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DILIsym User Training –

DILIsym Simulations with Exploratory Mitochondrial Biogenesis Parameters

DILIsym Development Team

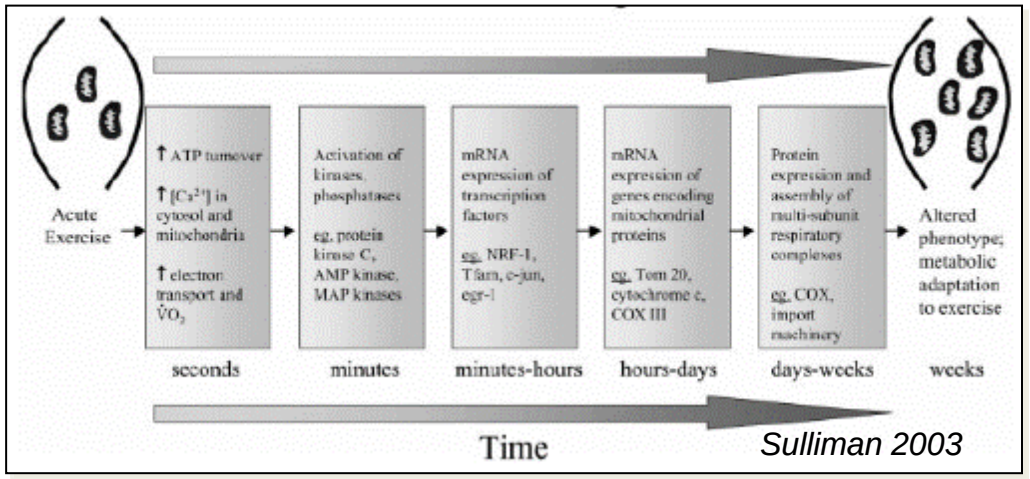
Goals for This Training Session

Participants should understand the following general concepts:

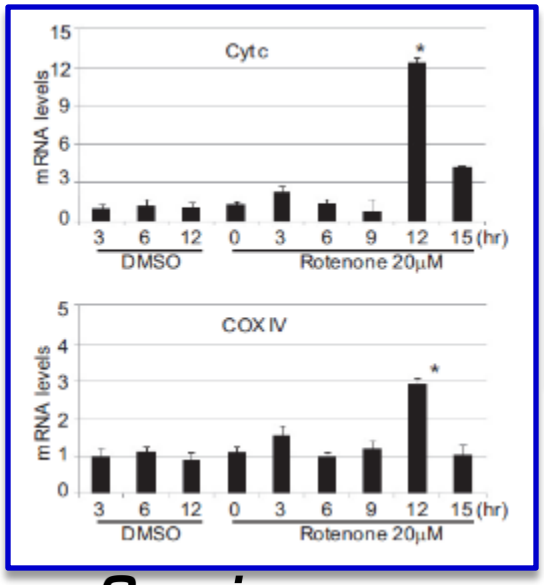
- Background, DILIsym design, and practical information for exploratory mitochondrial biogenesis within DILIsym

Mitochondrial Biogenesis Can Help Overcome Bioenergetic Duress

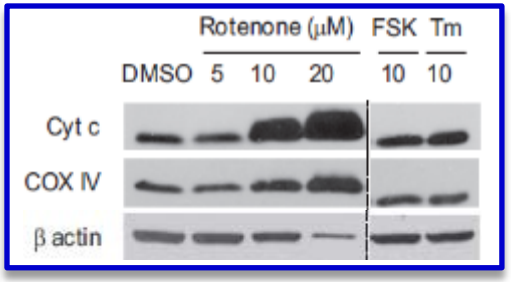
- Well documented that adaptive mitochondrial biogenesis helps compensate for bioenergetic stress in muscle
  - Multiple steps operate on different time scales (*Sulliman 2003*)
  - A primary initiating signal is ATP loss
- Some evidence that similar adaptations occur in liver
  - Than et al. treated primary mouse hepatocytes with rotenone for 3 h to reduce ATP
  - Measured increased expression of mRNA (left) and mitochondrial ETC proteins (right) after time delay
  - Unclear if kinetic response directly translates to *in vivo* situations
  - Have used these data to generate exploratory parameters for adaptive mitochondria biogenesis



