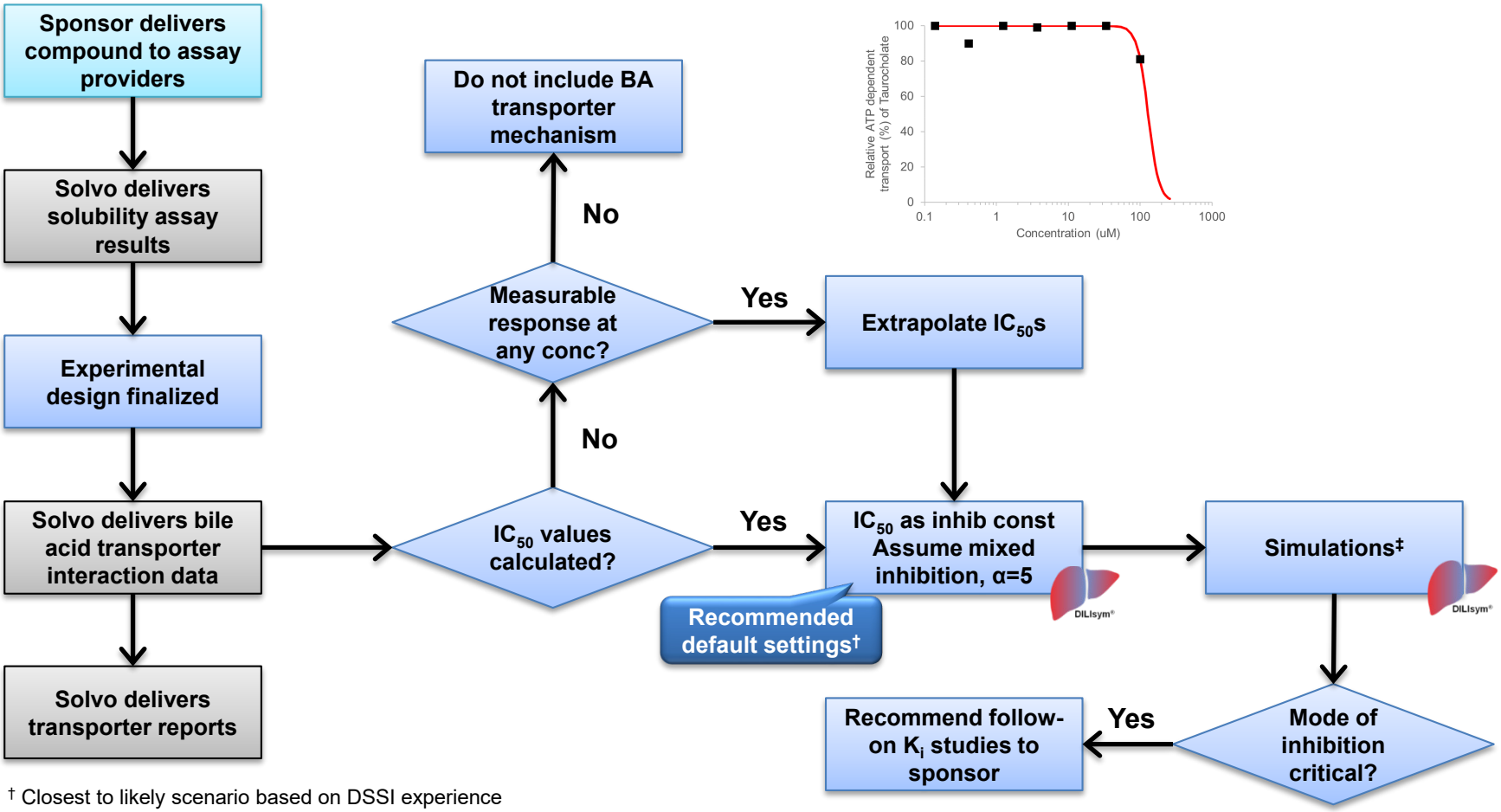
General Workflow for Use of Bile Acid Transporter Data



† Closest to likely scenario based on DSSI experience
‡ See training on exploration to determine contributors to simulated toxicity

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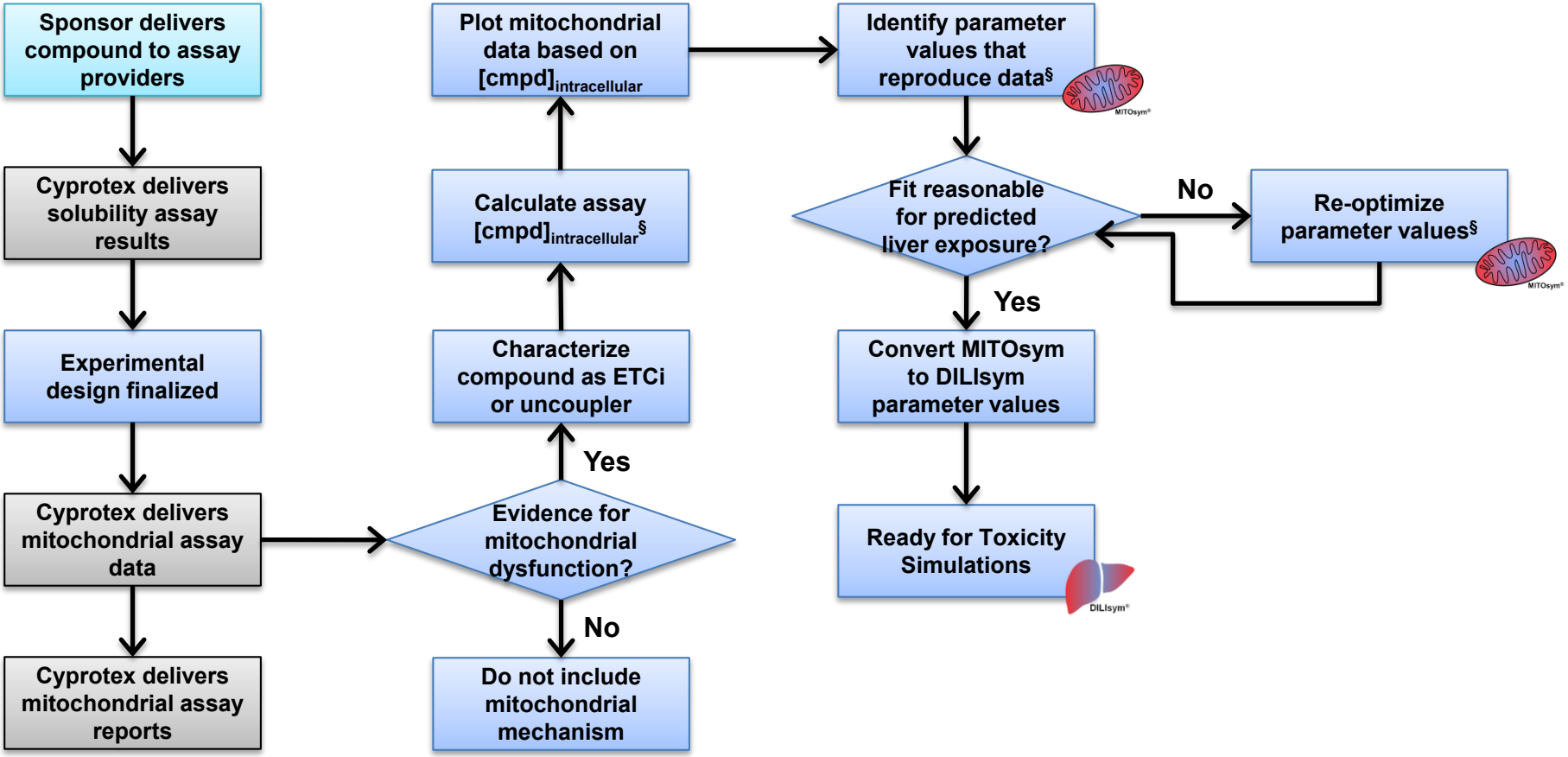
General Workflow for Use of Mitochondrial Function Data



