DILIsym Services

A SIMULATIONS PLUS COMPANY

DILIsym User Training – Mitochondrial DNA Depletion within DILIsym

DILIsym Development Team

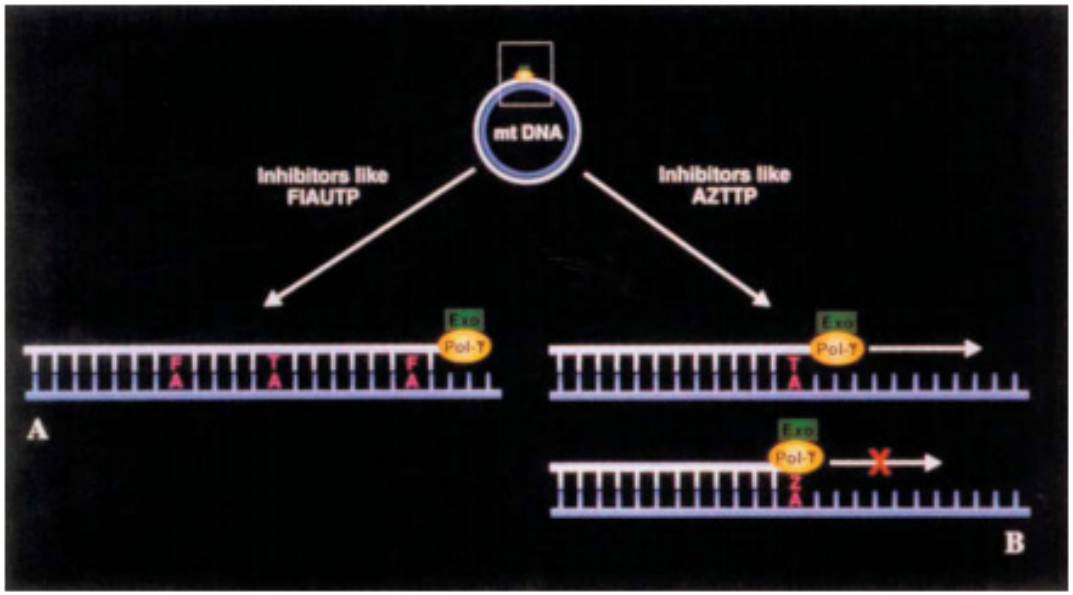
Goals for This Training Session

Participants should understand the following general concepts:

- Background, DILIsym design, and practical information for the DILI mechanism of mitochondrial DNA depletion within DILIsym

Multiple Mechanisms for Drug-Induced mtDNA Depletion

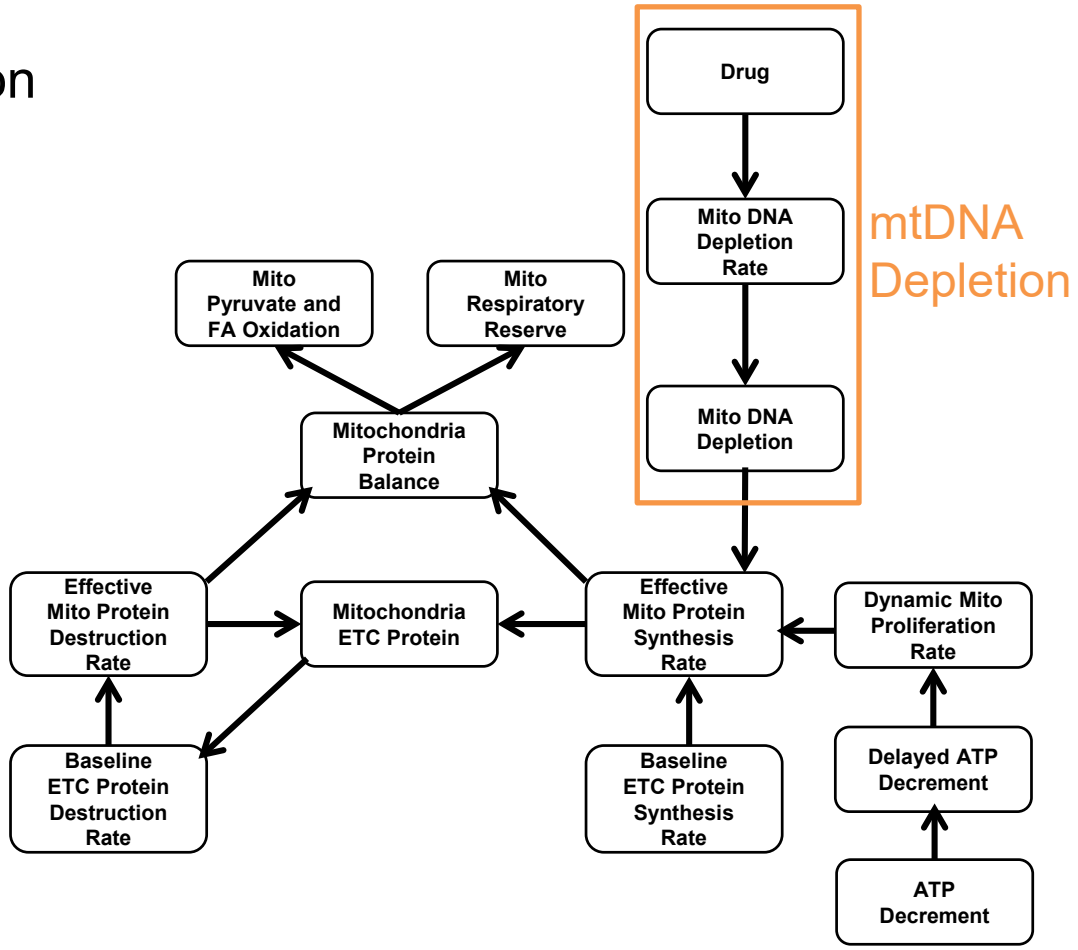
- Some nucleoside analogs integrate into mtDNA
  - FIAU is an example
  - Delays inhibitory effect on mtDNA synthesis and Mito DNA Depletion Rate
- Other nucleoside analogs terminate mtDNA chain elongation rapidly
  - AZTTP, ddC, and ddI are examples
  - Effectively inhibitors of DNA polymerase γ (*Lewis 1995*)
  - More rapid effect on mtDNA synthesis inhibition and Mito DNA Depletion Rate