Sulliman 2003



MOUSE HCs



Than 2011

Preclinical Data

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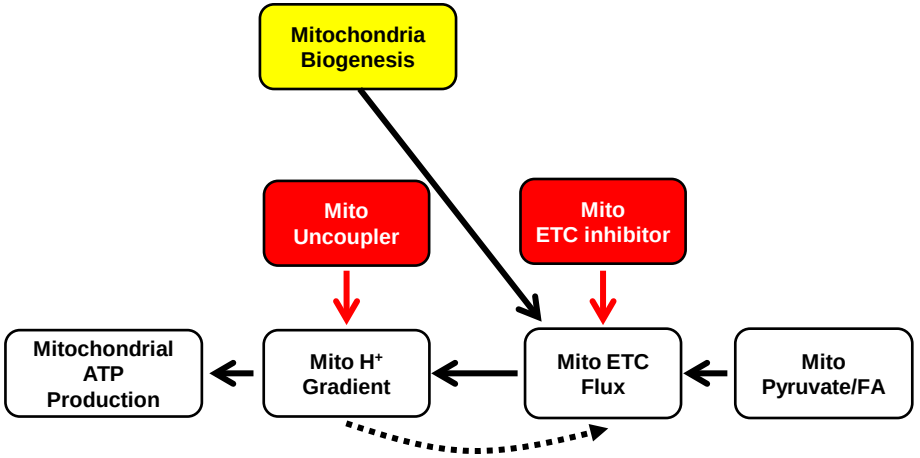
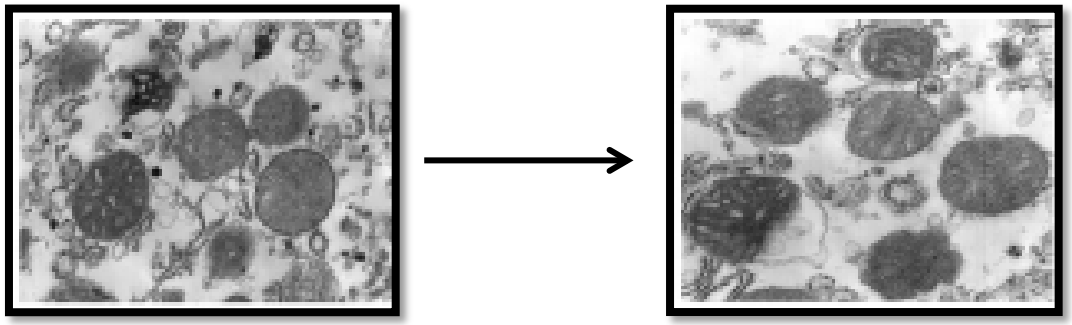
Mitochondrial Biogenesis Can Reduce Sensitivity to DILI

- Increased number of mitochondria can partially offset mitochondrial dysfunction
  - Need to be functional mitochondria
- Increased number of not-inhibited ETC complexes can act to preserve ATP synthesis
- Increased number of mitochondria can reduce degree of uncoupling in each mitochondria
  - Helps to preserve ATP synthesis

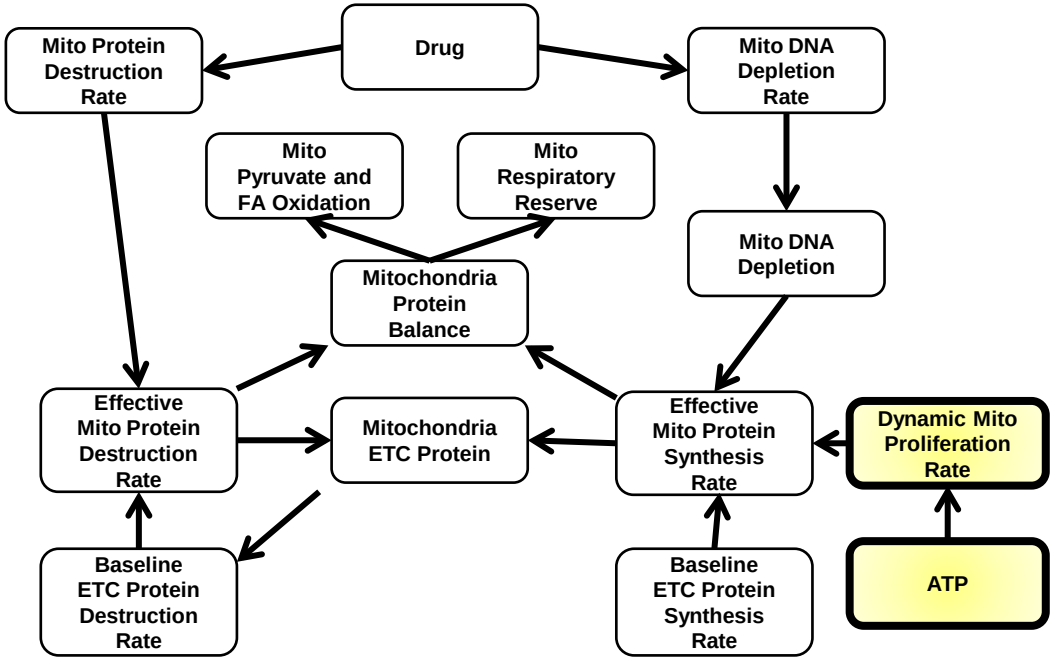




Justo 2005

Dynamic Mitochondrial Biogenesis Is Included in DILIsym

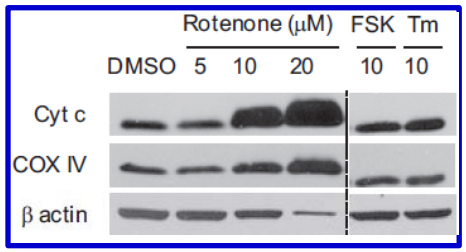
- Mitochondrial biogenesis equations are included in DILIsym
  - Enables exploration of hypothesis that mitochondrial adaptations can mitigate DILI
- Mitochondrial ETC protein content is determined by the balance of synthesis and destruction rates
  - At steady-state, baseline synthesis and destruction rates are assumed equal; parameters obtained from experimental data (Schwerzmann 1986, Price 2012)
  - The dynamic synthesis rate is driven by changes in liver ATP and mtDNA content



Parameter	Value	Unit
Basal mitochondria ETC protein content	9.56e-14	mmol
Rate constant for baseline mitochondria ETC protein synthesis	0.0069	1/hr

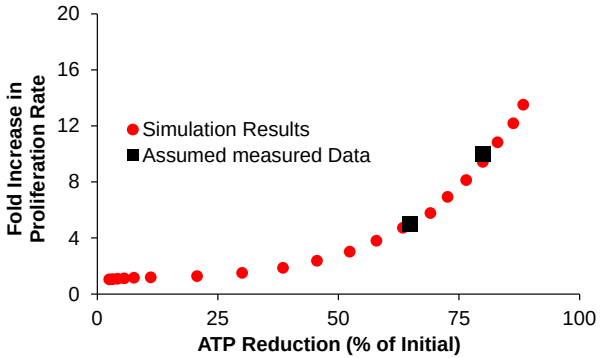
Exploratory Biogenesis Parameters Generated Based on *in vitro* Studies