§ see training presentations/videos on identification of parameter values for mitochondrial dysfunction

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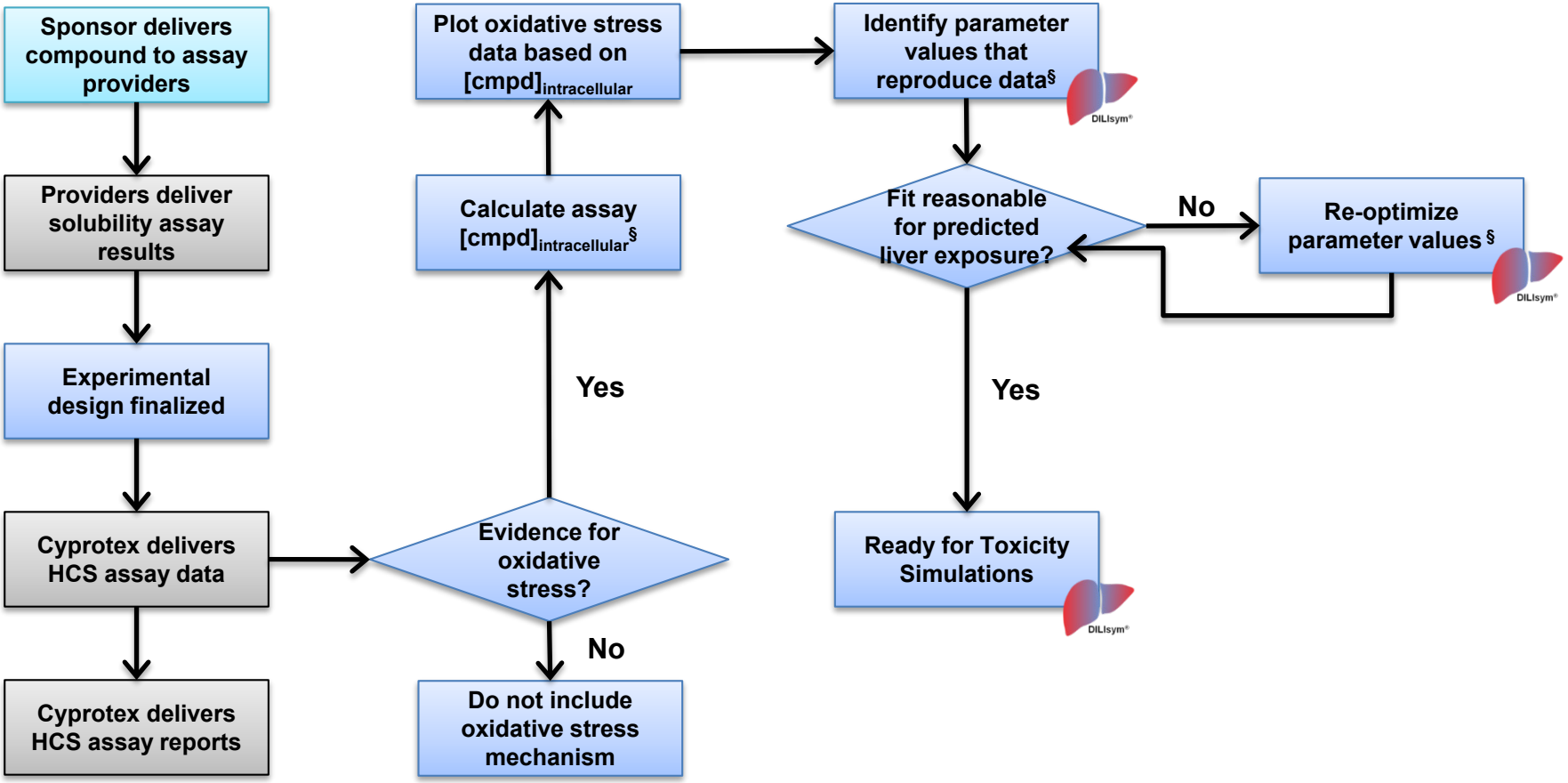
General Workflow for Use of Oxidative Stress Data



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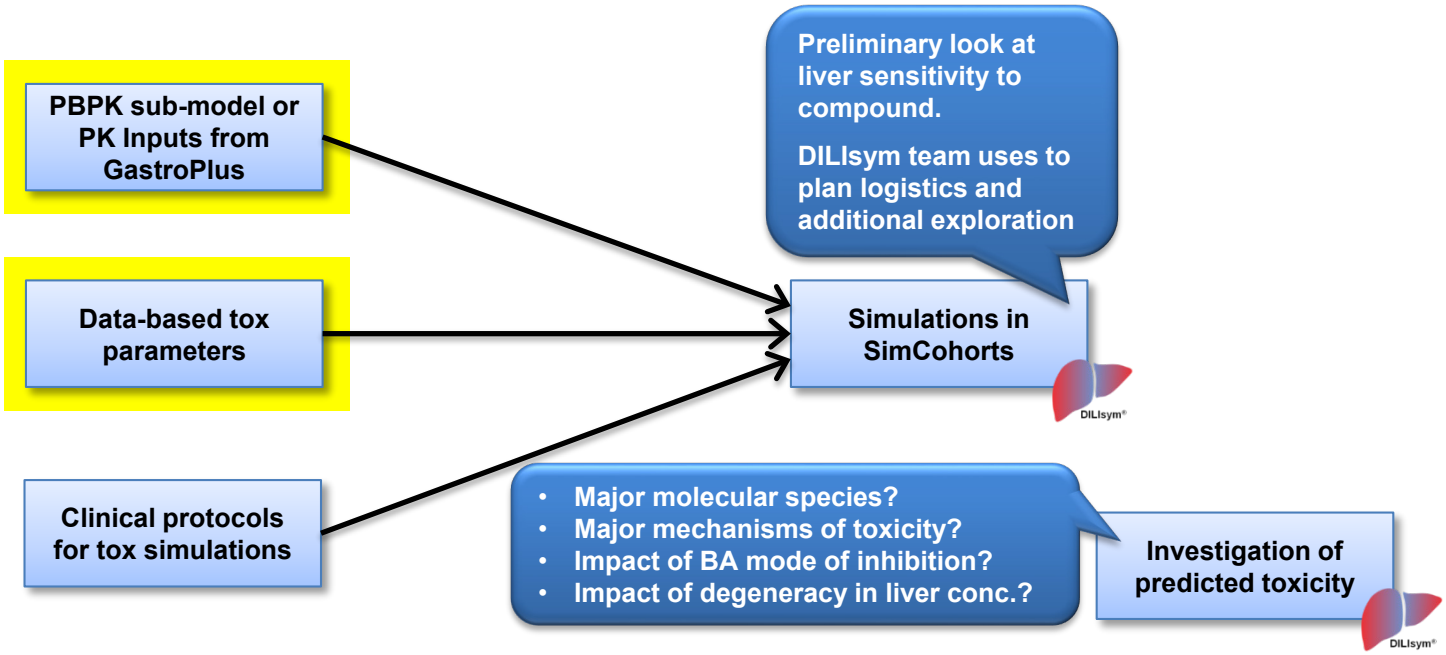
General Workflow for Toxicity Simulations



