From Bench to Computational Modeling: Integrating Mitochondrial Stress Data from a Rat Hepatic Ischemia-Reperfusion Injury Model into a Preclinical and Human Quantitative Systems Toxicology Platform

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BACKGROUND

- **Hepatic ischemia-reperfusion injury (IRI)** contributes significantly to liver dysfunction post-surgery and transplantation
- Mitochondrial, oxidative, and inflammatory processes are central to IRI pathology
- **Quantitative systems toxicology (QST)** modeling integrates mechanistic data to simulate injury dynamics and assess biomarkers or therapies

METHODS

- Mitochondrial respiration assessed in IRI and sham rat liver tissue using extracellular flux analysis
- **Electron transport chain (ETC)** function and mitochondrial integrity evaluated using substrate/modulator combinations
- **QST platform (DILIsym®)** used to model liver biochemistry and simulate IRI based on experimental data

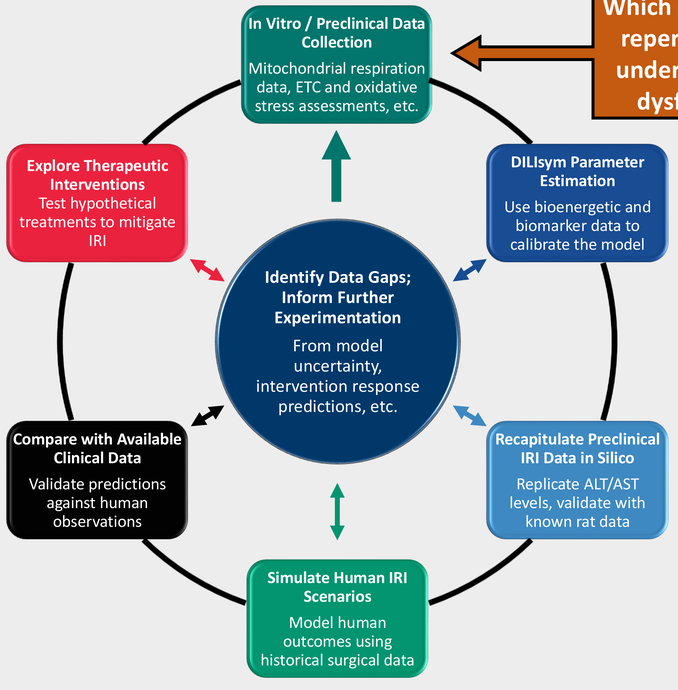
RESULTS

- IRI reduced pyruvate/malate-stimulated respiration by ~60%, suggesting impaired TCA cycle function
- Succinate/rotenone treatment yielded similar respiration between groups, indicating intact complex II and downstream ETC function
- NADH respiration was preserved in IRI, implicating upstream metabolic disruption rather than complex I failure
- DILIsym® simulations reproduced rat aminotransferase elevations and aligned with historical human IRI data

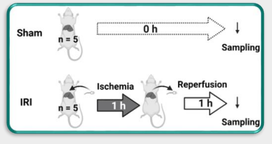
CONCLUSION

- Mitochondrial data from IRI models provide mechanistic insights into liver injury
- QST modeling enables translation of preclinical findings to clinical contexts
- This integrative approach serves as a bridge between preclinical findings and clinical trial design, supporting hypothesis generation and reducing the need for further animal studies

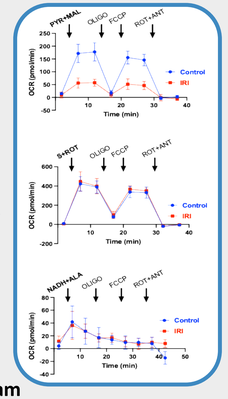
Which mechanisms drive ischemia-reperfusion injury, and how can understanding them reduce liver dysfunction post-transplant?