- Exploratory biogenesis parameters generated based on hepatocellular *in vitro* studies (Than 2011)
  - Mito ETC enzyme expression from primary mouse hepatocytes treated with varying levels of rotenone for 3 h; difficult to determine quantitative effects
  - Used MITOsym to simulate study and predict ATP reductions with rotenone exposure
  - Combined simulation results with data describing ETC protein expression
  - Baseline parameter values set to have NO effect



MOUSE HCs

Than 2011



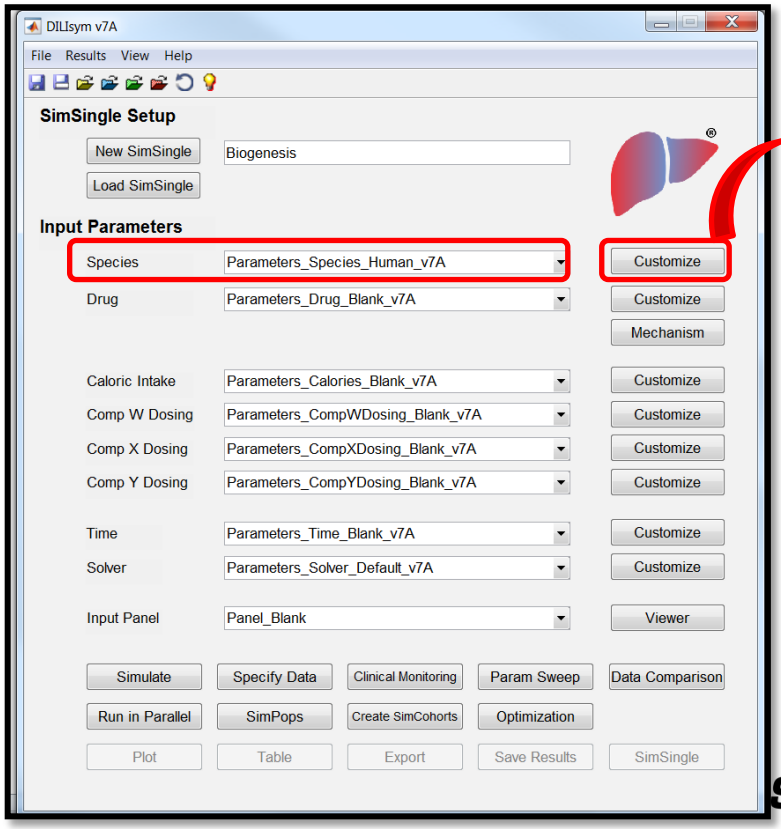
Parameter	Unit	Value
Mitochondria protein proliferation Vmax	mmol/hour	3.59e-13
Mitochondria protein proliferation Km	dimensionless	0.55
Mitochondria protein proliferation Hill	dimensionless	1.5
ATP decrement delay constant for mitochondria	hr	12

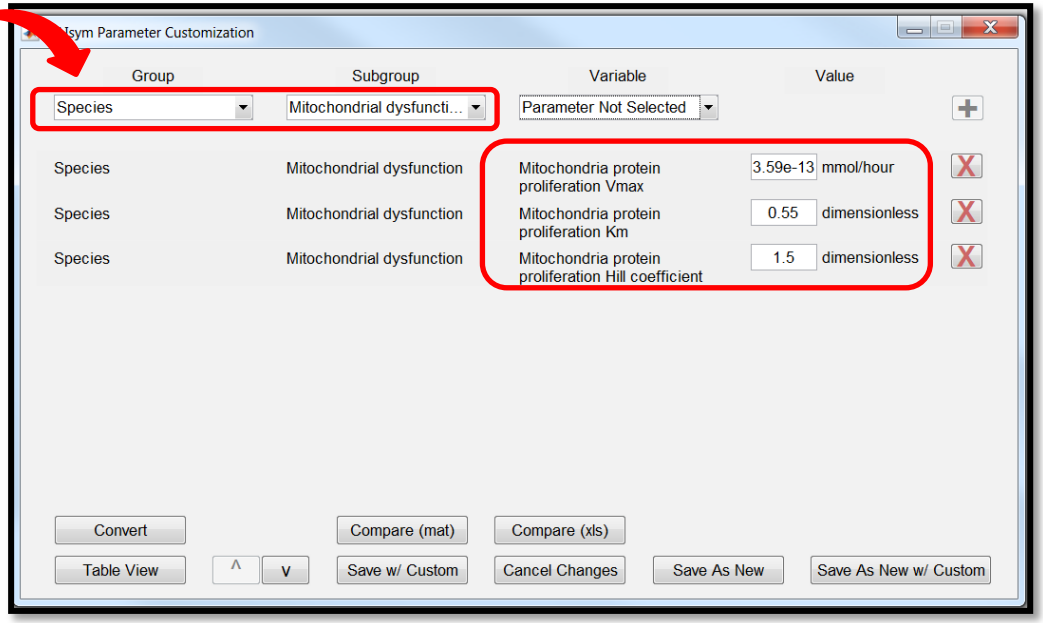
Preclinical Data

Biogenesis Parameters Can be Altered in The Species Parameter Set

- To activate biogenesis, alter the values of the DILIsym parameters shown in the table to the values in the table (Species parameter set)
  - *Species -> Mitochondrial Dysfunction*

