DILIsym® User Training - Gathering Mitochondrial Toxicity Data for DILIsym®

DILI-sim Team

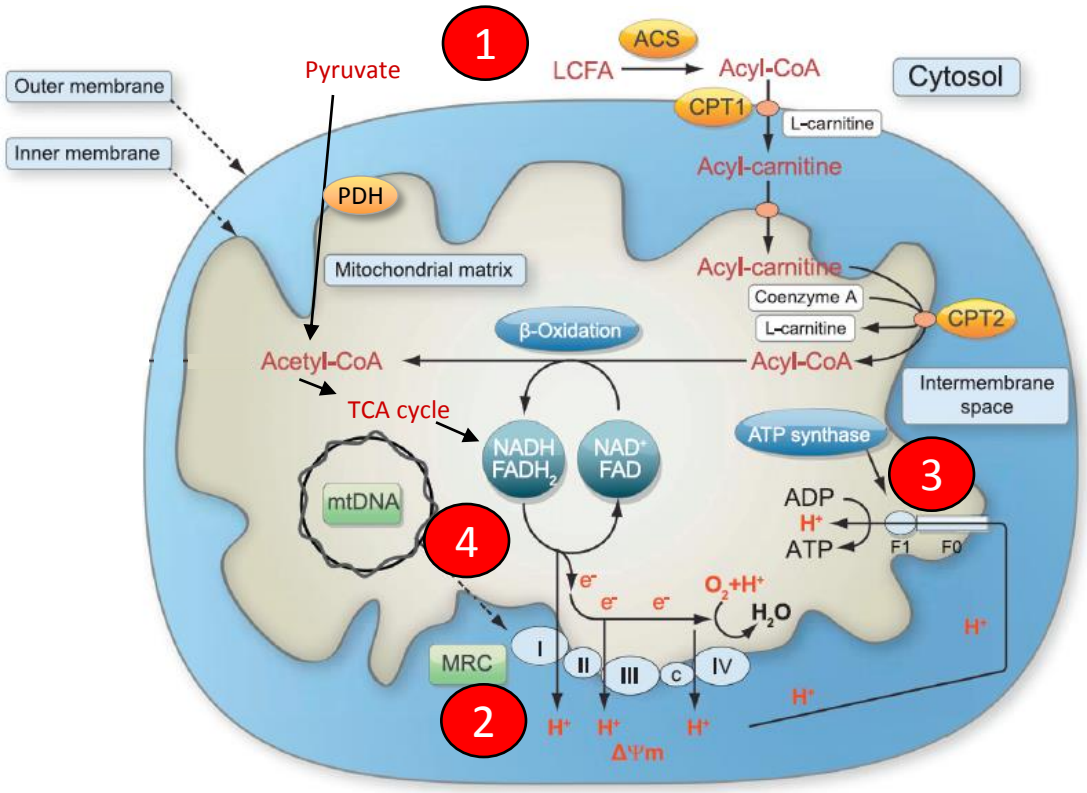
Goals for This Training Session

Participants should understand the following general concepts:

- The assay systems should be used to gather mitochondrial toxicity data for compounds to be represented in DILIsym®
- The conditions and general protocols for the mitochondrial toxicity assay used to provide data inputs for DILIsym®

