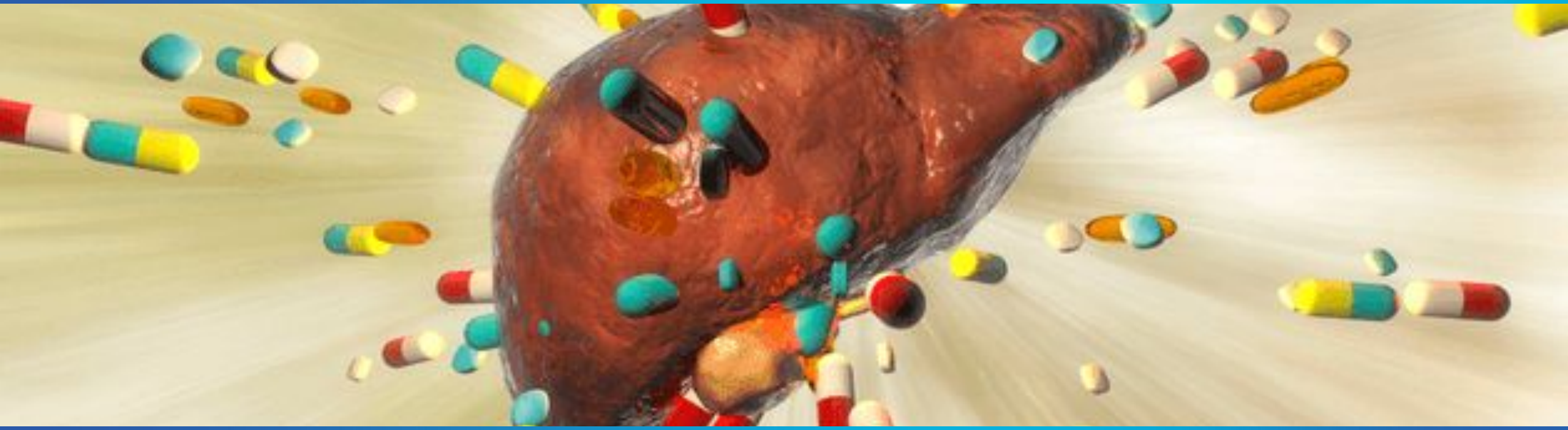

DILI: A Medicinal Chemist's Perspective

Drug Hunter Flash Talk

Anh Tran

June 6, 2024

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Outline

1. **Introduction**
2. **Mechanisms of DILI**
   - Inhibition of hepatic transporters
   - Mitochondrial impairment
   - Reactive metabolite formation
   - Immune-system mediated liver injury
   - *In silico* prediction of DILI: DILIsym®
3. **Progress towards a classification-based holistic assessment of DILI risks**
4. **DILI mitigation strategies: Case Studies**
5. **Balancing DILI risks and therapeutic opportunities: recent FDA approval**
6. **Conclusion**

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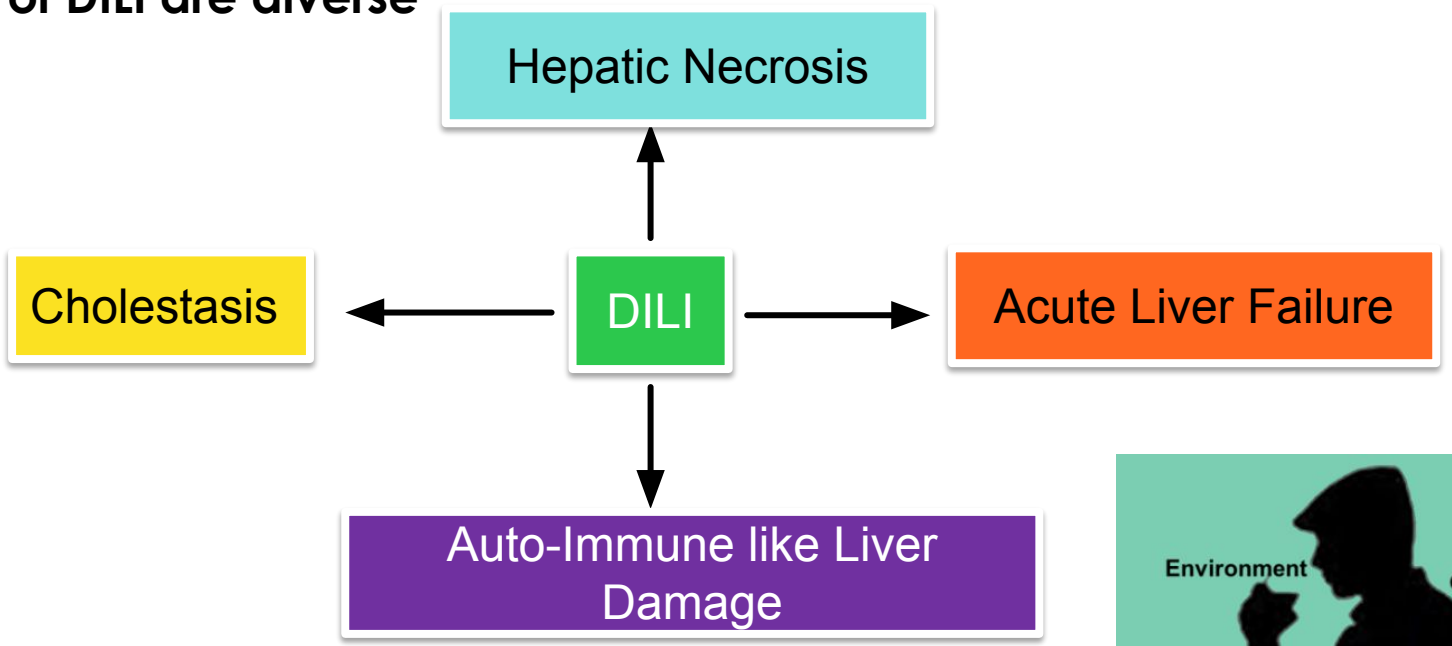
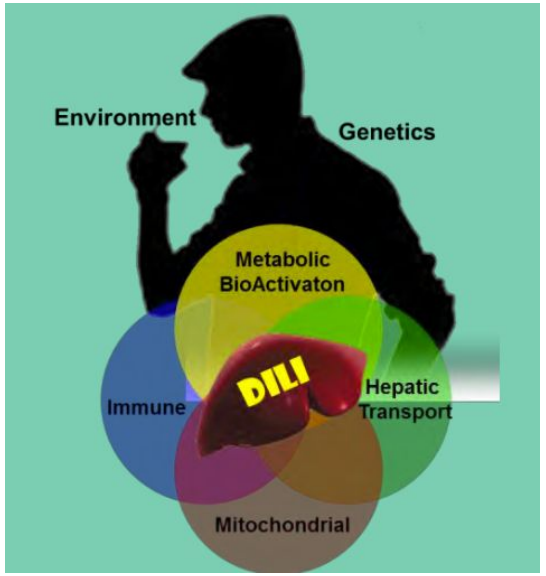
What is Drug-Induced Liver Injury (DILI)?