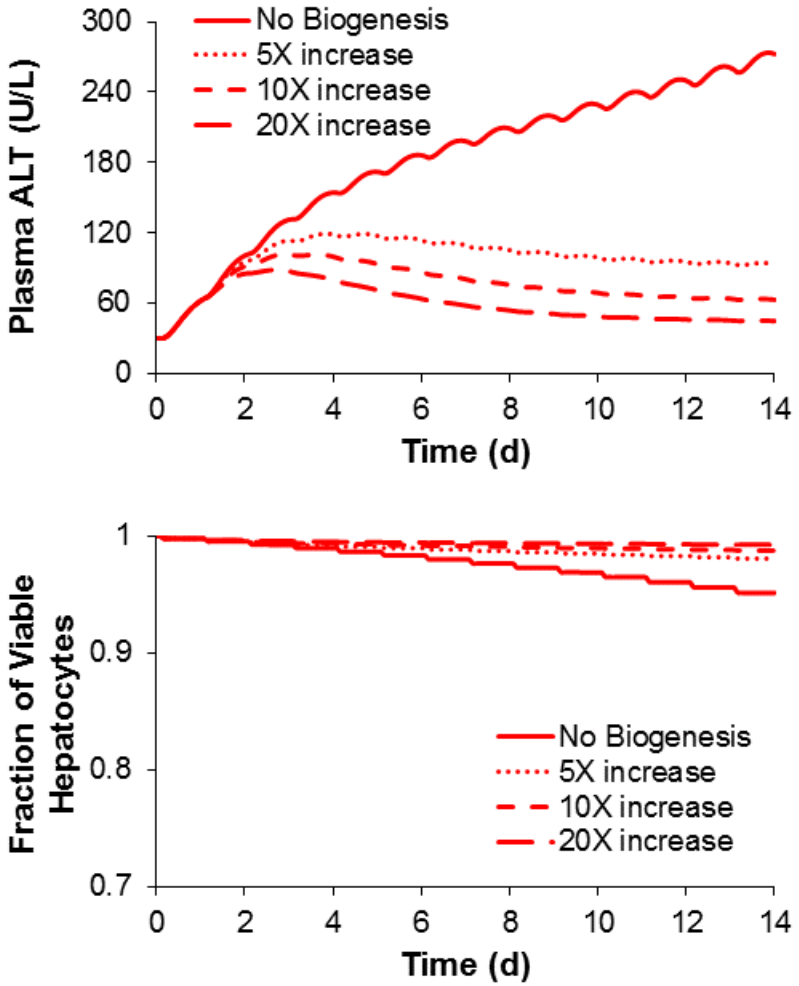
Parameter	Unit	Value
Mitochondria protein proliferation Vmax	mmol/hour	3.59e-13
Mitochondria protein proliferation Km	dimensionless	0.55
Mitochondria protein proliferation Hill	dimensionless	1.5
ATP decrement delay constant for mitochondria	hr	12





Adaptive Mitochondrial Biogenesis Can Mitigate DILI

- Tested exploratory biogenesis parameters by simulating injury in DILIsym with exemplar drug buprenorphine
  - 85 mg administered q.d. for 2 weeks
  - Mitochondrial uncoupling and ETC inhibition
- Adaptive mitochondrial biogenesis may mitigate mitochondrial-based DILI
  - Stronger biogenesis signals reduce magnitude of injury (ALT)
  - Simulations provide proof of concept that equations are functional and biochemistry could participate in observed DILI
- Not confident that biogenesis timing and magnitude in controlled hepatocyte studies translates to *in vivo* environment
  - Parameters can be established when *in vivo* studies have been conducted



Simulation Results

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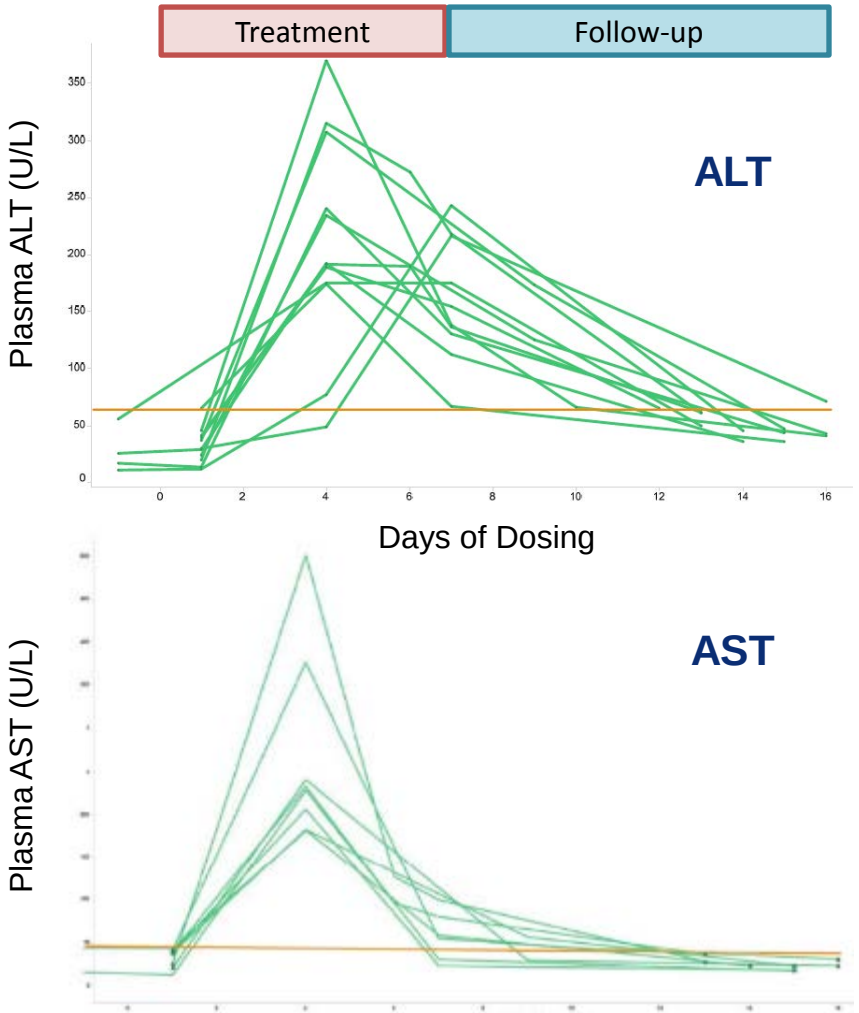
Apparent Adaptation in Patients Treated with Solithromycin