Evaluation of SimCohorts Results

SimCohorts	Protocol	Investigation	Mechanisms On	Mechanisms Off	ALT >3x ULN	Hy's Law cases
Multi16	1x (12 wks)	Baseline toxicity	Parent: ROS, ETCi Metabolite: ROS, ETCi	None	0	0
	2x (12 wks)	Baseline toxicity	Parent: ROS, ETCi Metabolite: ROS, ETCi	None	9	2

9/16 suggests high frequency of ALT elevations in SimPops

- Multi16 includes
  - 13 individuals sensitive to each of the 3 mechanisms of toxicity, plus the combination of BA accumulation and mitochondrial dysfunction
  - Baseline human
  - Two insensitive individuals
- Results provide early hints of liver injury
  - 0/16 with no ALT elevations >3x ULN sets expectation that SimPops results are likely clean
  - 1+/16 with ALT elevations >3x ULN sets expectation for liver signals in SimPops
  - 1+/16 Hy's law cases sets expectation for severe injury in the SimPops
  - Time reports (not shown) can be used to estimate duration for SimPops runs; if long, consider executing sensitivity analyses in SimCohorts

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	2x (12 wks)	Baseline toxicity	Parent: ROS, ETCi Metabolite: ROS, ETCi	None	9	2
		No ETCi toxicity	Parent: ROS Metabolite: ROS	Parent: ETCi Metabolite: ETCi	8	2
		No ROS toxicity	Parent: ETCi Metabolite: ETCi	Parent: ROS Metabolite: ROS	0	0

- Sensitivity analysis for contributing mechanisms
  - Re-run simulations with mechanism of interest turned OFF
  - Change in liver injury indicates contribution by mechanism that is OFF
- Results may point to additional investigations
  - Any uncertainties related to the toxicological parameter values can be investigated to determine robustness of prediction to variation in the *in vitro* assay
  - May consider additional analyses to identify putative biomarkers of response

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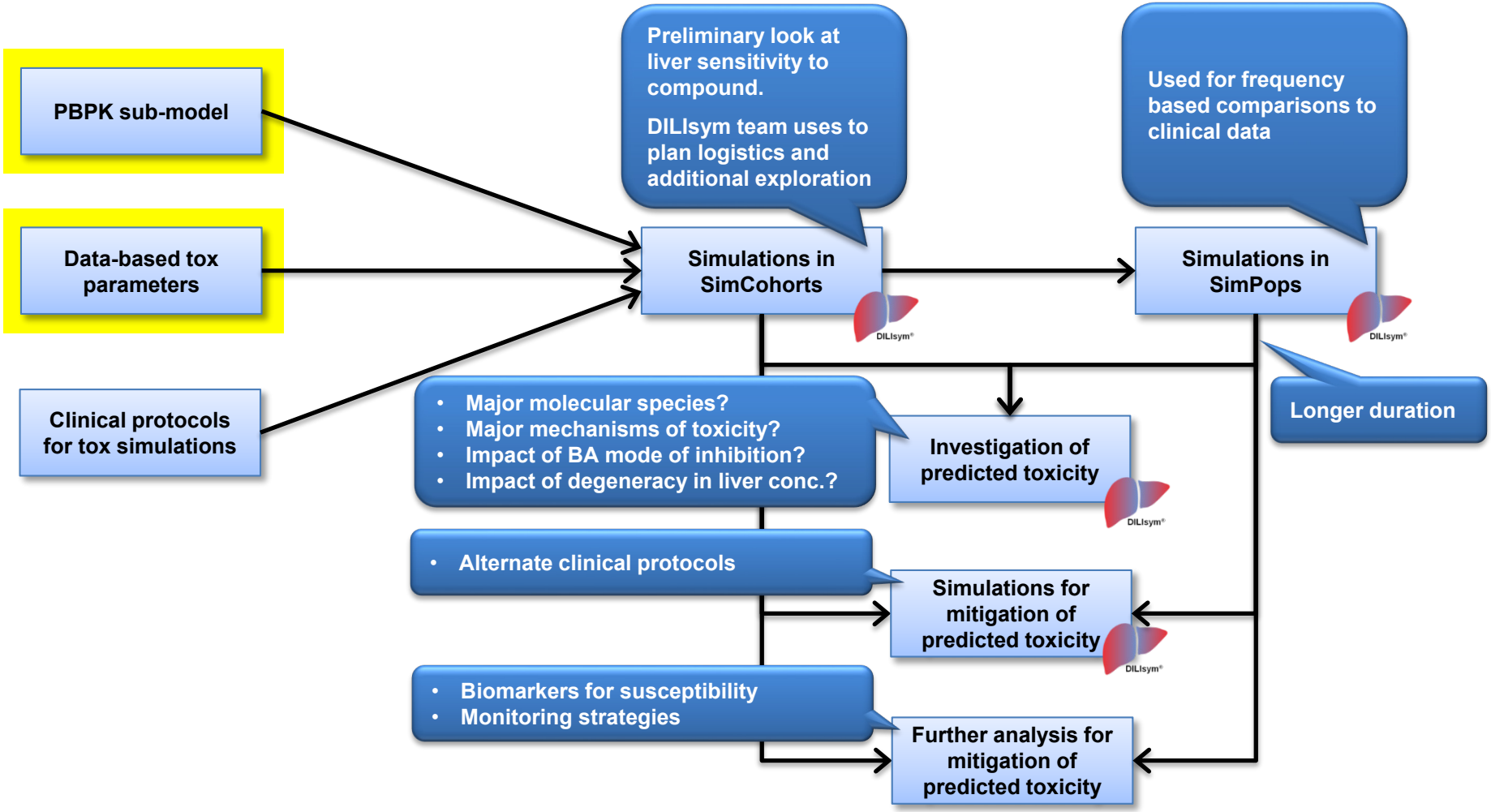
		No ETCi toxicity	Parent: ROS Metabolite: ROS	Parent: ETCi Metabolite: ETCi	8	2
		No ROS toxicity	Parent: ETCi Metabolite: ETCi	Parent: ROS Metabolite: ROS	0	0
		No parent toxicity	Metabolite: ROS, ETCi	Parent: ROS, ETCi	4	1
		No metabolite toxicity	Parent: ROS, ETCi	Metabolite: ROS, ETCi	0	0

- Sensitivity analysis for contributing molecular species
  - Re-run simulations with molecular species toxicity mechanisms turned OFF
  - Change in liver injury indicates contribution by the molecular species that is OFF
- Results may point to drug development considerations
  - Internal discussions related to potential toxicity by metabolites