Overview of Mitochondria Bioenergetics Biochemistry

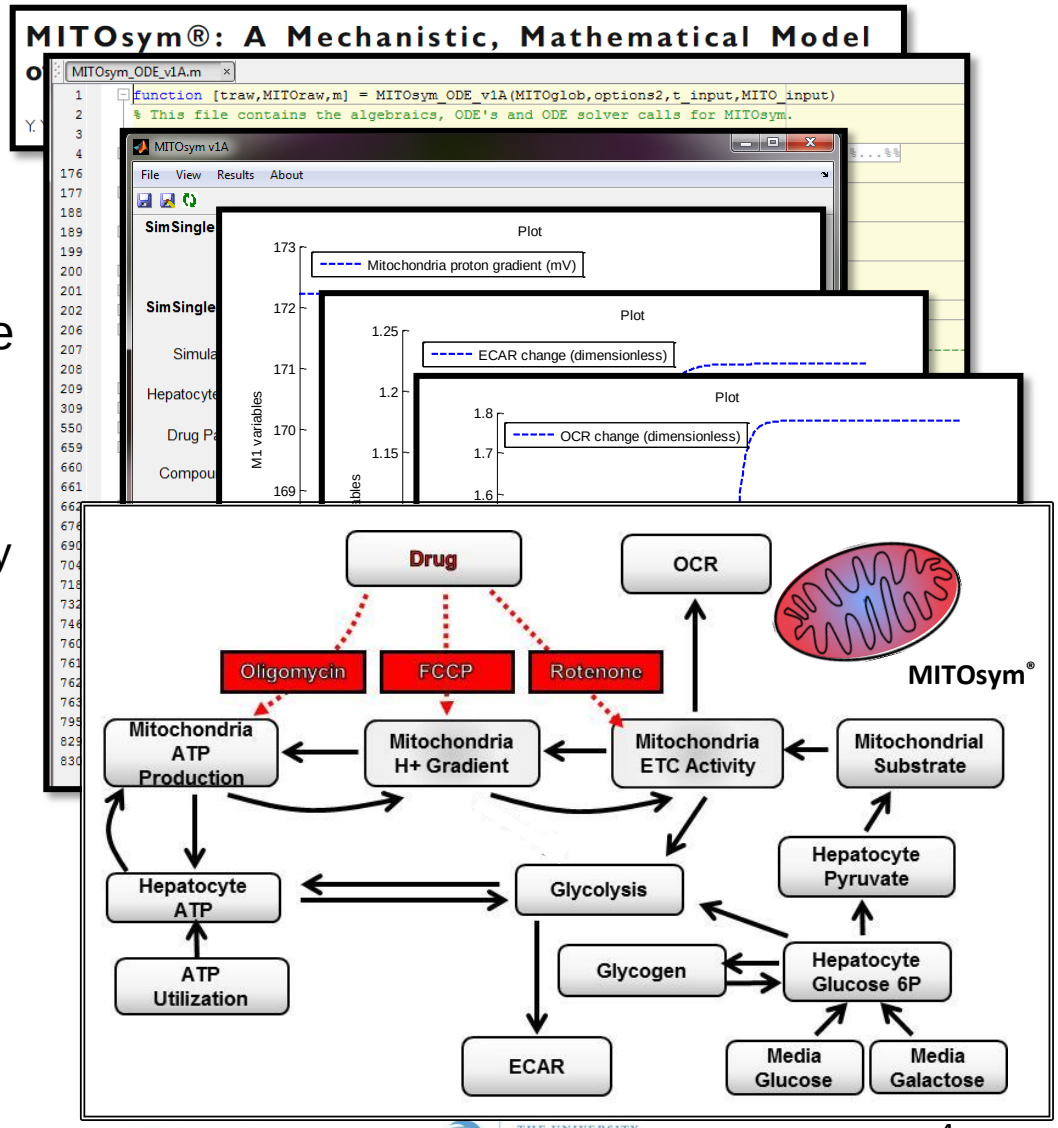
1. Metabolic substrate
   - Fatty acids via beta oxidation
   - Pyruvate via PDH
2. Electron Transport Chain (ETC)
   - Electrons donated from NADH and $FADH_2$
   - Protons pumped out of mitochondria, establishing H^+ gradient ($\Delta\Psi m$)
   - Electrons ultimately donated to O_2, accounting for energy-related respiration
3. ATP synthesis
   - H^+ gradient used to drive ATP synthesis via ATP synthase
4. Mitochondrial DNA encodes multiple mitochondria proteins
   - Within ETC