Rat IRI Study Design



Mitochondrial Flux Analysis in Rat Liver (Control vs. IRI)

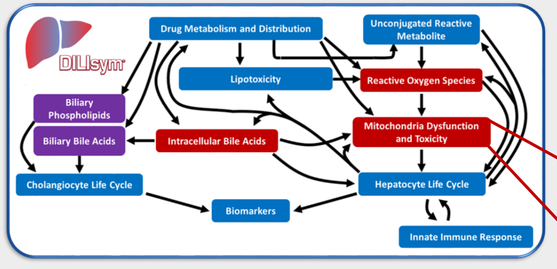


- **Top: Pyruvate + Malate (NADH-linked respiration)** Control (blue) shows higher oxygen consumption compared with IRI (red). Indicates impaired **NADH supply or Complex I–linked flux** in IRI.
- **Middle: Succinate + Rotenone ($FADH_2$-linked respiration)** Similar oxygen consumption in Control and IRI. Demonstrates preserved **Complex II → IV function** despite IRI.
- **Bottom: Alamethicin + NADH (direct Complex I assay)** Control and IRI overlap when NADH is provided directly. Confirms **Complex I catalytic activity is intact**; dysfunction arises **upstream (PDH/TCA metabolism or NADH generation)**.

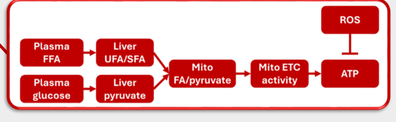
***Substrates & Modulators**

- **Pyruvate (PYR) + Malate (MAL)** → Drive the TCA cycle to generate **NADH**, fueling Complex I.
- **Succinate (S) + Rotenone (ROT)** → Succinate generates **$FADH_2$** at Complex II; rotenone blocks Complex I to prevent reverse electron flow.
- **Alamethicin (ALA)** → Permeabilizes the inner membrane, allowing **direct NADH access to Complex I**, bypassing upstream metabolism.
- **Oligomycin (OLIGO) / FCCP / Antimycin A (ANT)** → Standard inhibitors for defining leak, maximal respiration, and non-mitochondrial oxygen consumption (for assay calibration).

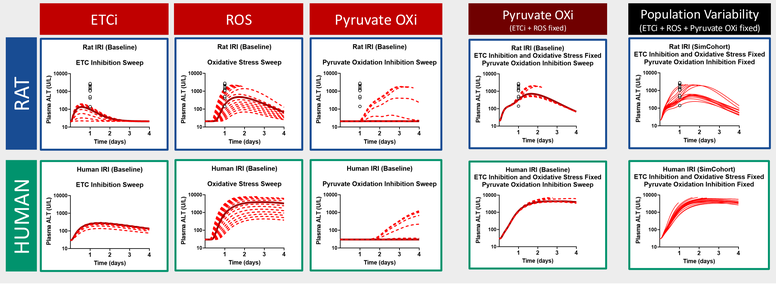
DILIsym® Model Diagram



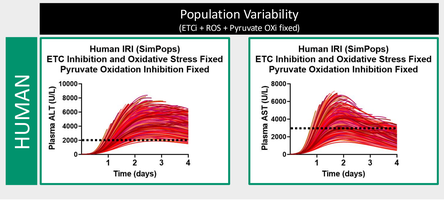
Mitochondria Dysfunction and Toxicity Submodel Diagram



DILIsym® Simulations of Plasma Aminotransferase Elevations After Rat and Human IRI



DILIsym simulations (red-hued profiles) in an average rat/human, and in population samples in the form of SimCohorts (n=16) and SimPops (n=285). Observed data are represented with open black circles. ETCi, electron transport chain inhibition; ROS, reactive oxygen species; OXi, oxidation inhibition.



Dotted black line: OPTN criteria for primary non-function (severe IRI requiring immediate re-transplantation).

Financial Disclosure
J.J.B. is an employee of Simulations Plus, Inc. and holds stock options in the company. J.K., S.K.H., and J.Z. have no conflicts of interest to disclose.