HUMAN

- Patients treated with solithromycin for 7 days
  - ALT and AST were measured at baseline (Day -1 or Day 1), on Days 4 and 7, and then 5-10 days after therapy (Days 12-17)
- ALT at day 7 was less than at day 4 for most patients
  - Despite continued dosing through day 7
- ALT and AST elevations were asymptomatic
  - No bilirubin elevations



Clinical Data

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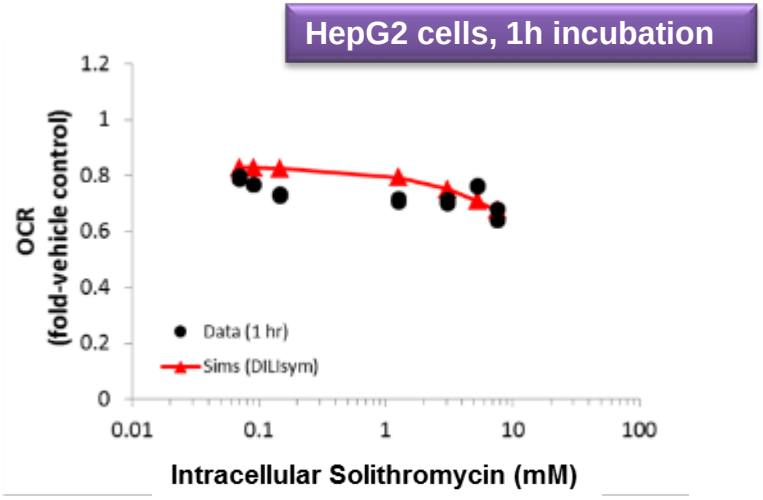
Parameters Identified for Solithromycin Mediated Mitochondrial Dysfunction

- Solithromycin is a mild inhibitor of mitochondrial electron transport chain
  - Cellular respiration via Seahorse XF analyzer
  - Mitochondrial function was normalized to cell viability and to vehicle control
- LC/MS/MS data used to estimate intracellular concentrations of solithromycin at each dose
- Solithromycin concentration-dependent changes in mitochondrial function were recapitulated in DILIsym
  - Parameter values determined and employed in subsequent simulations



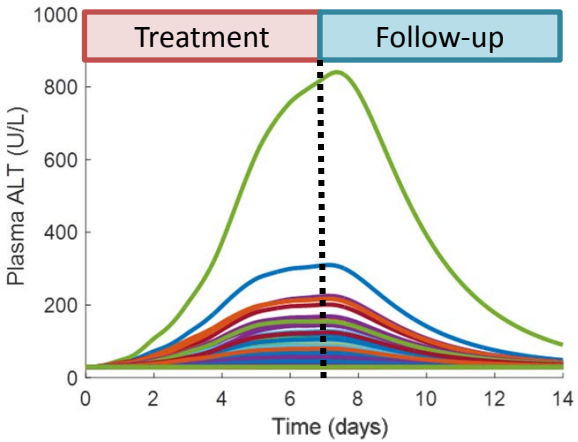
Biogenesis Parameters Optimized to Represent Clinically Observed Adaptation

- Biogenesis parameters based on the *in vitro* mouse HC data over-predicts adaptation
- Biogenesis parameters further optimized to represent clinically observed adaptation of solithromycin
  - With adaptation, ALT peaks on day 4 and resolves with continuing treatment, consistent with the clinical data

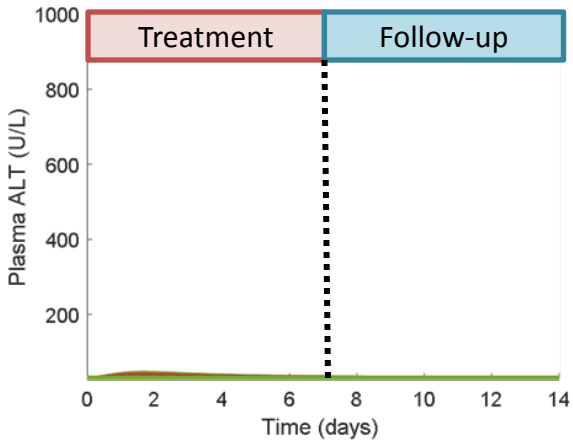
Parameter	Unit	In Vitro	Clinical
Mitochondria protein proliferation Vmax	mmol/hour	3.59e-13	4e-14
Mitochondria protein proliferation Km	dimensionless	0.55	0.8
Mitochondria protein proliferation Hill	dimensionless	1.5	1.5
ATP decrement delay constant for mitochondria	hr	12	96