Adapted from Lewis 1995

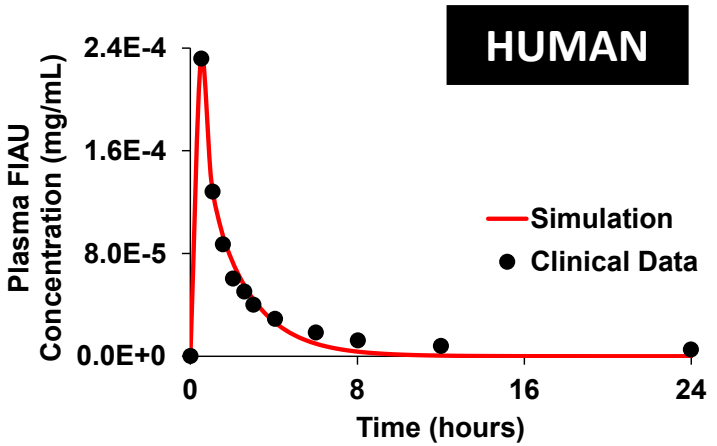
mtDNA Depletion Is Included in DILIsym

- Drug-induced mtDNA depletion can lead to severe hepatotoxicity
  - mtDNA encodes essential components of mitochondrial ETC
  - Several drugs have been shown to deplete mtDNA
  - Fialuridine is an exemplar compound

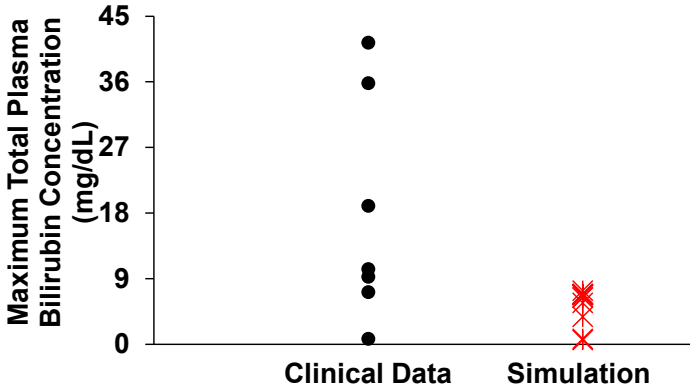


Mitochondria DNA (mtDNA) Depletion and Fialuridine (FIAU)

- FIAU treatment causes delayed mtDNA and enzyme loss
  - FIAU effects on hepatocyte function were based on reductions in mtDNA synthesis and subsequent disruptions in replication and mitochondrial function
  - Parameters describing the rate of FIAU-imposed mtDNA reductions were calculated based on in vitro data (*Lewis 1996*) and subsequently optimized based on clinical DILI responses (*McKenzie 1995*)
- DILIsym accurately estimates DILI due to FIAU administration
  - Accurately captures the plasma FIAU PK in humans
  - A comparable frequency of severe liver injury is observed in the SimCohort (11 out of 15 patients) as in the clinical patients (7 out of 10 patients).
  - The proportion of simulated patients with maximum total plasma bilirubin concentrations exceeding 3 mg/dL was also comparable with the clinical patients.
- Further investigation will be required to bridge the gap between the *in vitro* data and optimized data for rate of drug-imposed mtDNA reductions.