- **Clinical manifestations of DILI are diverse**





- **Current DILI clinical biomarkers:**
  - Alanine aminotransferase (ALT)
  - Aspartate aminotransferase (AST)
  - Total bilirubin

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DILI: The Business Case

- **Adverse toxicological findings account for 40% of program terminations**
  - Drug-induced liver injury (DILI), despite low frequency (2 in 10000 cases), accounts for more than 50% of acute liver failure cases
  - DILI often escapes detection until late-stage clinical trials incurring significant R & D cost: recent FDA clinical hold of multiple BTK inhibitors for multiple sclerosis
    - Orelabrutinib (Biogen, Phase II, 2022)
    - Tolebrutinib (Sanofi, Phase III, 2022)
    - Evobrutinib (Merck KGaA, Phase III, 2023)
    - Fenebrutinib (Genentech, Phase III, 2023)
- **Underlying mechanisms of DILI are complex**
  - Clinical toxicity stems from interplay between multiple mechanisms, patient-specific factors, and environment
  - Historically poor correlation between animal and human pathophysiology

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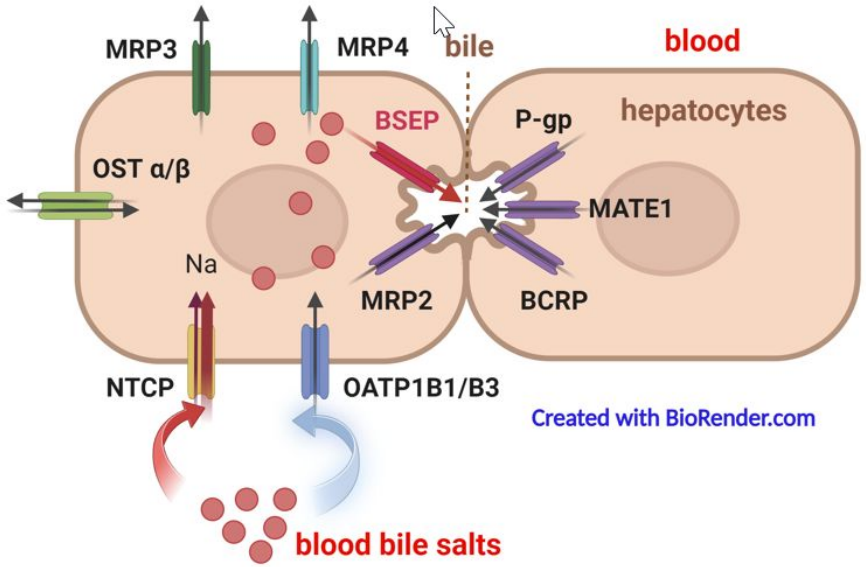
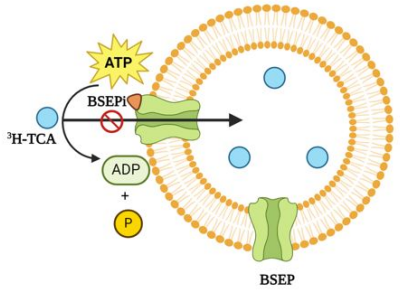
Inhibition of Hepatic Transporters

- Enterohepatic circulation of bile salts is regulated by intestinal and hepatic membrane transporters.
- Bile salt export pump (BSEP) mediates rate-limiting transport of bile salts from hepatocytes to bile duct.
- Reduced BSEP activities may result in bile salt accumulation leading to liver injury.
- No consensus on *in vitro* BSEP inhibition value as a flag for high clinical DILI risk:
  - Other contributory mechanisms include mitochondrial impairment, reactive metabolites, and immune activation are also at play.
- **Recent development:** two-tiered approach to determine potential *in vivo* BSEP inhibition from Merck

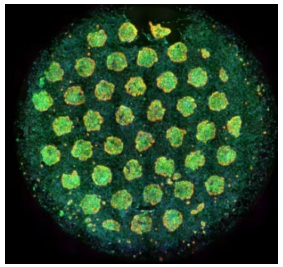


Tier 1: Inverted membrane vesicle assay (Sf19 insect cells)



IC_{50} < 5 µM

Tier 2: Biliary excretion index (BEI) determination in HepatoPac™
(*in vitro* co-culture system of primary hepatocytes + 3T3 murine fibroblasts)