Adapted from Begriche 2011

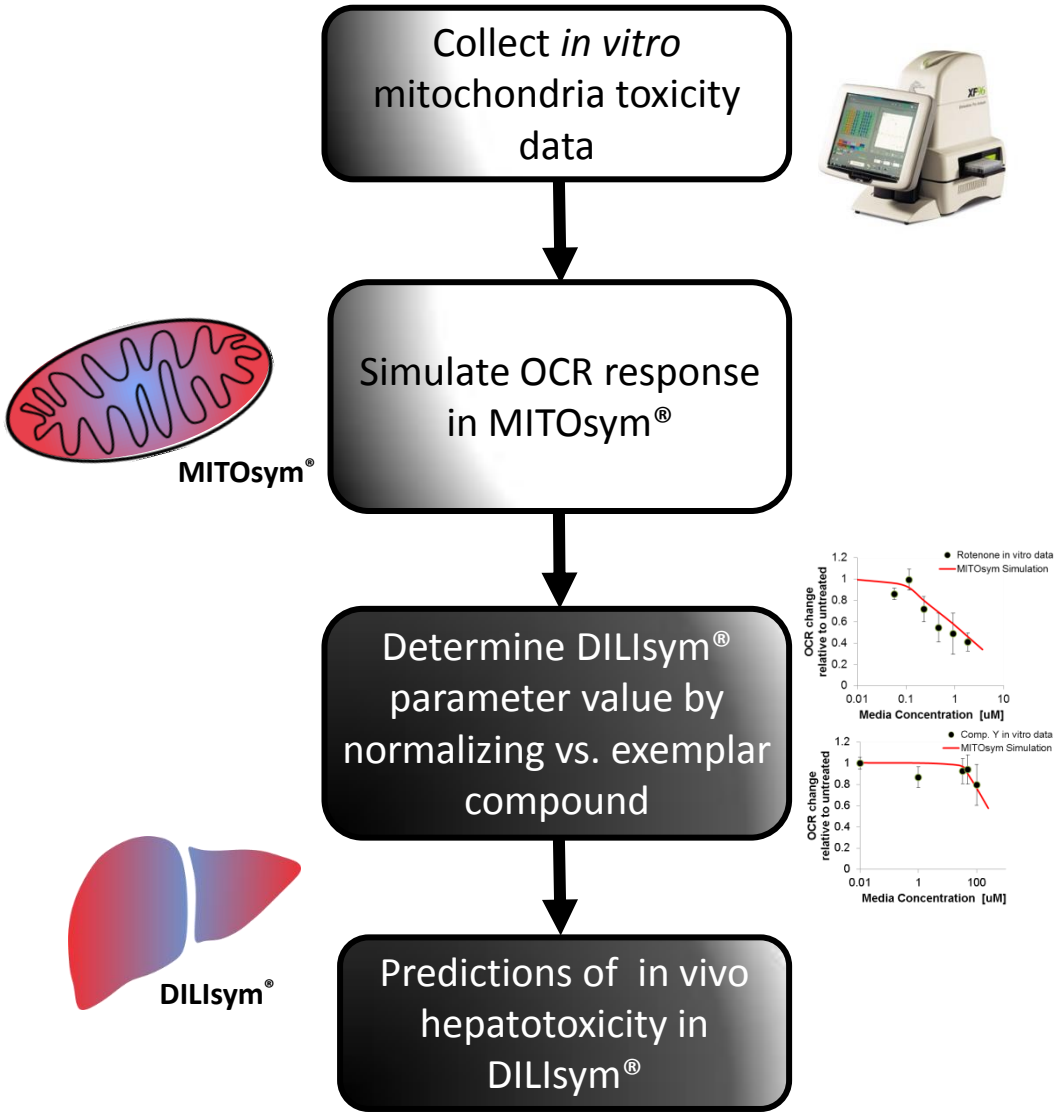
MITOsym® Is Designed to Support IVIVE DILI Predictions and Mechanistic Data Interpretation

- MITOsym® is a standalone model of hepatocyte bioenergetics
  - Yang et al. 2015
- MITOsym® can be used to facilitate predictions of hepatotoxicity based on *in vitro* cellular respiration data
  - Combine with DILIsym® model
  - Multiple cell types: HepG2, primary human hepatocytes, primary rat hepatocytes
- MITOsym® can be used to develop and explore hypotheses of the mechanisms underlying observed changes in respiration and glycolysis in hepatocytes
  - Comparison with exemplar drugs