General Workflow for Toxicity Simulations



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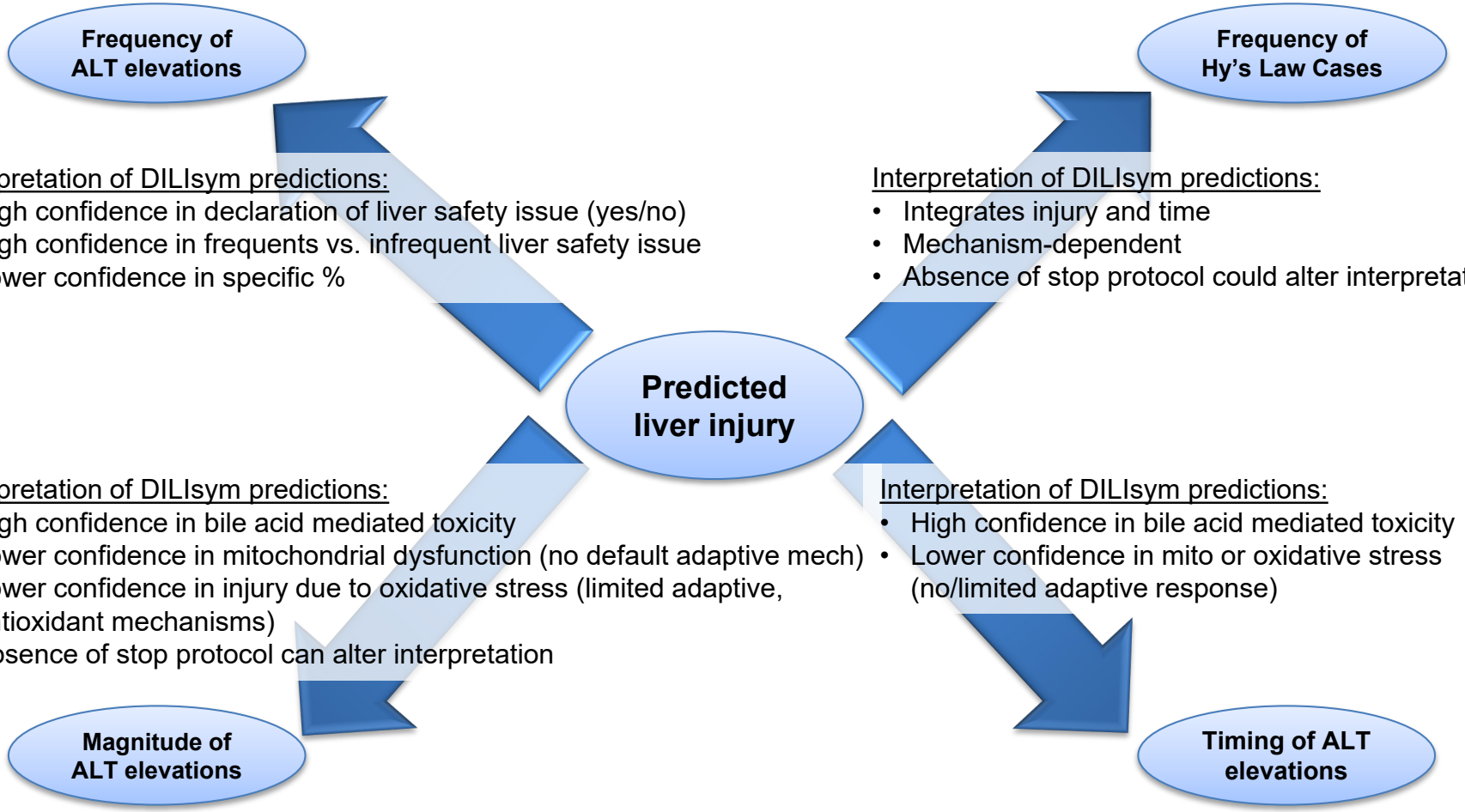
Evaluation and Presentation of Predicted Liver Injury



Interpretation of DILIsym predictions:
- High confidence in declaration of liver safety issue (yes/no)
- High confidence in frequents vs. infrequent liver safety issue
- Lower confidence in specific %

Interpretation of DILIsym predictions:
- Integrates injury and time
- Mechanism-dependent
- Absence of stop protocol could alter interpretation

Interpretation of DILIsym predictions:
- High confidence in bile acid mediated toxicity
- Lower confidence in mitochondrial dysfunction (no default adaptive mech)
- Lower confidence in injury due to oxidative stress (limited adaptive, antioxidant mechanisms)
- Absence of stop protocol can alter interpretation

Interpretation of DILIsym predictions:
- High confidence in bile acid mediated toxicity
- Lower confidence in mito or oxidative stress (no/limited adaptive response)

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Evaluation and Presentation of Contributors to Liver Injury

Simulations for mode of BA inhibition

Source	Mode of BA inhibition	ALT > 3x ULN (%)
Observed	-	**1-2**
SimPops v4A_1 (n=285)	Noncompetitive	15.4
	Mixed (α=5)	**2.8**
	Competitive	0.4

- Combining *in vitro* data with compound exposure in DILIsym could produce similar ALT elevations as observed
  - Only IC_{50} data available for BAi
- Results sensitive to mode of inhibition
  - Both mixed and competitive reproduce the clinical data

Simulations for degeneracy in liver concentrations

Source	L:B ratio	ALT > 3x ULN (%)
Observed	-	**9-22**
Customized SimPops (n=285)	1x (default)	4
	1.5x	**21**
	2x	45

- Combining *in vitro* data with compound exposure in DILIsym could produce similar ALT elevations as observed
  - No constraints on liver concentration
- Liver concentration strongly influenced simulated toxicity

DILIsym Regulatory Experience

DILIsym projects designed to support the following types of argument:

New compound exhibits different mechanisms of toxicity than a DILI compound in the same class.

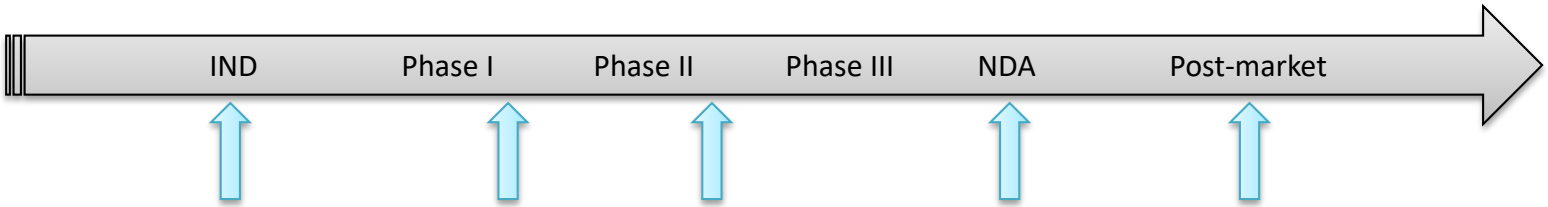
Combination of mechanisms and exposure predicts less toxicity by new compound, relative to DILI compound in the same class.

Simulation results increase confidence that observed liver injury will not occur in planned trials.

Simulation results increase confidence that observed liver injury was not due to drug.

Simulation results guide design of safer protocols for planned trials.

DILIsym projects intended to support regulatory interaction at multiple stages in the drug pipeline:



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Overview of the DILIsym Workflow and Mechanism Integration Training Session

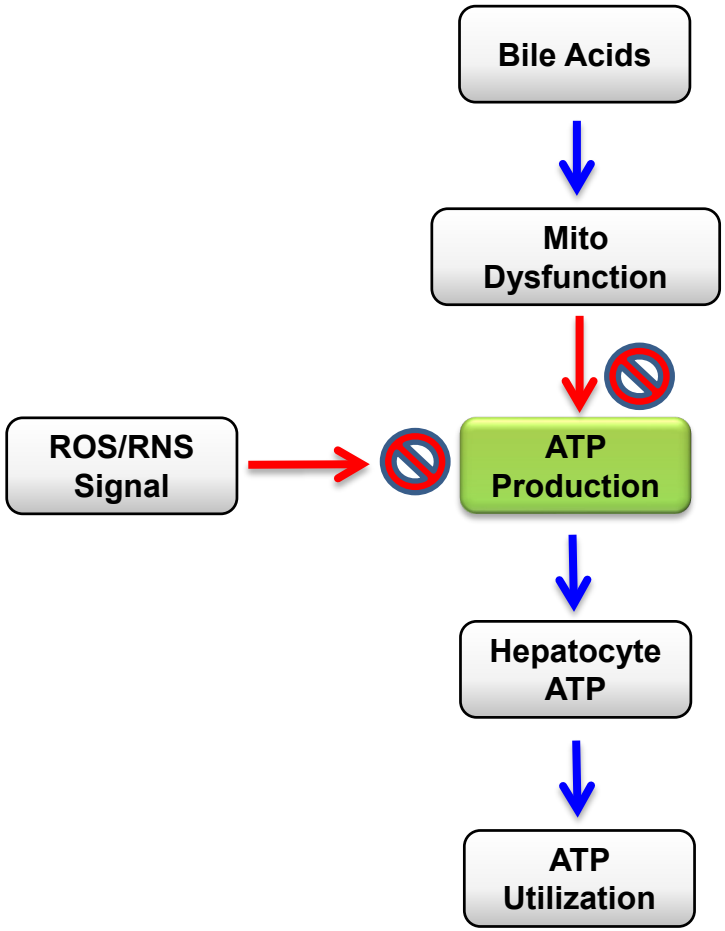
- How to approach mechanistic DILIsym projects from a workflow perspective
- The importance of integrating all mechanisms in DILIsym together for accurate predictions – background information
- The importance of integrating all mechanisms in DILIsym together for accurate predictions – hands-on example