$BEI\ IC_{50} < 10 \times I_{in,u}$

Potential to inhibit BSEP *in vivo*; Consider SAR to reduce liability or clinical monitoring for cholestasis

Hafey, M. J. *et al. Drug Metab. Dispos.* **2020,** *48*, 1147-1160

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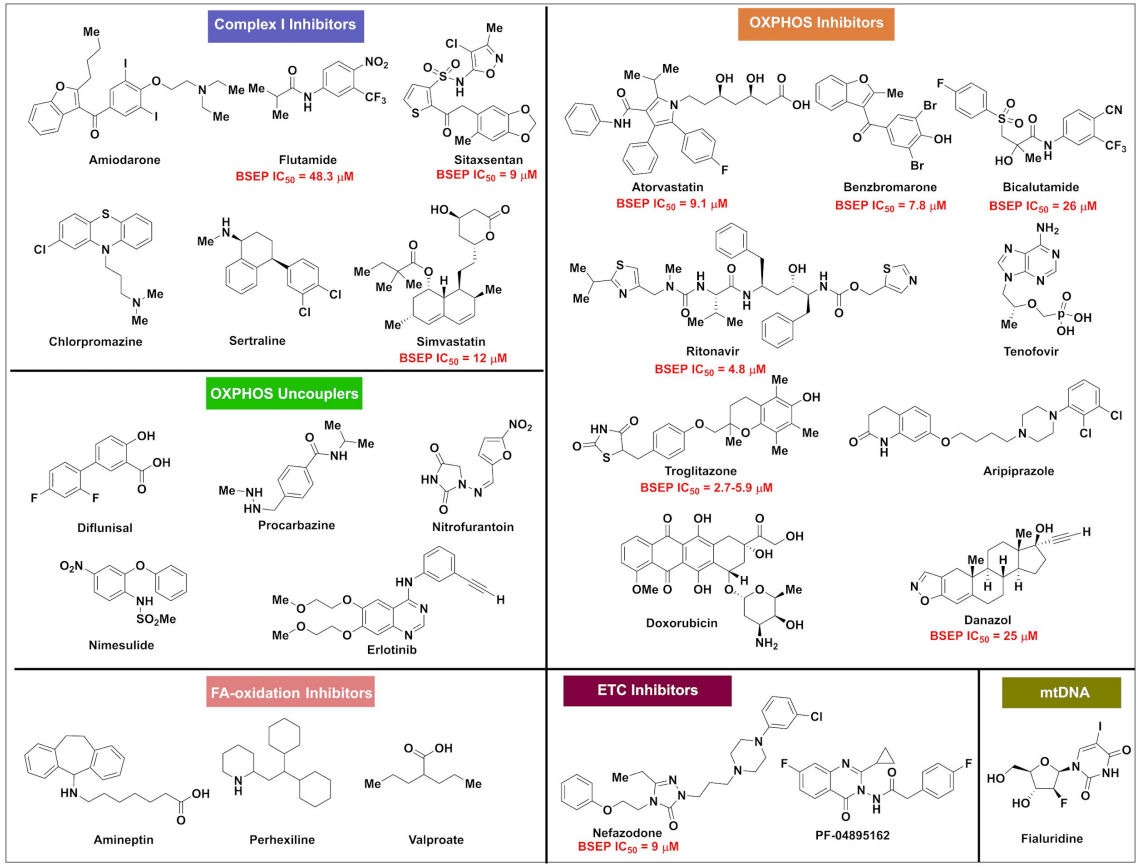
Mitochondrial Impairment



- 40% of hepatotoxicity-related ADRs associated with mitochondrial mechanism of toxicity
- Drug-induced mitotoxicity caused by several mechanisms leading to:
  - Impaired ATP synthesis
  - Increased ROS production
- Two high-throughput assays to detect mitochondrial toxicants:
  - Cytotoxicity IC_{50}(Glu)/IC_{50} (Gal) in HepG2 cells
  - Respiratory screening technology in freshly isolated rat mitochondria
- Best practice: mitotoxicity screening in early optimization phase of drug discovery programs
- Delayed presentation of mitochondrial toxicity in the clinic due to innate compensatory mechanisms

Fialuridine is a mitochondrial toxicant which resulted in delayed fatal liver failure after 13 weeks of dosing in human

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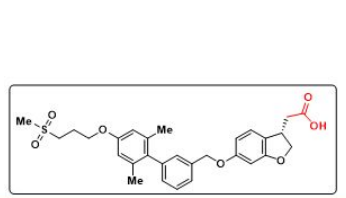
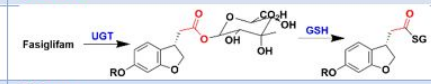
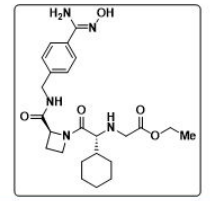
Reactive Metabolite Formation

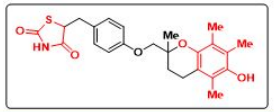
- Xenobiotics may be metabolized to soluble and benign metabolites or undergo bioactivation to produce electrophilic reactive metabolites (RM)
- RM can react with nucleophiles in endogenous proteins resulting in direct or immune-mediated toxicity *via* hapten formation: both mechanisms are relevant in DILI
- Multiple methods have been developed to assess RM formation:
  - Covalent binding (CVB) studies of radiolabelled drugs in primary human hepatocytes
  - MS/MS based *in vitro* MetID of non-radiolabelled compounds incubated in HLMs or primary human hepatocytes in the presence of cysteine, GSH or KCN
  - **Recent development:** Merck teams developed an *in vivo* and *in vitro* rat liver transcriptional signature (BA-LRA) measuring transcriptional activation of NRF2/KEAP1 & NRF1 to determine bioactivation potential of NCEs