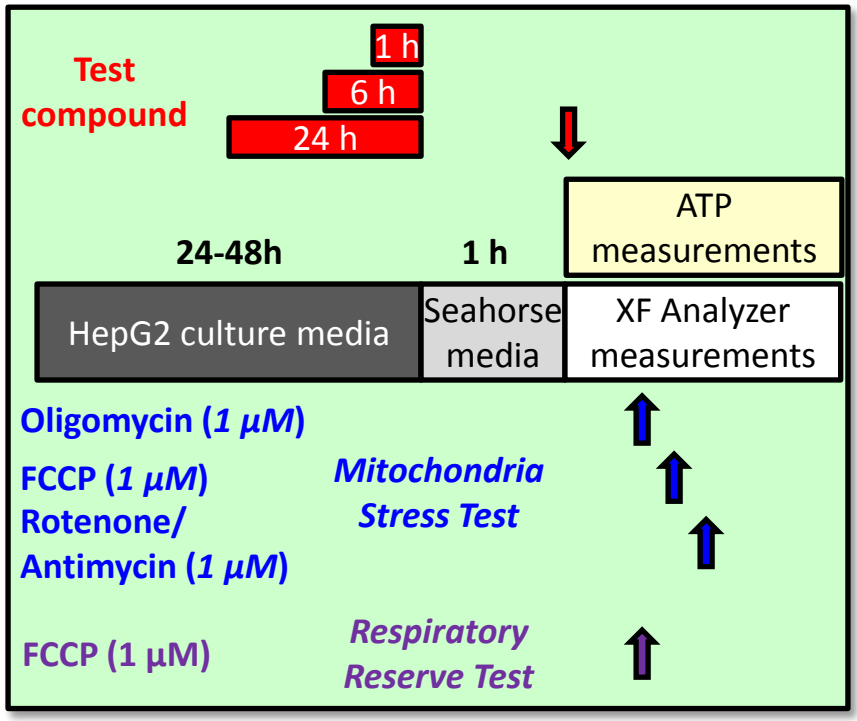
MITOsym® Developed to Support Identification of DILIsym® Toxicity Parameter Inputs

- Simulate Seahorse-based measurements of oxygen consumption rate (OCR) with MITOsym®
- Determine appropriate parameter values for mitochondria toxicity to be used in *in vivo* predictions with DILIsym®
- MITOsym® can be used to develop and explore hypotheses of the mechanisms underlying observed changes in respiration and glycolysis in hepatocytes
  - ETC inhibition, Δψm uncoupling, F1F0 ATPase inhibition, glycolysis inhibition



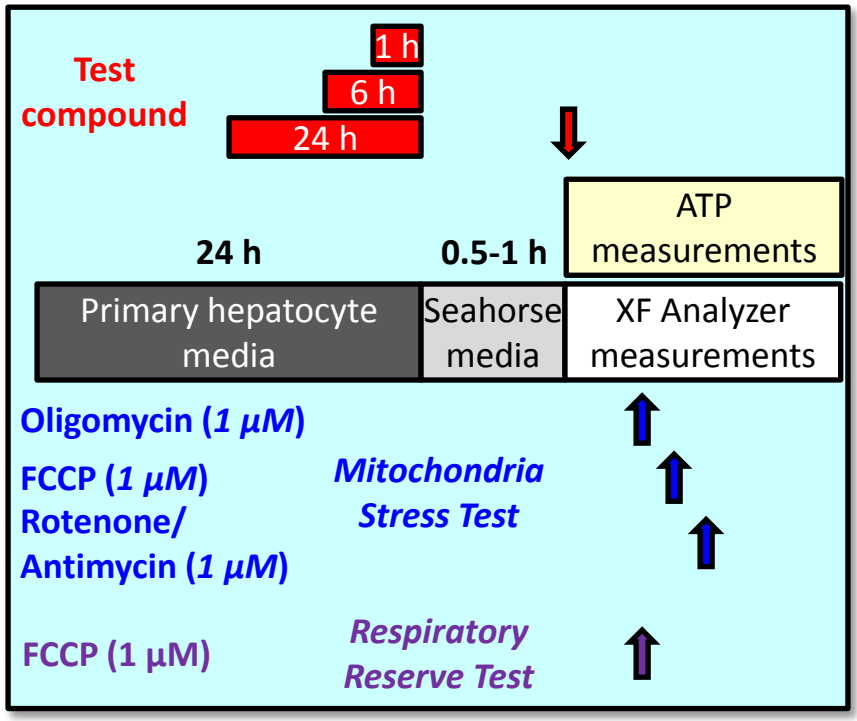
General XF Analyzer Protocol for DILIsym® Input Panel Mitochondria Measurements: HepG2

- HepG2 cells lack drug metabolism capacity
  - Exposure with parent, metabolites
  - Cells seeded into plates and cultured for 24-48 h
- Cells incubated in Seahorse media for 1 h
- Several possibilities for administering test compound
  - Direct injection, 1 h, 6 h, 24 h incubation
  - Depends on compound characteristics
  - Need to measure cell count with longer incubations
- Mitochondria Stress Test provides information on mitochondria toxicity mechanisms
- Respiratory Reserve Test can reveal modest ETC inhibition effects
- Measure ATP levels to validate OCR measures
- Measure intracellular compound concentrations coincident with OCR and ATP measures
  - Can estimate intracellular concentrations via PBPK methods