The Interaction Between Various DILI Mechanisms is Critical to Assess

- DILIsym includes multiple mechanisms of DILI
- The combination of mechanisms, often times inconsequential in isolation, has proven absolutely critical to the DILIsym predictions made in many cases
- Bile acid and mitochondrial dysfunction interaction effects have been most notable
- Simulation projects have also shown that bile acids, mitochondrial dysfunction, and oxidative stress are collectively required to predict a DILI response in some cases
- ***Viewing in vitro results in isolation and drawing conclusions is misleading and NOT recommended***
  - ***Drug exposure also complicates the interpretation***

ATP is the Common Link Between ROS/RNS, Bile Acids, and Direct Mitochondrial Toxicity

- Oxidative stress, direct mitochondrial dysfunction, and bile acid effects on mitochondrial function have a common intersection point at ATP Production
- Oxidative stress effects on ATP production do not necessarily always combine with mito/BA effects in a linear way, since ATP production inhibition is nonlinear
- Effects of ATP inhibition, including apoptosis and necrosis, are also nonlinear in nature and include thresholds, making it difficult to predict how two or more effects will combine without running the simulations



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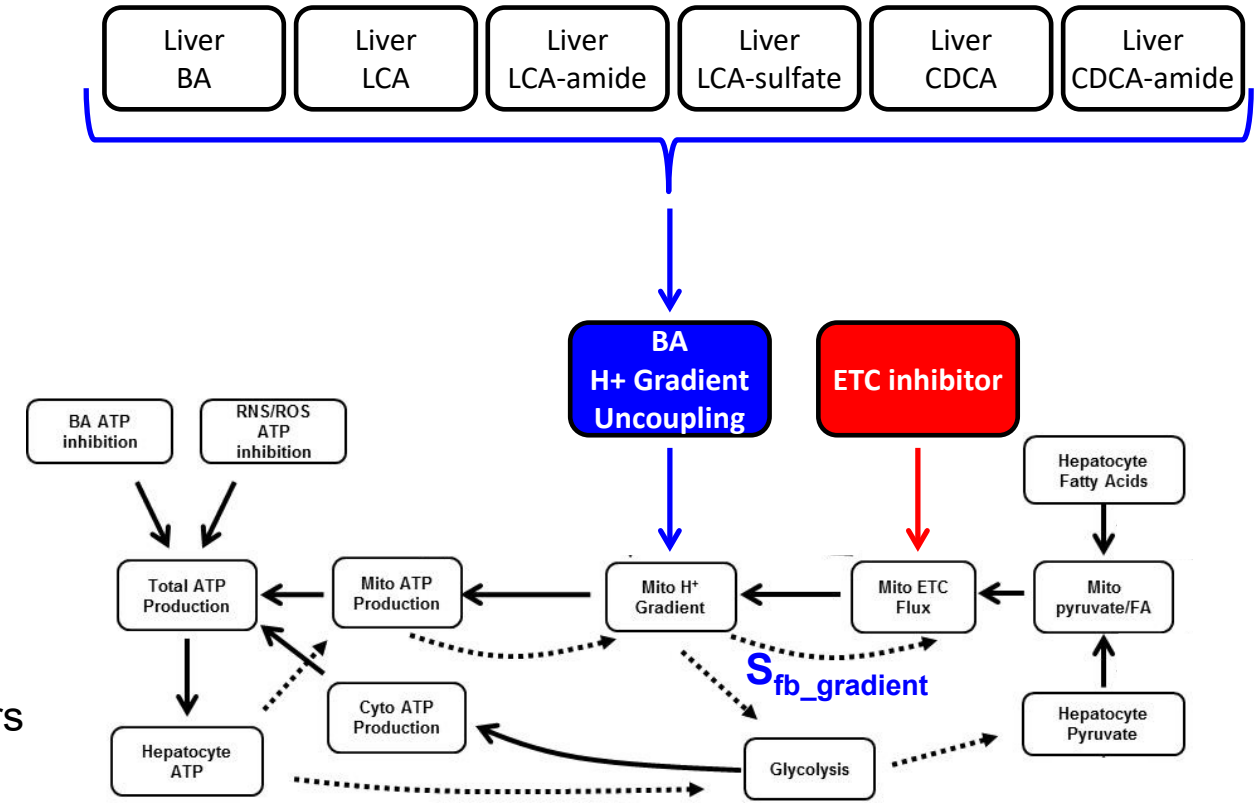
Mechanistic Interactions Reveal Interaction Effect between Bile Acids and ETC Inhibitors

- Hepatic bile acids cause reductions in mitochondria proton gradient
  - BA's have been shown to invoke MPT and reduce Δψm (Rolo 2000, Schulz 2013)
- Uncoupling causes adaptive increase in ETC flux via S_fb_gradient
  - Acts to preserve Δψm to some extent
- Potential for negative interaction with ETC inhibitors
  - Restricts adaptive increase in ETC flux



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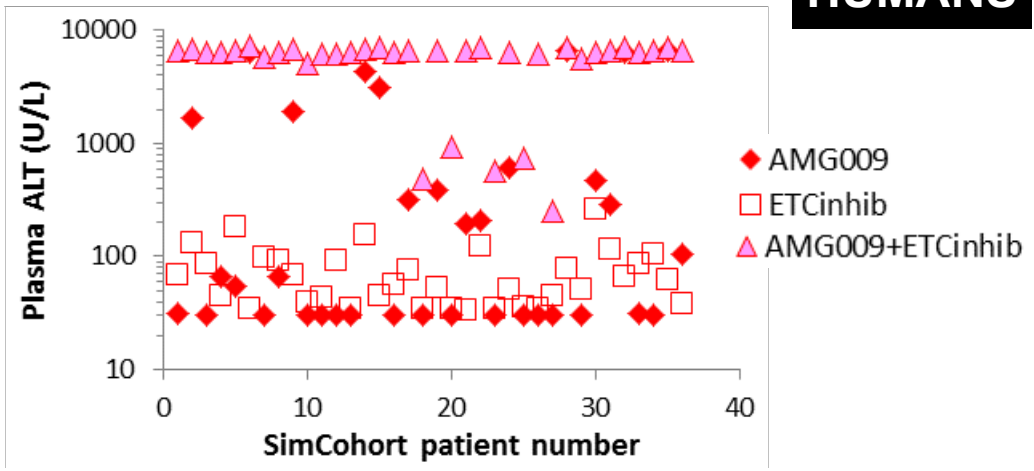
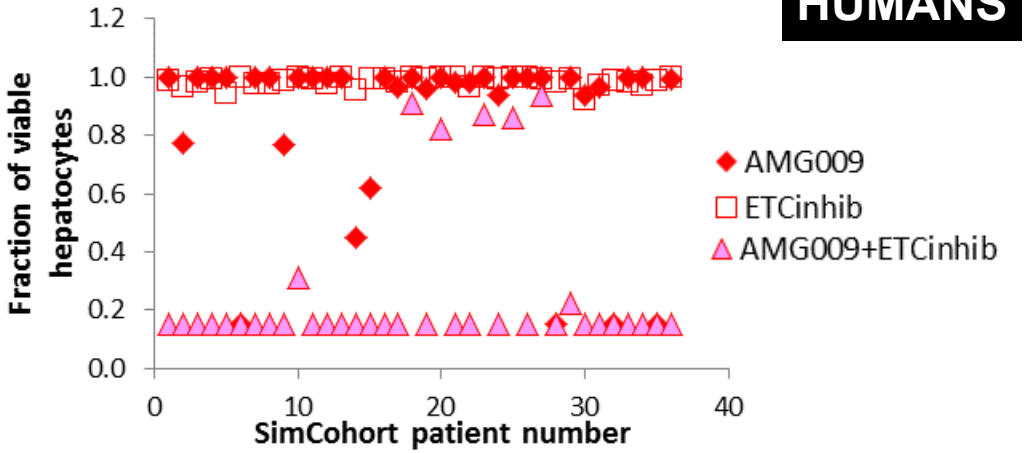
Combining BA Toxicity with ETC Inhibition Reveals Hepatotoxic Interaction Effect