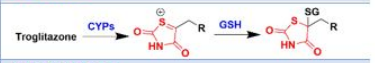


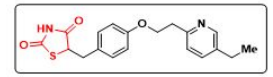
Fasiglifam	DILI Positive
GSH HLM Trapping	Positive
Bioactivation Mechanism	
Daily Dose	50 mg



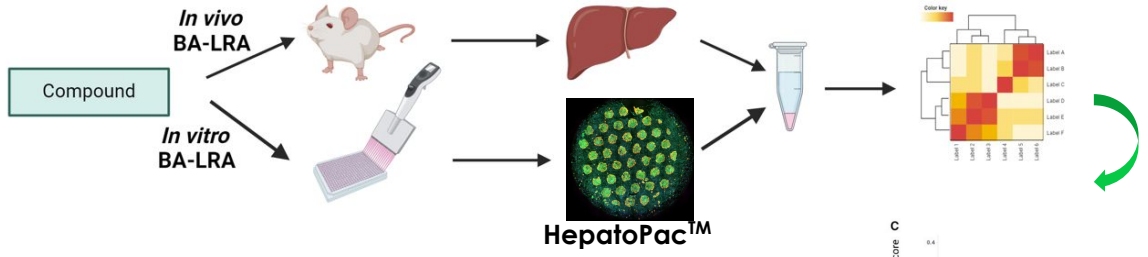
Ximelagatran	DILI Positive
GSH HLM Trapping	Negative
Bioactivation Mechanism	N/A (possibly immune-mediated DILI)
Daily Dose	36 mg BID

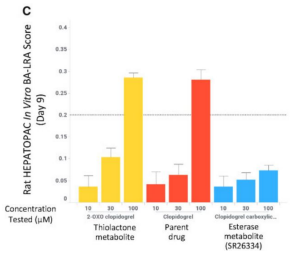


Troglitazone	DILI Positive
GSH HLM Trapping	Positive
Bioactivation Mechanism	
Daily Dose	200-600 mg



Pioglitazone	DILI Negative
GSH HLM Trapping	Positive
Bioactivation Mechanism	Similar to Troglitazone
Daily Dose	30 mg



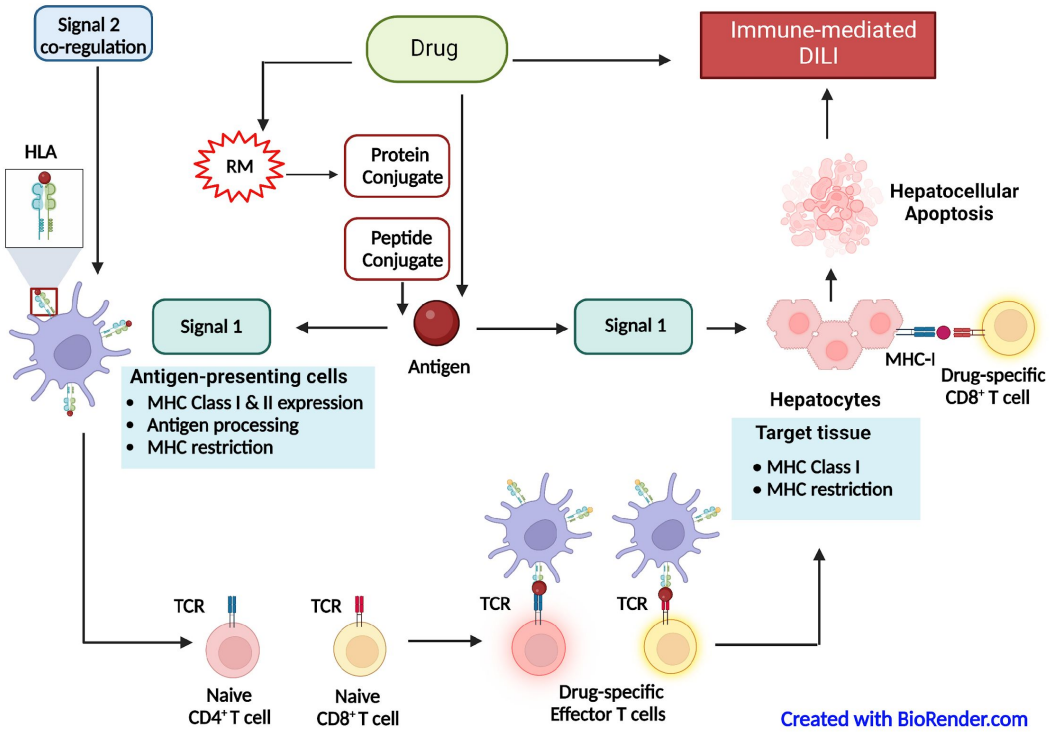


BA-LRA differentiates DILI-positive and DILI-negative drugs

Kang, W. *et al. Toxicol. Sci.* **2020,** *177*, 121-139
Monroe, J. *et al. Toxicol. Sci.* **2020,** *177*, 281-299