Bowsher 1994



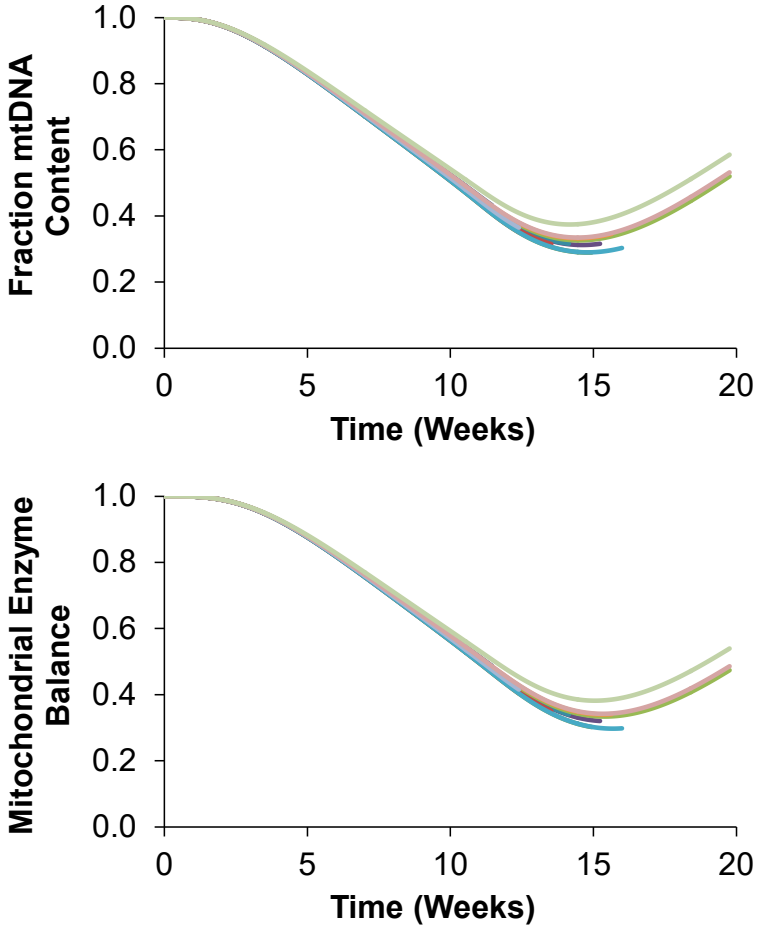
McKenzie 1995

Clinical Data and Simulation Results

DILIsym Services
A SIMULATIONS PLUS COMPANY

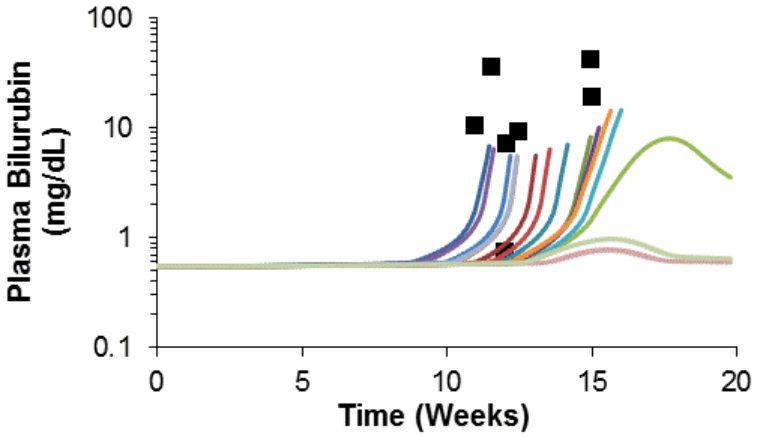
FIAU Reduces mtDNA Content and Mitochondrial Enzymes

