# Mechanistic modeling of drug-induced liver injury due to mtDNA depletion in DILIsym®

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## ABSTRACT

**OBJECTIVE**: To simulate drug-induced liver injury (DILI) due to mitochondrial DNA (mtDNA) depletion in DILIsym using Fialuridine (FIAU) as an exemplar compound.

**METHODS:** FIAU-induced mtDNA depletion and the subsequent effects on mitochondria function, hepatocellular bioenergetics, and liver injury were modeled in DILIsym by combining predictions of compound exposure with compound-induced reductions in mtDNA synthesis. A simplified physiologically-based pharmacokinetic (PBPK) model was employed to simulate FIAU exposure. All PBPK parameters were calculated based on physio-chemical properties of FIAU or optimized to clinical PK data [1]. FIAU effects on hepatocyte function within DILIsym were based on reductions in mtDNA synthesis and subsequent disruptions in mitochondrial function. Parameters describing the rate of FIAU-imposed mtDNA reductions were calculated based on in vitro data and subsequently optimized based on clinical DILI responses [2,3]. The FIAU dosing protocol described by McKenzie et al. [3] was simulated with a SimCohort, a group of simulated patients with variability in selected system-level parameters.

**RESULTS**: DILIsym accurately captures the plasma FIAU PK in humans. DILIsym also recapitulates the hepatotoxicity reported for extended treatment with FIAU [3]. A comparable frequency of severe liver injury is predicted in the SimCohort (11 out of 15 patients) as was reported for clinical patients (7 out of 10 patients). Delayed presentation of severe liver injury (>9 weeks ) is also predicted in the simulated patients. The proportion of simulated patients with maximum total plasma bilirubin concentrations exceeding 3 mg/dL is comparable with the clinical patients [3].

**CONCLUSION**: DILIsym accurately simulates DILI due to FIAU administration and can be used to evaluate the DILI risk of compounds that have the potential to deplete mtDNA. Further investigation will be required to translate in vitro data into DILIsym input parameters for drug-imposed mtDNA reductions.

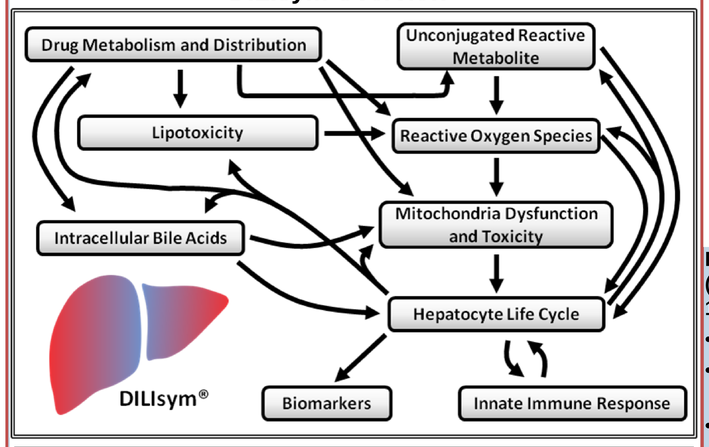
## INTRODUCTION

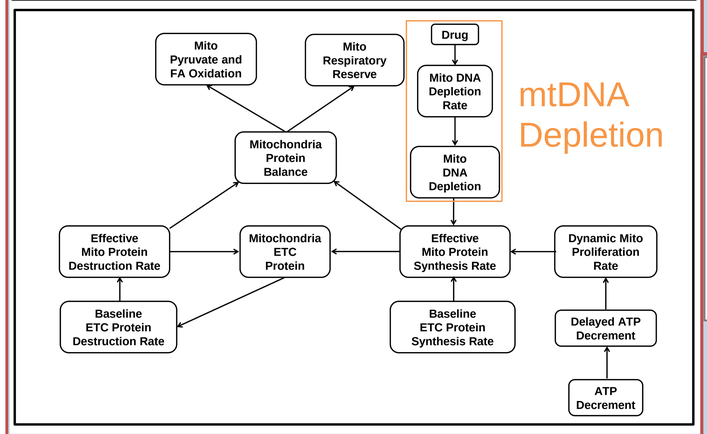
- Drug-induced mtDNA depletion can lead to severe hepatotoxicity as mtDNA encodes essential components of mitochondrial electron transport chain (ETC).
- Fialuridine (FIAU), a nucleoside analogue, was developed for the treatment of chronic hepatitis B viral infection. FIAU development was terminated after severe hepatotoxicity was observed in a pivotal phase 2 clinical trial [3]. Subsequent mechanistic studies identified mtDNA depletion as the mechanistic cause [4].
- DILIsym is a mechanistic, multiscale model of DILI that integrates compound exposure, hepatotoxicity mechanisms and inter-patient variability.