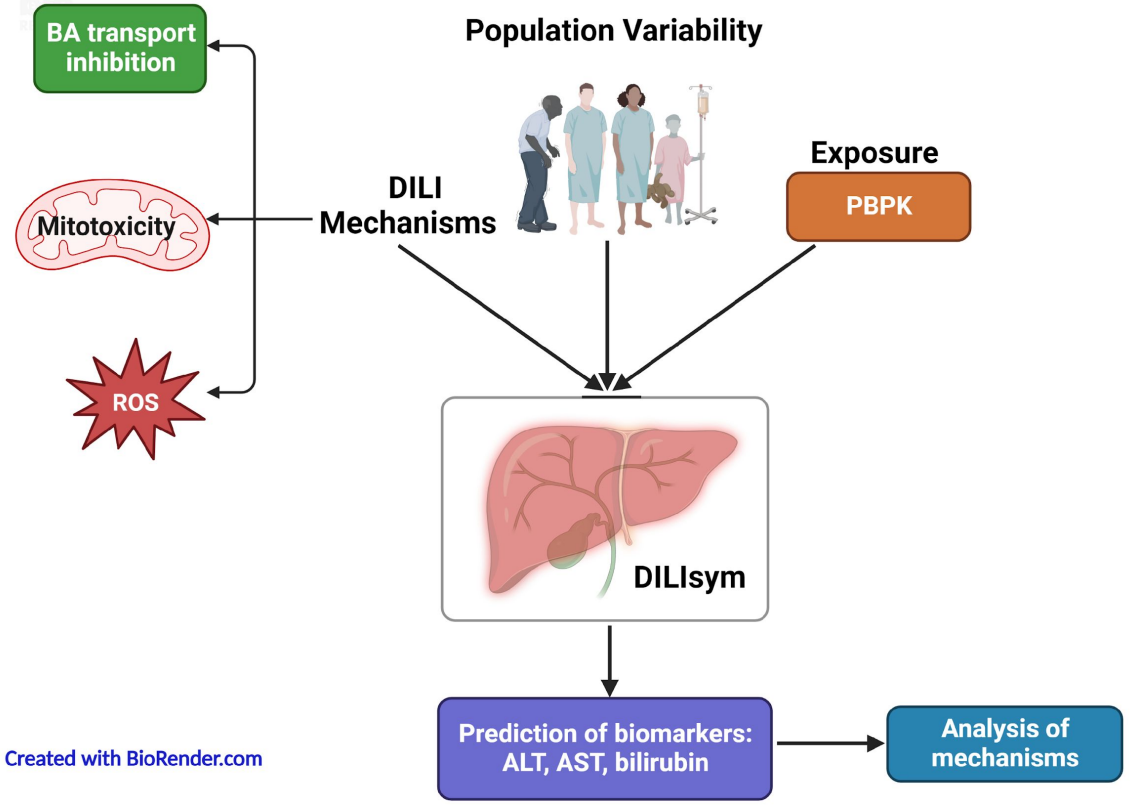
Immune-System Mediated Liver Injury

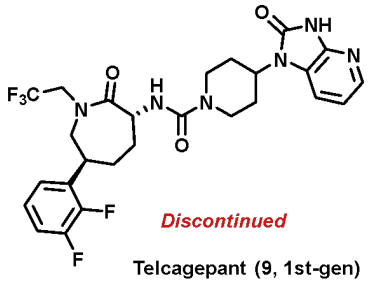
- Immune-mediated DILI is rare and complex
  - Involves both innate and adaptive immune systems
  - Drug-specific T cells isolated from patients with DILI
  - HLA alleles detected as important susceptibility factors
- Checkpoint inhibitors (CPI) are associated with hepatotoxicity
  - Increased risk of all-grade and high-grade hepatitis
  - Combination therapies have higher risk of toxicity: lower predicted human dose is recommended if CPI combination is planned in the clinic
- Need for screening strategies to detect immune-mediated DILI risk
  - Move towards long-term 3D hepatocyte culture or co-culture systems consisting of liver and dendritic cells



In Silico Prediction of DILI

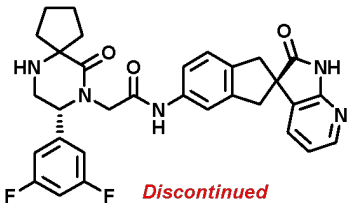
- DILIsym is a quantitative systems toxicology model developed in 2010 to integrate various DILI mechanistic approaches
  - Graphical user interface allows users to specify new variables
  - Predicts drug/metabolite hepatic concentrations using PBPK modeling
  - Assesses concentration-dependent impact on multiple DILI mechanisms
- Captures cumulative effect of drug or metabolites on pathways to predict hepatocyte death and release of serum biomarkers





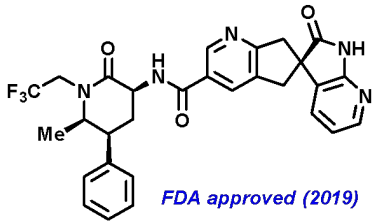
Discontinued

Telcagepant (9, 1st-gen)
DILI positive
DILIsym prediction: DILI-positive



Discontinued

MK-3207 (10, 2nd-gen)
DILI positive
DILIsym prediction: DILI-positive



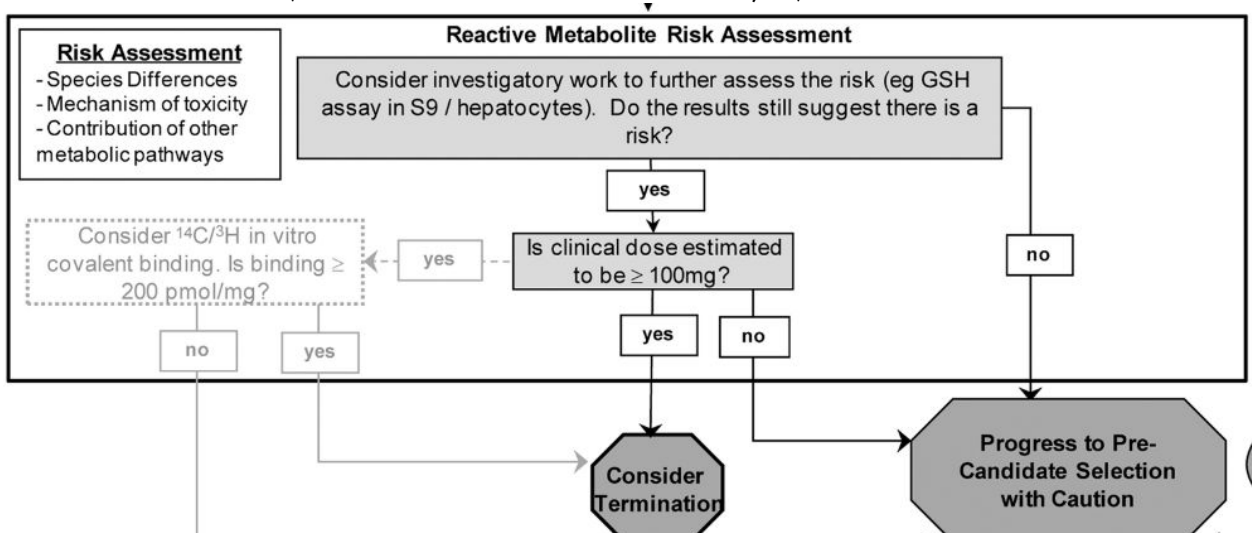
FDA approved (2019)