HUMAN

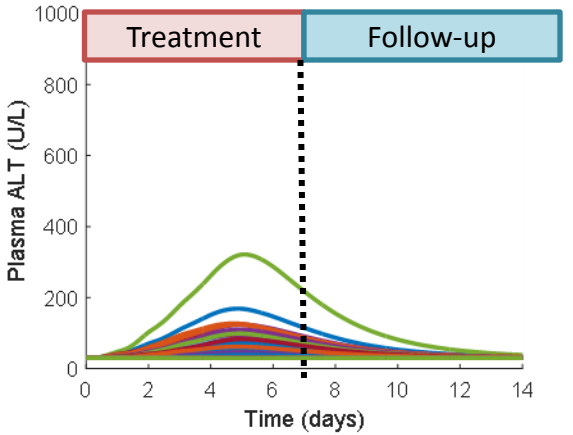
Without biogenesis



With *in vitro*-based biogenesis parameters



With *in vivo*-based biogenesis parameters



Simulation Results

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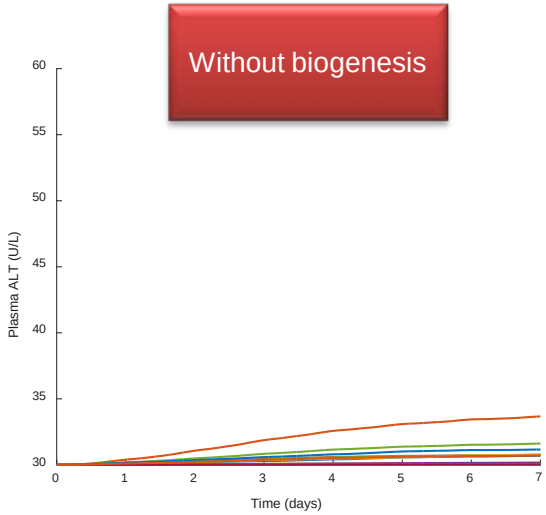
Important Considerations When Evaluating the Simulation Results with Adaptation

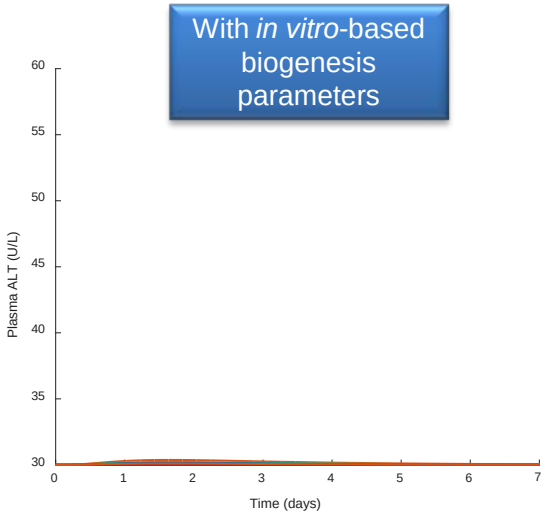
- Adaptation to liver injury onset through mitochondrial biogenesis was explored through optimization of parameters to clinical data after short term dosing
- The safety margin for longer term solithromycin dosing may be significantly improved through adaptation; this was the case in the simulations
- Simulations of adaptation are exploratory and preliminary and this phenomena must be proven through clinical experience in a large number of patients
  - No inter-individual variability built into these exploratory simulations
  - Assumption made that adaptation observed during short-term treatment extends to long-term treatment
  - Not known what percentage of treated individuals will adapt versus which will not
  - Disease state and age could impair ability to adapt (not built into these early simulations)

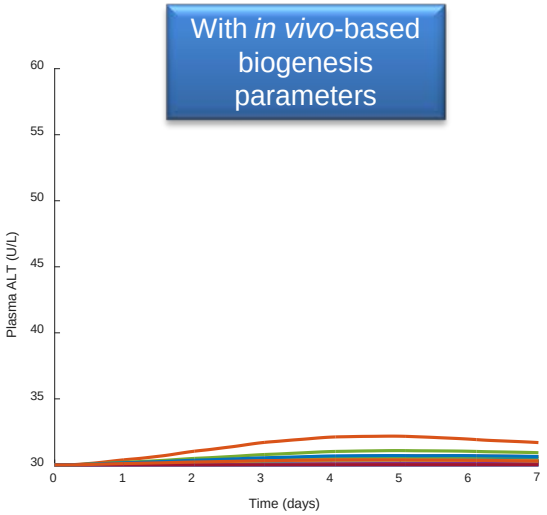
Biogenesis Alleviates ALT Elevations Following Phenformin Administration

- Phenformin imposes modest mitochondrial ETC inhibition at clinically relevant concentrations
  - ALT elevations not reported for phenformin
  - Exploratory simulations performed with phenformin (50 mg BID) using two different sets of biogenesis parameters
- Early, small increase in ALT is predicted in some patients without biogenesis
- Simulations of phenformin monotherapy including mitochondrial biogenesis show no risk of DILI