General XF Analyzer Protocol for DILIsym® Input Panel Mitochondria Measurements: Hepatocytes

- Primary hepatocytes are metabolically competent
  - Difficult to delineate effects of parent vs. metabolites
  - Culture condition requirements differ
- Cells incubated in Seahorse media for 0.5-1 h
- Several possibilities for administering test compound
  - Direct injection, 1 h, 6 h, 24 h incubation
  - Depends on compound characteristics
  - Need to measure cell count with longer incubations
- Mitochondria Stress Test provides information on mitochondria toxicity mechanisms
- Respiratory Reserve Test can reveal modest ETC inhibition effects
- Measure ATP levels to validate OCR measures
- Measure intracellular compound concentrations coincident with OCR and ATP measures
  - Active compound transporters can make it challenging to estimate intracellular concentrations via PBPK methods



The Recommended Extracellular Exposure Range is Based on Relevant Plasma Concentrations *in vivo*

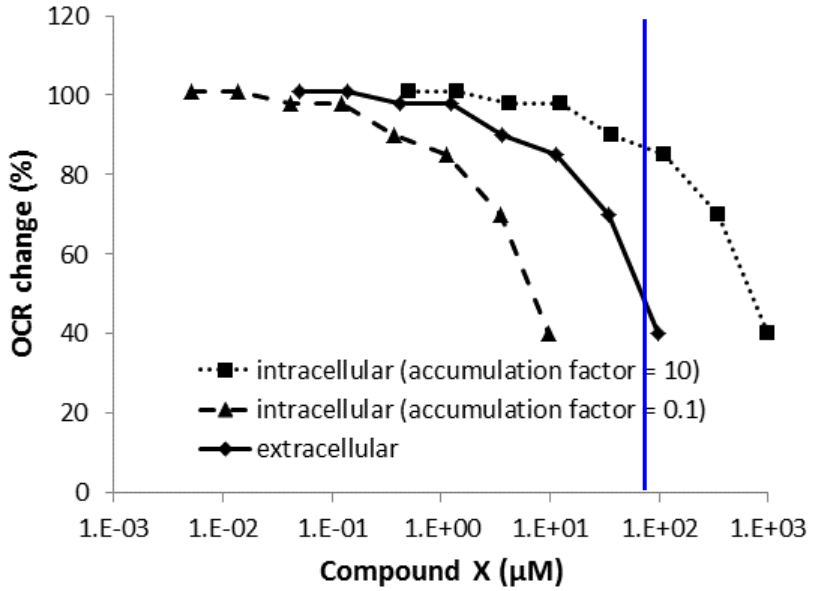
- Extracellular exposure ranges should be relative to the plasma concentrations, or predictions thereof
  - **This is critical to gathering useful data!**
  - *In vitro* studies are often done at extremely high concentrations
  - Contrary to the approach often taken, pushing the cells to a maximal response is not the best approach for this application
- Example table shown that blankets the predicted C_{max}
  - This example is applicable when clinical PK data are unavailable
- Range should be adjusted if more information is available
  - Clinical PK data can help set lower and upper bounds of plasma levels
  - High anticipated variability in exposure may warrant a broader range

Extracellular Exposure Level
100x plasma Cmax
10x plasma Cmax
Plasma Cmax
0.1x plasma Cmax
0.01 plasma Cmax
Vehicle control

Improved DILIsym® Parameter Values When Intracellular Compound Is Measured

- Most Seahorse oxygen consumption rate (OCR) or ROS/RNS data are expressed in an exposure-response relationship
  - OCR change on y-axis
  - Extracellular compound concentration on x-axis
- Numerous compounds have been shown to accumulate in liver
  - Potency relative to intracellular concentrations different than relative to extracellular
  - Intracellular ≠ extracellular
- Basing parameter values on extracellular concentrations introduces inaccuracy for compounds that accumulate in hepatocytes
- Recommend measuring intracellular compound concentration for cell based assays used to provide DILIsym® parameter values
  - OCR, ROS production
  - For compounds that are known to have liver:blood ratio ≠ 1 (or not known)

HepG2



	extracellular	intracellular
10X accumulation	74.4 uM	744 uM
1x accumulation	74.4 uM	74.4 uM
0.1x accumulation	74.4 uM	7.29 uM

Theoretical Preclinical Data