Ubrogepant (8, 2nd-gen)
DILI negative in clinical trials
DILIsym prediction: DILI-negative

Classification-Based Holistic Assessment of DILI Risks

GSK (2012): improved prediction of DILI risk by combining reactive metabolite risk **(Merck, 2004)** with predicted human dose

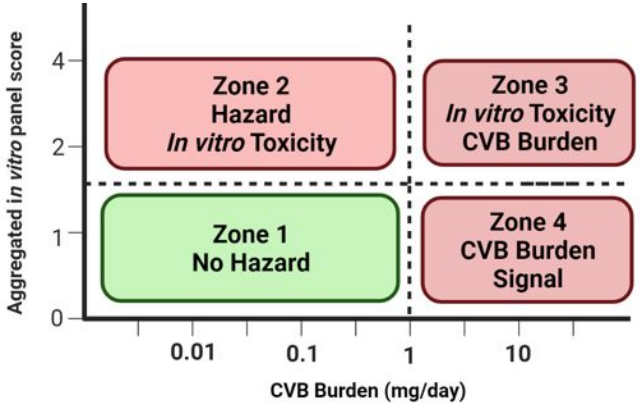
Sakatis, M. Z *et al. Chem. Res. Toxicol.* **2012,** *25*, 2067-2082



AZ (2012): combination of CVB burden with predicted human dose (**Daiichi Sankyo, 2004**) and 5 DILI mechanistic assays

Nakayama, S. *et al. Drug Metab. Dispos.* **2009,** *37*, 1970-1977

Thompson, R. A *et al. Chem. Res. Toxicol.* **2012,** *25*, 1616-1632



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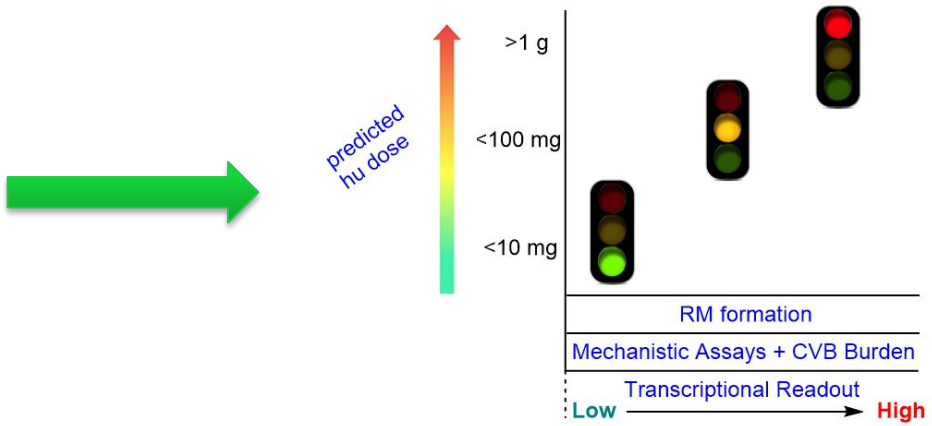
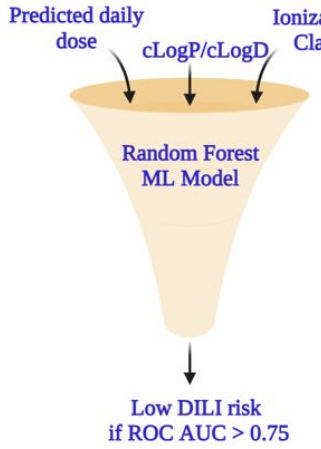