- Predicted DILI in response to either theoretical ETC inhibitor or AMG009
  - AMG009 is bile acid transport inhibitor
  - N=36 SimCohort
  - Some predicted DILI for each drug (ALT, loss of hepatocytes)
- Combined ETC inhibitor + AMG009
  - Substantial increase in magnitude and frequency of predicted DILI with combination of two simulated drugs
- Simulations reveal clear interaction effect between bile acid-induced DILI and ETC inhibition
- ETC inhibitor is restricting adaptive increase in ETC flux
  - via S_fb_gradient
  - Loss of $\Delta\psi m$

HUMANS



HUMANS



Simulation Results

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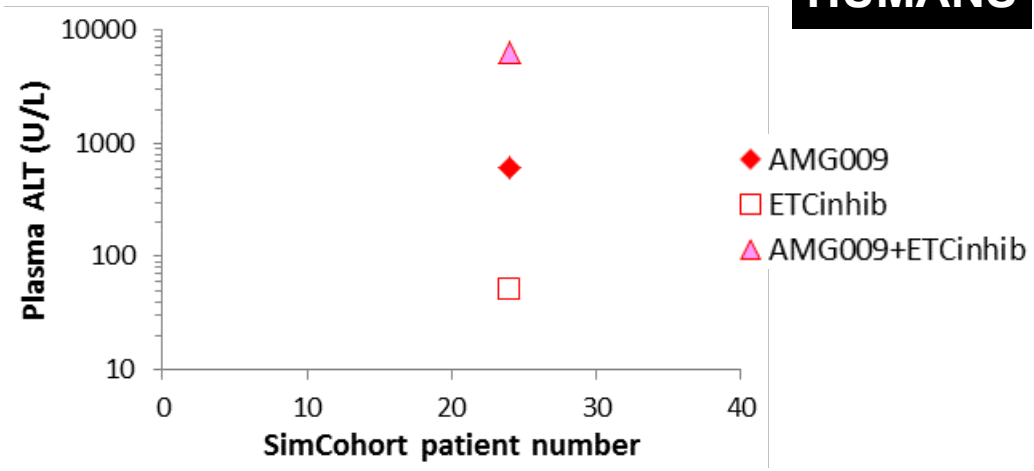
Combining BA Toxicity with ETC Inhibition Reveals Hepatotoxic Interaction Effect

- Predicted DILI in response theoretical ETC inhibitor and AMG009, alone and in combination
  - Patient #24 from n=36 SimCohort
  - Simulated 4 weeks of dosing

HUMANS



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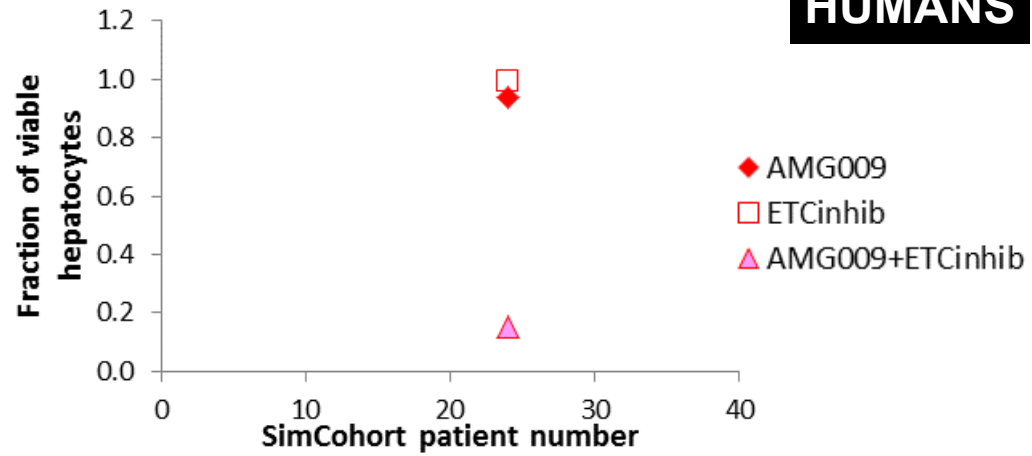

Simulation Results

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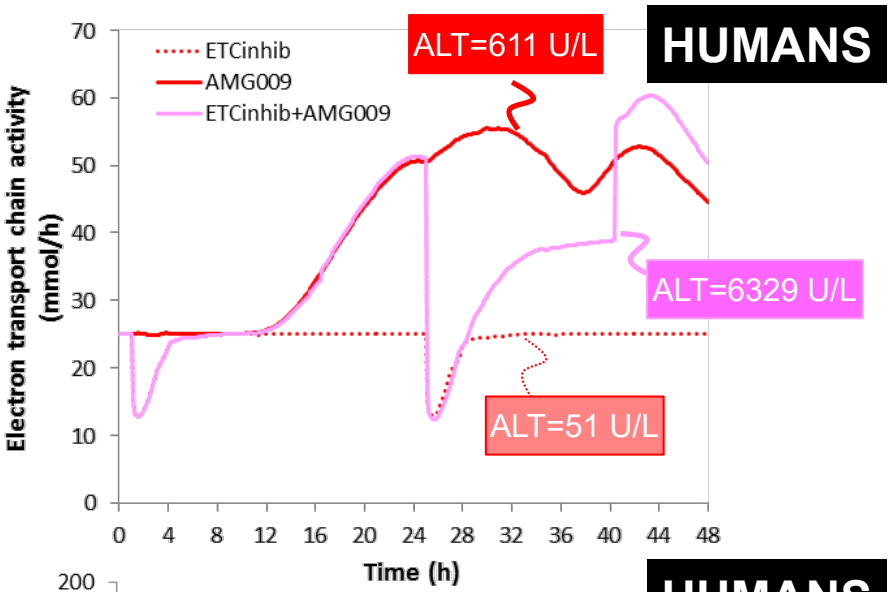
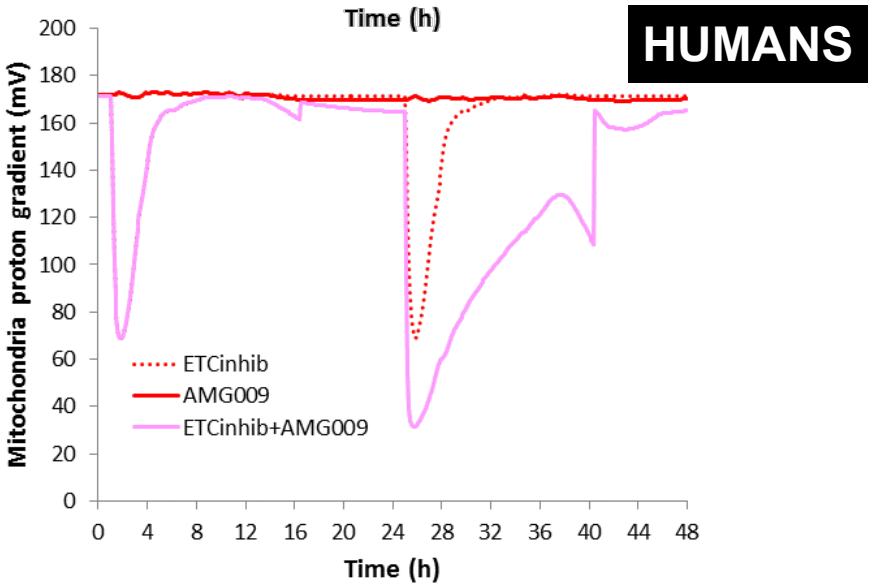
ETC Inhibition Restricts Adaptive Response to Bile Acid-Induced Mitochondrial Uncoupling