HUMAN







Simulation Results

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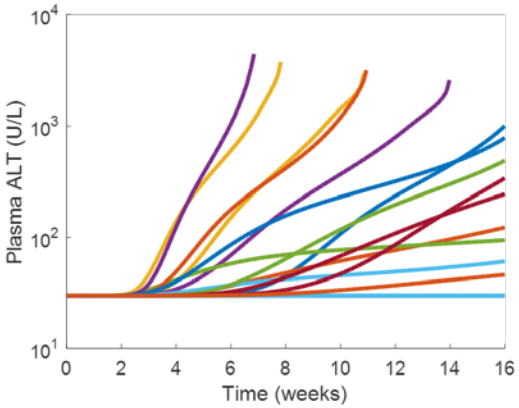
Biogenesis Alleviates ALT Elevations Following Compound Y Administration

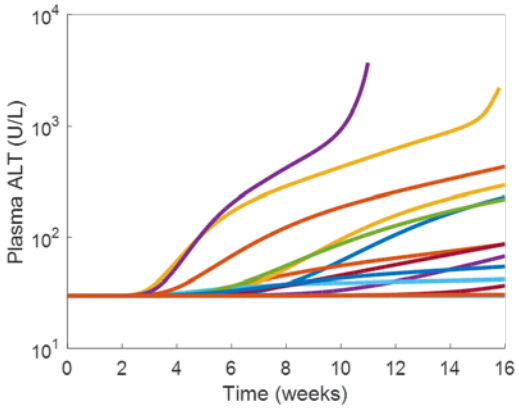
- Compound Y imposes mitochondrial ETC inhibition and oxidative stress
  - ALT elevations reported for Compound Y
  - Exploratory simulations performed with Compound Y using two different sets of biogenesis parameters
- Significant ALT elevations are predicted in some patients without biogenesis
- Simulations of Compound Y including mitochondrial biogenesis show reduced risk of DILI

HUMAN

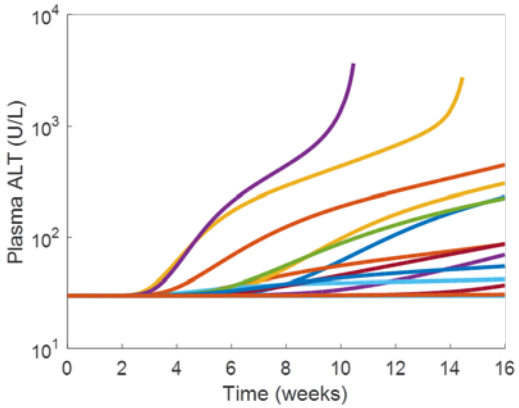
Without biogenesis



With *in vitro*-based biogenesis parameters



With *in vivo*-based biogenesis parameters



Simulation Results

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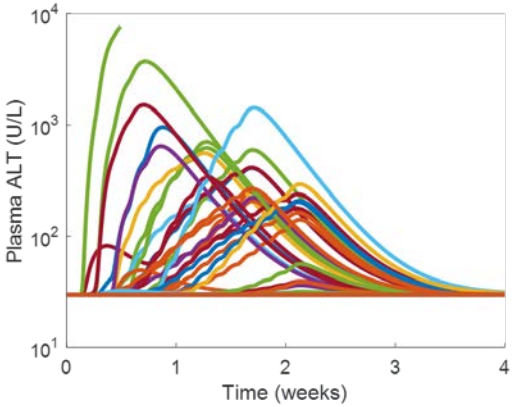
Biogenesis Does Not Alleviate AMG 009-Mediated ALT Elevations

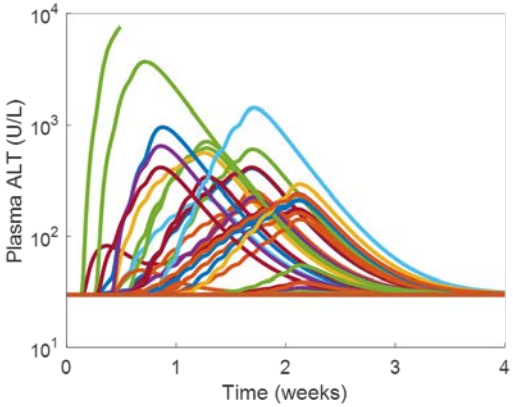
- AMG 009, an inhibitor of bile acid transporter, caused ALT elevations
  - Exploratory simulations performed with AMG 009 using two different sets of biogenesis parameters
- Significant ALT elevations are predicted in a subset of patients without biogenesis
- Simulations including mitochondrial biogenesis parameters indicate the activation of biogenesis but it has minimal impact on serum ALT levels
  - Impact of biogenesis on adaptation to DILI depends on multiple factors such as underlying mechanisms, extent and timing of injury