Current assessment of DILI risks

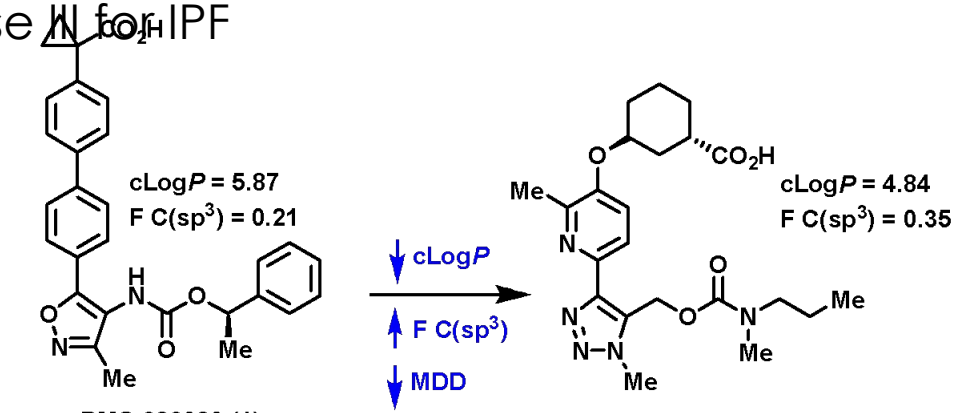


Martin, M. T. *et al. Toxicol. Sci.* **2022,** *188*, 208-218

Pfizer (2022): Machine learning early DILI screening

DILI Mitigation Strategies Case Study 1: LPA1 Antagonists

- LPA1 is a key mediator of lung fibrosis and idiopathic pulmonary fibrosis (IPF)
- BMS-986020 demonstrated proof-of-concept efficacy in a 6-month Phase II trial at 600 mg BID
  - Voluntarily terminated due to elevated ALT & AST (3 × ULN) and cholestasis in 3/96 patients
- DILIsym analysis suggests a combination of bile acid transporter inhibition and mitochondrial dysfunction as key contributing factors to clinical DILI
- **Mitigation strategies**: abolish above liabilities and reduce maximum daily dose (MDD) to give BMS-986278, currently in Phase III for IPF



BMS-986020 (1)

LPA_1 K_b = 6.7 nM

BSEP IC_{50} = 4.8 μM

MRP4 IC_{50} = 6.2 μM

MDR3 IC_{50} = 7.5 μM

MDD: 600 mg BID

BA transporter inhibition & mitochondrial toxicant

BMS-986278 (2)

LPA_1 K_b = 6.9 nM

BSEP IC_{50} > 100 μM

MRP4 IC_{50} = 47 μM

MDR3 IC_{50} > 100 μM

MDD: 125 mg BID

No heptatobiliary toxicity

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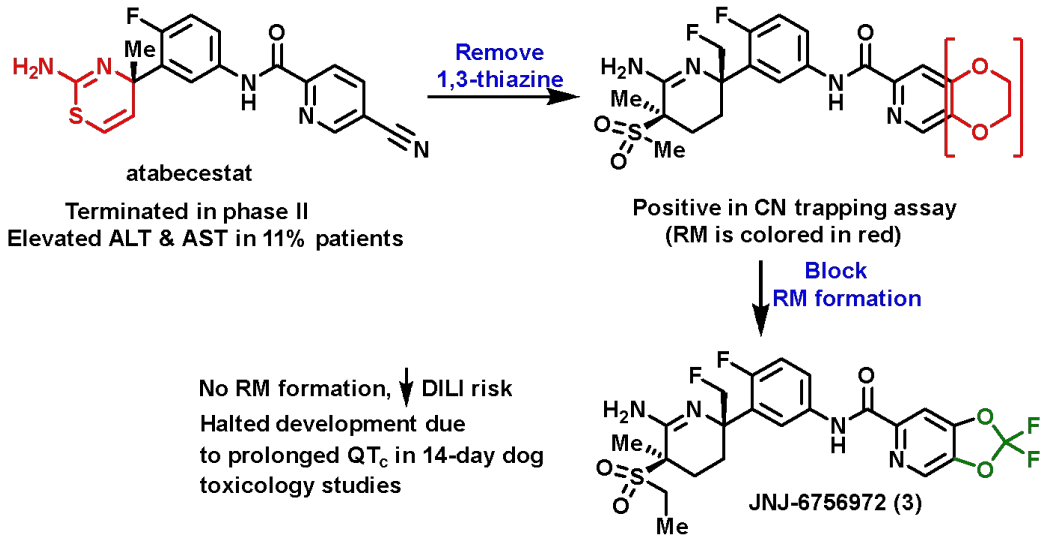
DILI Mitigation Strategies Case Study 2: BACE1 Inhibitor

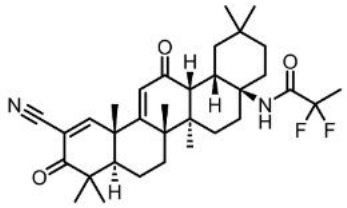
- Atabecestat was terminated in Phase II due to elevated ALT & AST (3 × ULN) in 11% patients, likely the result of immune-mediated DILI
- **Mitigation strategies**: reduce RM formation and maximum daily dose (MDD) to give JNJ-6756972 with predicted lower DILI risk



- **Recommended best practices with the Rule of Two: MDD < 100 mg/day + LogP < 3**

Balancing DILI Risks with Therapeutic Opportunities

Omaveloxolone



Nrf2 activator

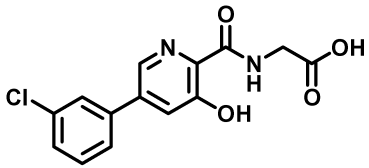
REATA PHARMA., TX / BIOGEN, MA

FDA approval: Feb 2023

Omaveloxolone is a Nrf2 activator approved for the treatment of Friedreich's ataxia (FA)-an aggressive neurodegenerative disease with no approved therapy

Omaveloxolone treatment resulted in elevated liver injury biomarkers-FDA recommends ALT, AST and bilirubin assessment before treatment

Vadadustat



HIF-PH inhibitor for CKD anemia

Akebia Therapeutics

FDA CRL: March 2022

FDA approval: March 2024

FDA issued a CRL in 2022 for Akebia over safety concerns including increased risks of thromboembolic events and DILI. Vadadustat was approved for use in Japan since June 2020

In March 2024, FDA approved Vadadustat based on safety data collected from Japan in which no DILI case was reported in the 2 year-post-marketing period.

https://drughunter.com/molecule/omaveloxolone/

https://www.fiercepharma.com/pharma/akebia-therapeutics-vadadustat-gets-its-shot-us-market-fda-win-two-years-post