- FIAU treatment causes delayed mtDNA and enzyme loss
  - FIAU interferes with mtDNA synthesis due to its nature of being a nucleotide analog
  - There is an inherent initial delay of FIAU effects due to DNA synthesis kinetics
  - Mitochondrial enzyme balance directly affects both respiratory reserve and pyruvate/fatty acid oxidation rates (i.e., ETC function)
  - Liver toxicity is not observed until mtDNA is reduced to <50% of initial content
- When FIAU is withdrawn, mtDNA replication and mitochondria function can be restored

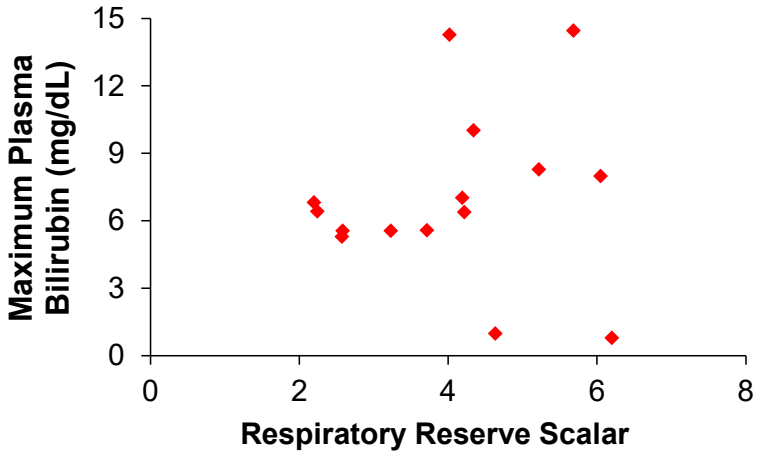


Simulation Results Show Delayed Plasma Bilirubin Increases and Severe Liver Injury

- FIAU elicited severe hepatotoxicity in phase II trials
  - 7/10 patients receiving FIAU for ≥9 weeks had fulminant liver injury
  - Cumulative doses ranged from 551 to 1753 mg
  - Delayed presentation of injury
  - Clinical data for individual patients plotted with black squares
- Large proportion of simulated patients have liver failure within 20 weeks in simulations of FIAU phase II clinical trial
  - n=15 SimCohort patient having initial respiratory reserve scalar between 2.2 and 6.02
  - Elevated bilirubin indicates severe liver injury in >80% of patients in SimCohort
  - Patients having low initial respiratory reserve scalar parameter are at higher risk of liver injury
  - Simulated ALT levels do not agree with clinical data; could be due to type of patients used in simulations
  - Continuing to optimize FIAU representation



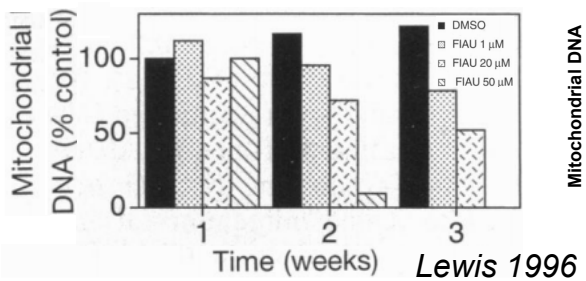
McKenzie 1995



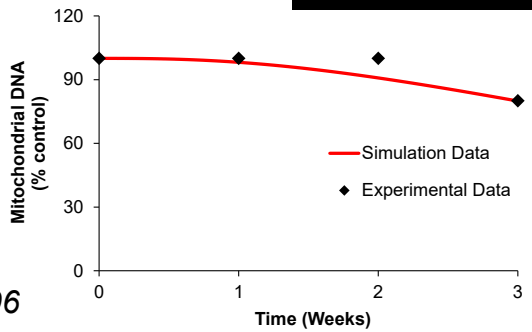
HepG2 Based mtDNA Data May Provide Inappropriate Parameter Values

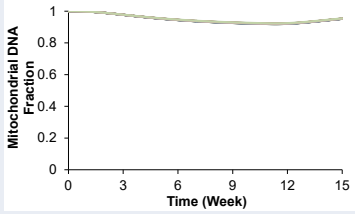
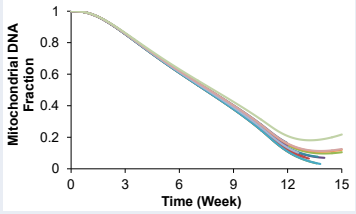
HepG2