HUMAN

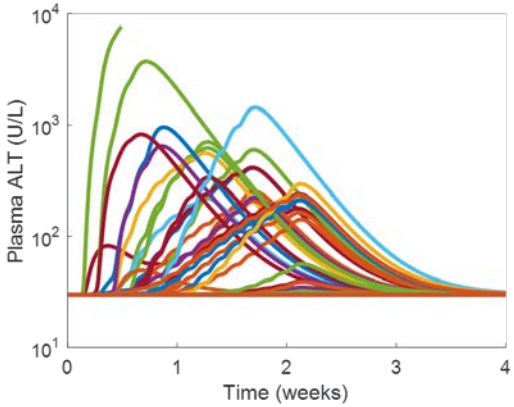
Without biogenesis



With *in vitro*-based biogenesis parameters



With *in vivo*-based biogenesis parameters



Simulation Results

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HUMAN

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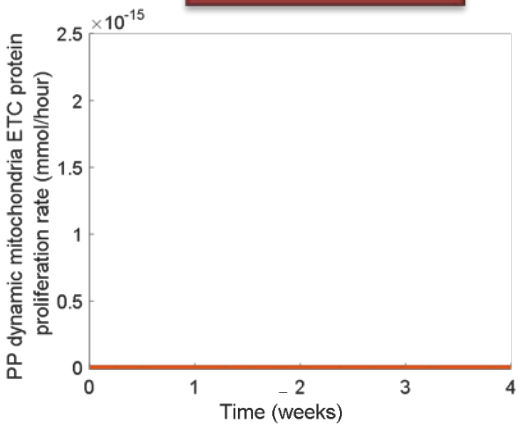
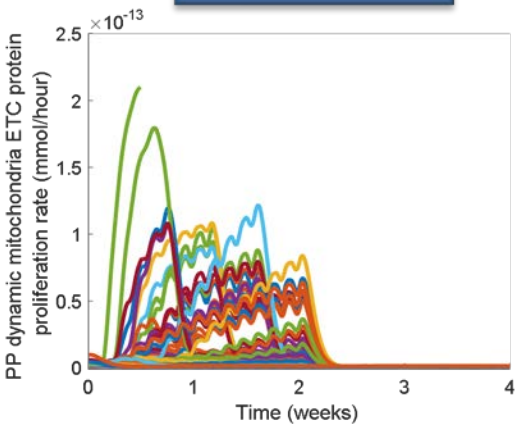
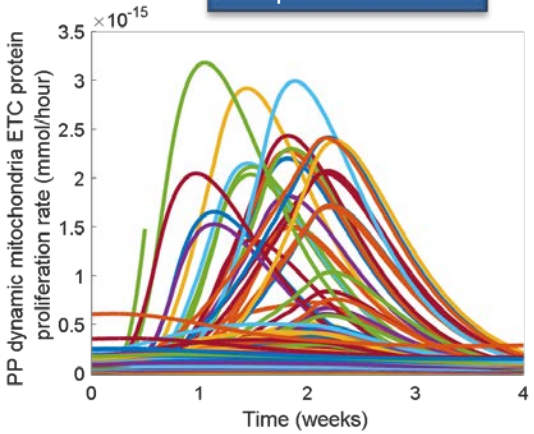


With *in vitro*-based biogenesis parameters



With *in vivo*-based biogenesis parameters

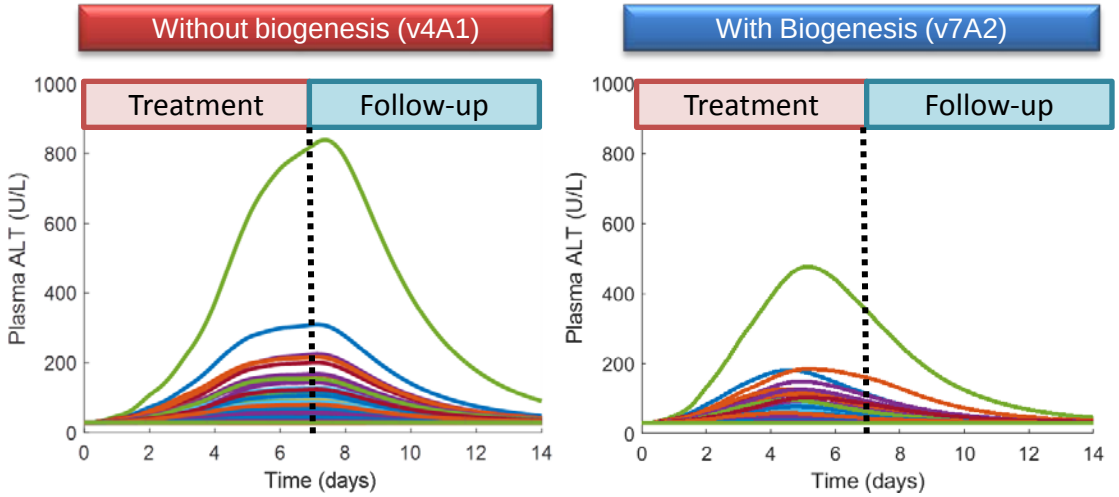


Simulation Results

SimPops Including Variability in Mitochondrial Biogenesis Added to DILIsym v7A

- Human mitochondrial biogenesis SimPops added to DILIsym v7A
  - Human ROS apop mito BA Biogenesis v7A_2 (n=285); <u>for exploration only</u>
  - Generated using general toxicity parameters from the SimPops v4A_1 combined with mitochondrial biogenesis parameters
  - Variability added to “Mitochondria protein proliferation Vmax” assuming 30% CV
- Solithromycin simulations with biogenesis SimPops recapitulate clinically observed ALT normalization during treatment

Parameter	Unit	Baseline Value	SimPops value
Mitochondria protein proliferation Vmax	mmol/hour	$4e^{-14}$	$1e^{-14} – 7e^{-14}$
Mitochondria protein proliferation Km	dimensionless	0.8	0.8
Mitochondria protein proliferation Hill	dimensionless	1.5	1.5
ATP decrement delay constant for mitochondria	hr	96	96



Simulation Results

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Biogenesis Conclusions and Perspectives

- Preclinical data support the activation of mitochondrial biogenesis in response to bioenergetic stress
- Exploratory simulations using DILIsym suggest that mitochondrial biogenesis can mitigate DILI caused by mitochondrial dysfunction
  - May be one of the mechanisms underlying frequent observation of adaptation during DILI
- Further work is required to ensure that use of mitochondrial biogenesis for prospective predictions is appropriate
  - Default DILIsym parameter sets do not include mitochondrial biogenesis
  - More *in vitro* and clinical data needed to identify reliable biogenesis parameters and relevant variability