- Predicted DILI in response theoretical ETC inhibitor and AMG009, alone and in combination
  - Patient #24 from n=36 SimCohort
  - Simulated 4 weeks of dosing
- ETC activity
  - AMG009: adaptive increase with BA uncoupling
  - ETCinhib: decrease due to inhibition
  - AMG009+ETCinhib: direct ETC inhibition prevents adaptive increase
- Δψm
  - AMG009: proton gradient preserved through 48 h
  - ETCinhib: transient decrease due to ETC inhibition
  - AMG009+ETCinhib: sustained reduction due to ETC inhibition preventing adaptive increase
- Substantial increase in DILI risk due to interaction between ETC inhibitor and bile acid mitochondrial effects





Simulation Results

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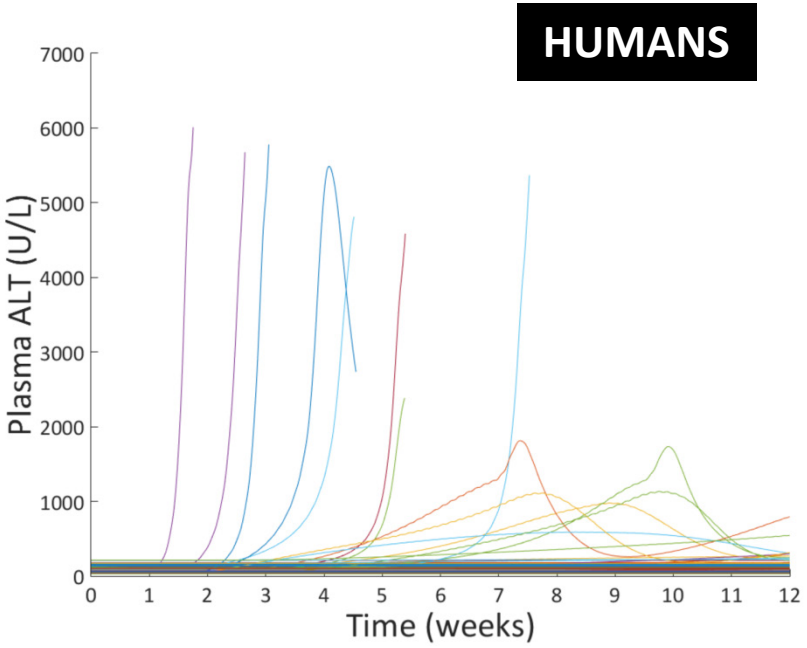
Synergy Between BA Accumulation and ETC Inhibition Can Explain Delayed-Onset Toxicity

- Delayed-onset liver toxicity is often thought to be indicative of an adaptive immune response
  - Unclear why delay would be required for adaptive immune activation in most circumstances
- ALT time course simulation results in T2D patients (right) demonstrate that delayed-onset liver toxicity can be explained by slow synergy between bile acid accumulation and ETC inhibition
  - Simulated ALT elevations occur anywhere from 1 week to 12 weeks after beginning of dosing
  - Adaptive immune attack still plausible explanation for TAK-875 toxicity; delay could be explained by delay in the development of cellular stress that would lead to immunogenic damage signals
  - Delay in manifestation of ALT elevations should not be taken as evidence of immune-mediated toxicity on its own
- Note overprediction of severity of TAK-875 toxicity
  - May be due to protective mechanisms not yet included in DILIsym
  - Lack of stop protocol in the simulations may also contribute to overprediction of severity