- Dose-dependent decreases in mtDNA abundance in HepG2 cell due to FIAU
  - FIAU inhibits DNA polymerase γ sites and restricts DNA chain elongation after incorporating into mtDNA (*Lewis 1996*)
- Setup an "*in vitro*–like" simulation within DILIsym using compound Y based on the Lewis 1996 protocol
  - For 1uM FIAU medium concentration, mtDNA degradation rate = 124,000 mol/mL/hour
  - Poorly predicts the clinical outcomes
- HepG2 mtDNA degradation rate parameter values required a correction factor to align with values inferred from clinical data



Lewis 1996



	Based on HepG2 Data	Based on Clinical Data
mtDNA degradation rate parameter (mL/mol/hour)	124,000	291,000
Time delay parameter (hour)	168	168
Predicted clinical incidence	0% (70%)	73% (70%)
Predicted range of time of onset (weeks)	NA (11-17)	12-14 (11-17)
Correction factor	2.3	
Predicted mtDNA fraction		

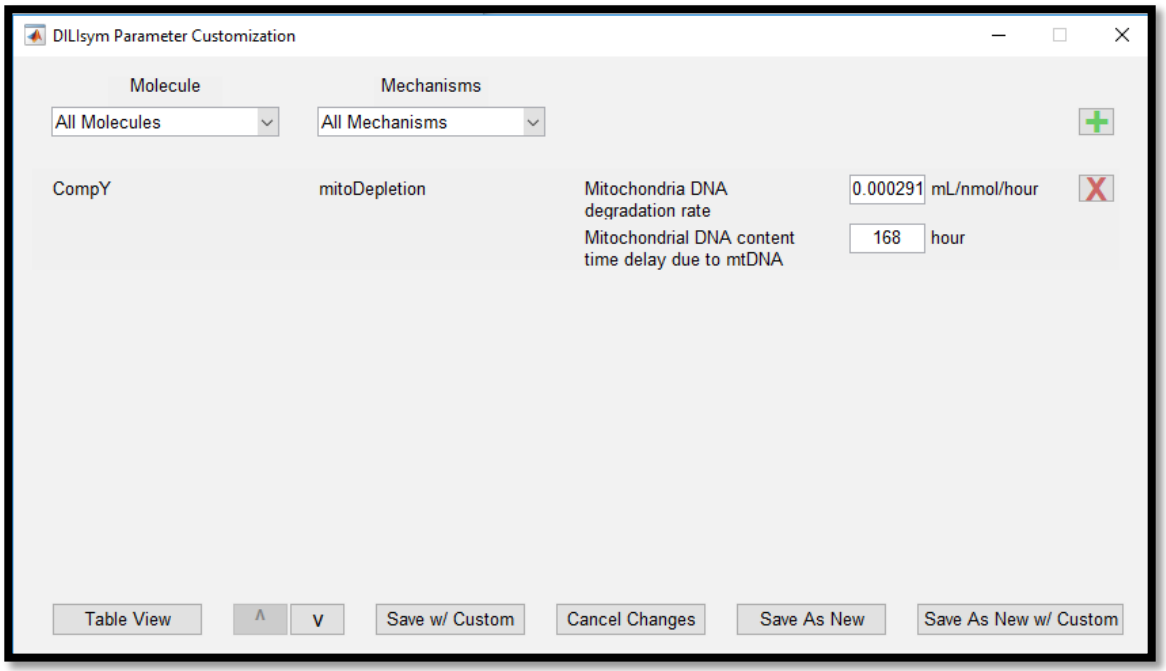
Preclinical Data and Simulation Results

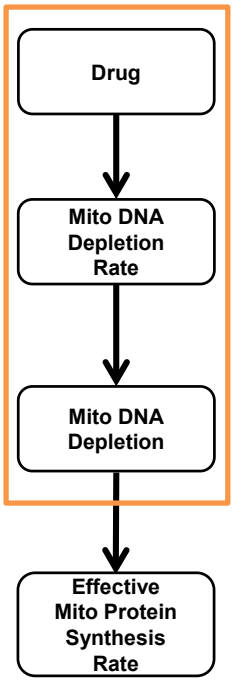
DILIsym Services

A SIMULATIONS PLUS COMPANY

Mitochondria DNA (mtDNA) Depletion DILIsym Setup

- mtDNA depletion based drug toxicity mechanism is included in DILIsym as part of toxicity mechanisms panel
  - Baseline parameter values set to have NO effect
  - Parameters describing the rate of mtDNA degradation and time delay need to be calculated from *in vitro* data





Preclinical Data

DILIsym Services

A SIMULATIONS PLUS COMPANY