DILI-sim Initiative

## RESULTS

### DILIsym Overview

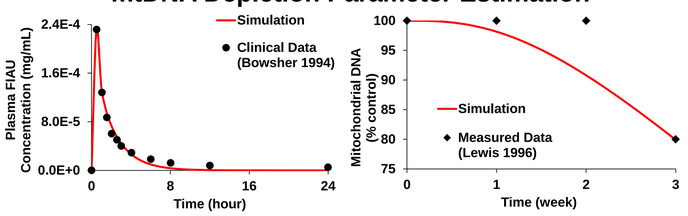



**Diagrams showing the overall structure of DILIsym (top) and key components of the mtDNA depletion mechanism (bottom)**

- Several drugs have the potential to inhibit DNA polymerase γ, restrict mtDNA chain replication, and reduce mtDNA and ETC enzyme content.
- Mitochondrial enzyme balance directly affects both respiratory reserve and pyruvate/fatty acid oxidation rates (i.e., ETC function)

### PBPK Simulation Results and mtDNA Depletion Parameter Estimation

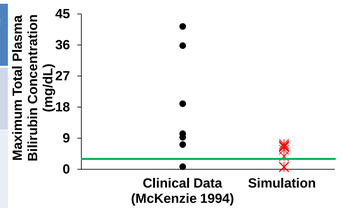



**Plasma FIAU pharmacokinetics in human after a single 5 mg oral dose (left) and fraction of mtDNA content when HepG2 cells are exposed to 1 μM of FIAU over three weeks (right).**

- DILIsym accurately simulates the plasma FIAU PK in humans [1].
- DILIsym recapitulates the *in vitro* FIAU-induced reduction in mtDNA content.
- The mtDNA degradation rate corresponding to 1 μM media concentration was utilized to estimate mtDNA depletion rates, based on the correspondence to the maximum clinical plasma FIAU concentration [2].

### Prediction of Clinical Outcomes

| | Clinical Data | Simulation Results |
|---|---|---|
| DILI Incidence | 70% (7/10) | 73% (11/15) |
| Time of DILI onset (weeks) | 11-16 | 12-14 |



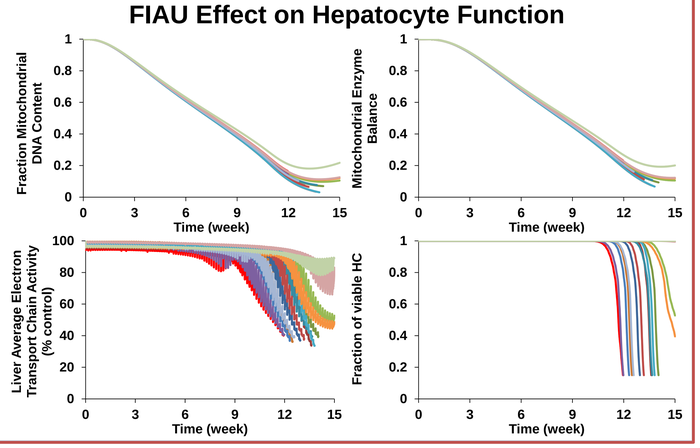

**DILIsym toxicity predictions (left) and maximum total plasma bilirubin concentration of the clinical patients before death and simulated patients after 15 weeks of simulation where a total 1030 mg of FIAU was administered to each simulated patient over almost 11 weeks (3 doses/day) (right).**

- DILIsym accurately recapitulates both the magnitude and delayed onset of severe liver injury in the SimCohort as was reported for clinical patients [3].
- Predicted maximum total plasma bilirubin concentrations frequency in simulated patients exceeding 3 mg/dL is comparable with the clinical patients [3].

**FIAU effects on hepatocyte function within DILIsym were based on drug-induced reductions in mtDNA synthesis and subsequent disruptions in mitochondrial function.**

- FIAU treatment caused delayed mtDNA and enzyme loss.
- FIAU possesses a 3'-hydroxyl groups that serves as competitive, alternative substrate for thymidine triphosphate for DNA polymerase γ site in mtDNA synthesis and therefore incorporated into the nascent chain [5]. Hepatocyte function was unaffected until the threshold for mtDNA content was achieved where the unaffected mtDNA were unable to maintain adequate synthesis of mitochondrial gene products [6].
- Reductions in mtDNA fractional content led to diminished generation of and maintenance of the mitochondria proton gradient and ATP synthesis.
- Liver toxicity was not observed until mtDNA was reduced to <50% of initial content.
- When FIAU was withdrawn, mtDNA replication and mitochondria function were restored (*not shown*).

### FIAU Effect on Hepatocyte Function




## METHODS

**PBPK model of FIAU** A PBPK model of FIAU was developed based on human plasma FIAU concentrations after single 5-mg oral dose administration to 16 healthy subjects [1]. A reduced-PBPK model was used where renal and hepatic clearance of FIAU were considered. All PBPK parameters were calculated based on physio-chemical properties of FIAU or optimized to clinical PK data [1].

**mtDNA depletion parameters** Parameters describing the rate of FIAU-imposed mtDNA reductions were initially determined using DILIsym "in vitro" like simulations based on the *in vitro* study where dose-dependent decreases in mtDNA levels in HepG2 cells due to FIAU were reported [2]. The parameter values included the inherent initial delays of FIAU effects on mtDNA content due to DNA synthesis kinetics [2]. The parameter values were subsequently additionally optimized utilizing the timing and magnitude of liver injury in patients, as reported by McKenzie et al. [3]. The restoration process of mtDNA replication and mitochondria function upon FIAU withdrawal was also included based on a report by Litoshenko et al. [7].

**Simulated hepatotoxicity** A SimCohort, a group of 15 simulated patients with a range of values for the respiratory reserve scalar, was used to mimic the clinical protocol described by McKenzie et al. [3]. This provides variability in the key mechanistic areas affected by FIAU. Moreover, each simulated patient in this SimCohort has approximately similar body weight. This ensures that simulated variability in liver injury is due to underlying hepatocellular mechanisms rather than differences in compound exposure.

## CONCLUSION

- DILIsym modeling can be used to predict DILI due to mtDNA depletion.
- Further investigation will be required to translate *in vitro* data into DILIsym input parameters for drug-imposed mtDNA reductions.

## REFERENCES

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## ACKNOWLEDGEMENTS

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