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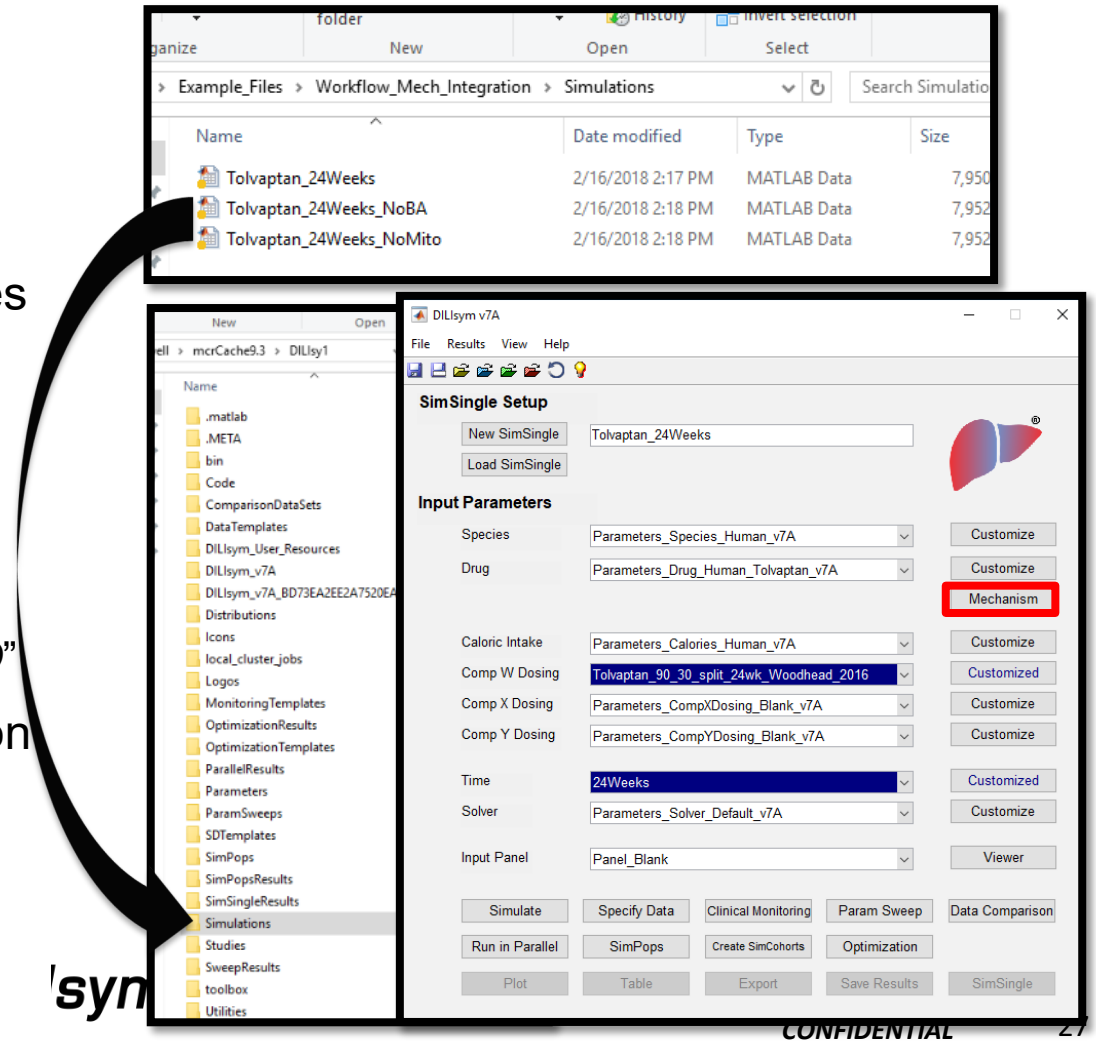
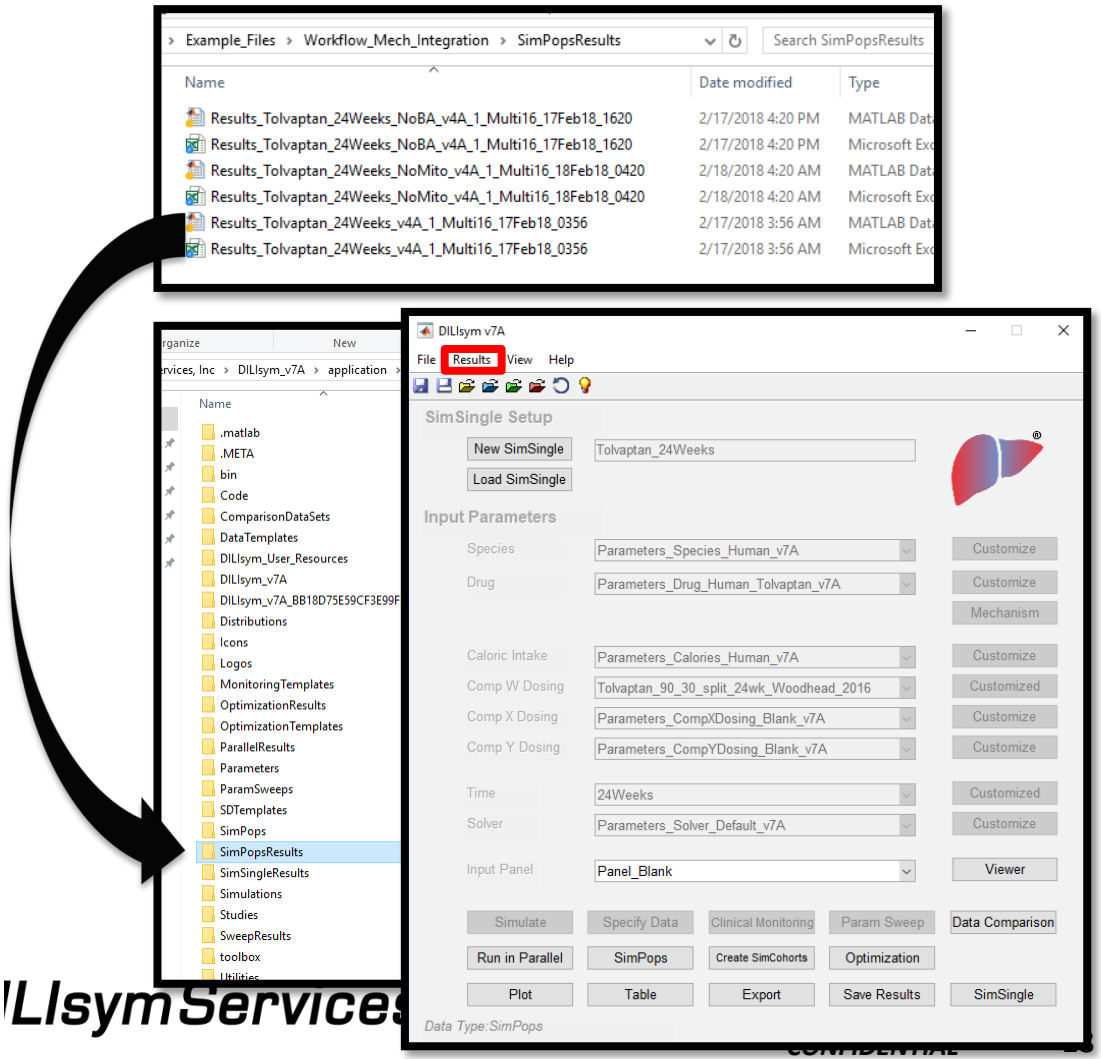
Hands-on DILI Mechanism Integration Example – *Step 1 – Place Provided Tolvaptan SimSingles in Simulations Folder and Review*

- Find Simulations directory by clicking any load option within DILIsym and copying location from Windows Explorer
- Copy three provided SimSingles into your Simulations directory
- Explore SimSingles
  - "***Tolvaptan_24Weeks***"
  - "***Tolvaptan_24Weeks_NoBA***"
  - "***Tolvaptan_24Weeks_NoMITO***"
- Review the mechanisms active on the Mechanism panel for each one



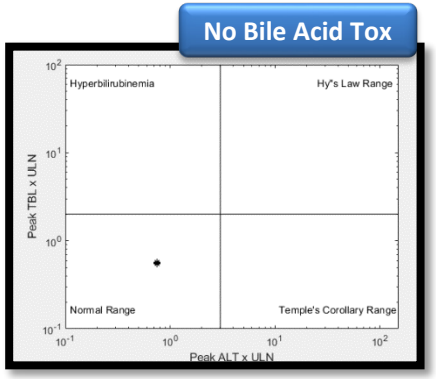
Hands-on DILI Mechanism Integration Example – *Step 2 – Place Provided Tolvaptan SimPops Results in SimPopsResults Folder*

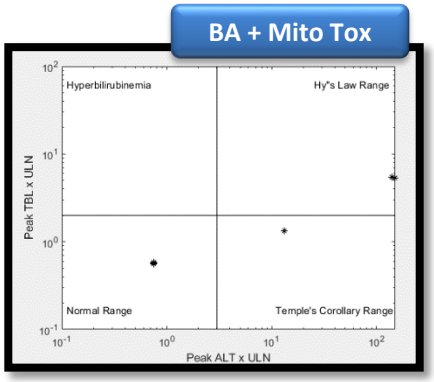
- Find SimPopsResults directory by clicking any load option within DILIsym and copying location from Windows Explorer
- Copy three provided SimPops results files into your SimPopsResults directory
- Load "***Results_Tolv……Multi16_17Feb18_0356***" results file using the *Results* menu -> ***Load SimPops Setup and Results*** option
  - Tolvaptan with all mechanisms active

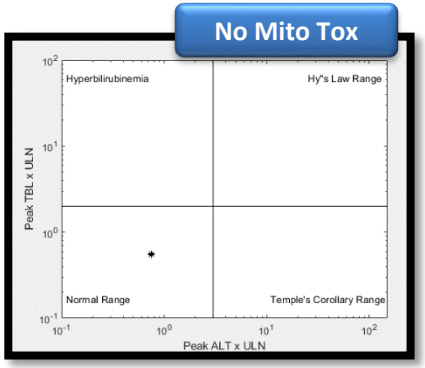


Hands-on DILI Mechanism Integration Example – *Step 3 – Analyze SimPops Results and Load Other Two Tolvaptan Results Files*

- Note response in liver ATP levels
  - *Mitochondria -> Bioenergetics -> **Liver average ATP***
- Note eDISH plot
- Repeat process for other two tolvaptan SimPops results files
- ***Which mechanism is responsible for the ALT elevations?***







Simulation Results

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