Is there a common mechanism of DILI, do we need to know?
IS THERE A COMMON MECHANISM OF DILI?

YES & NO

ANSWER
Mechanisms of DILI: 40 thousand feet view

Intrinsic Drug Toxicity
- Stress responses
- Cell death
- Impaired bile secretion

Idiosyncratic
- Adaptive immune response

Mechanisms of DILI:
- hapten
- drug metabolites produced in liver

Exceptions
- Chronic steatohepatitis and fibrosis
- Biologic DILI
- Mitochondrial DNA poisons

RARE

DILI
If there is a common mechanism of DILI, Why do pre-clinical studies predict IDILI?

- Severe IDILI is rare vs. “predictors” are common but perform surprisingly well.
  Cell culture, isolated mitochondria, ROS, ATP, covalent binding, transcriptomics, cell death, stress responses (mito, ER, signaling) collaborative cross, humanized mice.

- Do pre-clinical studies really inform about mechanism of IDILI?

  YES
  - Covalent binding (stress inducer and hapten)
  - Toxic stress responses – lethal or sublethal
    (DANGER SIGNALS)

  NO
  - Surrogate for drugs that undergo hepatic metabolism

- Not all IDILI drugs are metabolized in liver or require covalent binding to induce an adaptive immune.
- Many drugs are metabolized to reactive metabolites in everyone, but cause DILI in very few or none at all.
Why does IDILI occur in so few susceptible patients?

- Adaptations dampen stress responses (e.g. ER and mitochondrial UPR, mitochondrial plasticity, antioxidant responses).

- Immune tolerance (IT) dampens immune response
  - Rapid and strong IT, no DILI (normal ALT)?
  - Delayed onset IT, mild DILI (ALT)?
  - Weak or very delayed IT, severe DILI?
Do we need to know the mechanism of DILI?

YES!

**Drug Development**

- Preclinical prediction – select best candidate
- Clinical trials – predict mild and/or severe DILI early (specific biomarkers for surveillance and to distinguish DILI from other causes based on mechanism)
Do we need to know the mechanism of DILI?

Post-marketing Clinical practice

• **Monitoring**: Optimize for early evidence of DILI due to approved drugs with liability.

• **Treatment**
  – prevent danger signals
  – identify and dampen stress signaling pathways before or after ALT increases*

  *mitochondria* – targeted antioxidants, biogenesis

  *ER stress* – molecular chaperones

  *MAPK* – ASK1 inhibitor, block the binding of JNK to mitochondrial Sab

  – attack pathophysiology of liver injury (cell death, inflammation, cholestasis) in early or later stages of injury.
Regulated Cell Death in DILI and Liver Injury

Hy’s law and ALF reflect parenchymal extinction.

**Unequivocal**
- Apoptosis
- Mitochondrial necrosis (APAP)

**Uncertain**
- Necroptosis – AIH \* immune DILI?
- Ferroptosis – HCC treatment - ? variant of regulated mitochondrial necrosis
- Pyroptosis – innate immunity -?
TNF receptor-mediated cell death
The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 in AIH

RIPK3 in NPC (not hepatocytes)

T-Cell Hepatitis → Necrosis (noncanonical necroptosis)

Gasdermin (GSDM) - Mediated Programmed Necrosis: Pyroptosis

PAMPs/DAMPs → Inflammasomes → Caspase 1 → GSDM-D → GSDM-N → membr. pores → Necrosis

Intracellular LPS → Caspase 11/4/5 → GSDM-N → Necrosis

Death Receptor → Caspase 8 → Mitochondria → Caspase 3

Intrinsic stress (e.g. Chemo Rx) → Caspase 3 → GSDME → GSDME-N → Necrosis (high GSDM) e.g. intestines

Apoptosis (low GSDM) → caspase 8 → mitochondria → caspase 3 → GSDME → GSDME-N → Necrosis (high GSDM) e.g. intestines
Iron-dependent, non-apoptotic form of regulated cell death (MLKL+GSDM independent)

Mixture of GSH depletion, Fe^{+2} dependence, mitoch fragmentation + collapse, ROS, ER stress, signal transduction and LPO.

Probably a form of mitochondrial necrosis.
Model of the Interplay of MAPK cascade, Sab and ROS in Regulated Mitochondrial Necrosis and Apoptosis

- Triggers: ER Stress, Mitoch Stress, TNF-R, DNA damage
- Potential Therapeutic Targets: ASK1, MKK4, JNK, Sab, Antioxidants

Diagram:
- ASK1 → MKK4 → P-JNK → Sab → ETC → ROS → Necrosis
- Metabolic effects, Inflammation (transcriptional)
- MOMP, Bcl mediation, Apoptosis
- Self sustaining /amplification
Therapeutic Strategies in Cell Death

**Mitochondrial necrosis, ferroptosis, apoptosis**
Antioxidants – global or targeted to mitochondria
MAPK inhibitors - target ASK1, JNK binding to mitochondria
MPT inhibitors

**Apoptosis and Pyroptosis**
Pancaspase inhibitors

**Necroptosis**
RIPK1 inhibitor (necrostatin-1s)
MLKL blocker (necrosulfonamide)
TNF and IFNα inhibitors

**Ferroptosis**
Ferrostatin
Iron Chelators
GSH precursors
Conclusions

1. A working model for a common mechanism of DILI is plausible but encompasses many unique drug specific stress responses in the liver and genetic factors which promote or inhibit the immune system.

2. Applications of mechanistic understanding of DILI include identification of specific biomarkers for diagnosis and prognosis as well as therapeutic interventions to dampen stress responses and cell death.
As Cayal noted: “nature seems unaware of our intellectual need for convenience and unity, and ... takes delight in complication and diversity”

However, we can reasonably anticipate that the future will be more exciting than the past and will offer great opportunity for ... bridging science and medicine.

*Taken from Aria’s Master's Perspective
Hepatology 65:1785-88, 2017