Conclusion and Additional Resources

- DILI arises from a complex interplay between multiple mechanisms
  - Consideration in preclinical space depends on risk-therapeutic benefit analysis
- Successful mitigation strategies involve reduction of MDD and compound lipophilicity
  - Integrating read-outs from multiple DILI risk factors informed by suites of *in vitro* assays
- Recent advances in technologies include HepatoPac cultures, transcriptomic profiling, and DILIsym
- **Additional resources:**

Two-part ACS webinar on DILI:

Part 1: https://www.acs.org/acs-webinars/library/liver-injury.html

Part 2: https://www.acs.org/acs-webinars/library/liver-injury-2.html

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Acknowledgement

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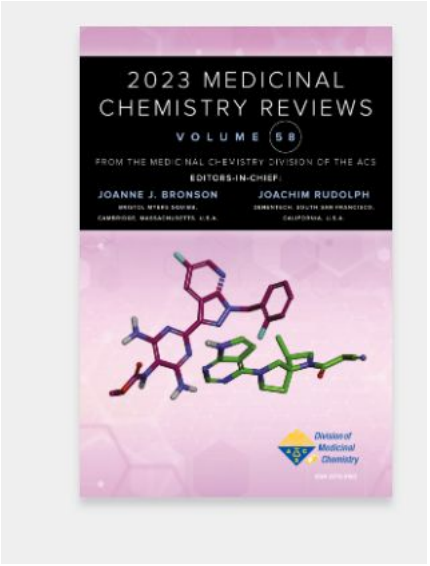

DILI chapter link: https://pubs.acs.org/doi/10.1021/mc-2023-vol58.ch19

***Current Affiliation: Altos Labs**

Supplementary Slides: HepatoPac vs Other *In Vitro* Models

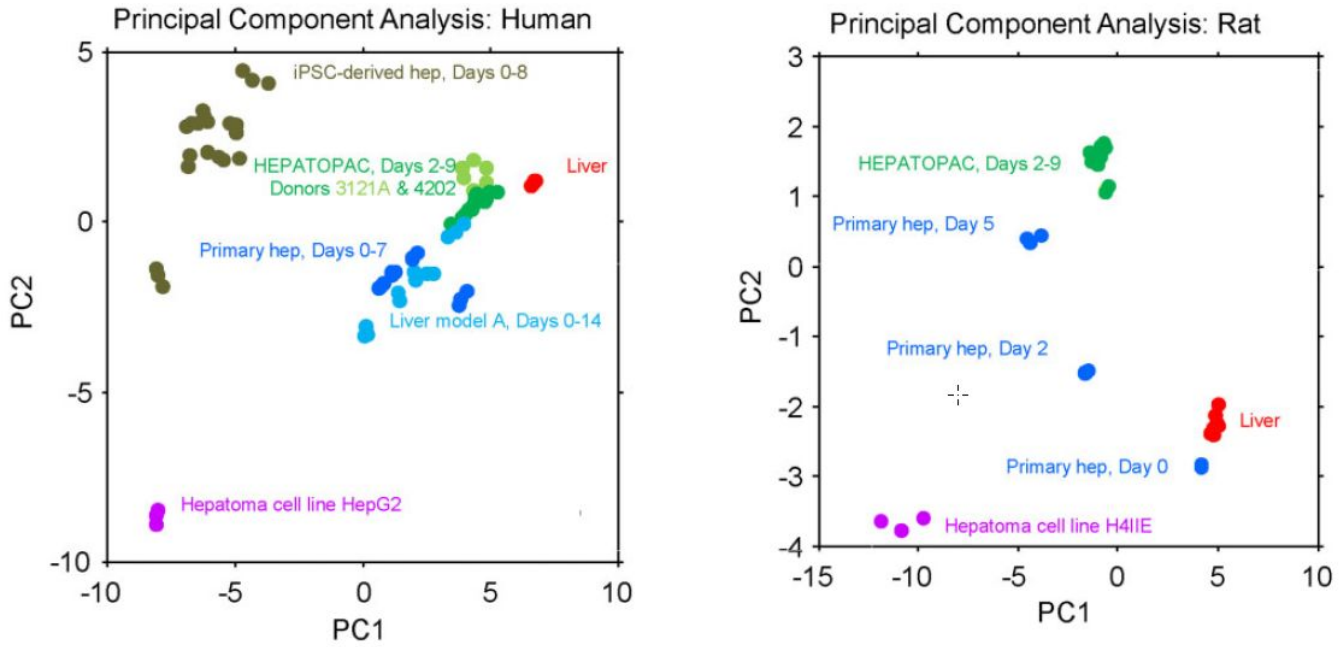

Figure 1. Principal component analysis (PCA) in baseline gene expression of *in vivo* liver and *in vitro* liver models. Genome-wide RNA-Seq data were generated using RNA harvested from human and rat liver, HEPATOPAC, primary hepatocytes, hepatoma cell lines, and other proprietary *in vitro* liver models as described in the Materials and Methods section. PCA in the basal expression was conducted using 68 prioritized genes from human and 72 prioritized genes from rat. The selected rat genes came from HNF4A, AHR, CAR, PXR+CAR, NRF2 biomarker signatures described elsewhere (Podtelezhnikov *et al.* 2020) and the human genes are orthologs of the rat genes (see Supplementary Figure 5 for gene list). For the PCAs of the human models, PC1 accounts for 58% and PC2 accounts for 18% of the total variance. For the PCAs of the rat models PC1 accounts for 74% and PC2 accounts for 15% of total variance.

Kang, W. *et al. Toxicol. Sci.* **2020,** *177*, 121-139.

Table 2. Albumin and Urea Production Rates in the HEPATOPAC

Production Rate	Human HEPATOPAC	Human *In Vivo* Liver	Reference (*In Vivo* Liver)
Albumin (μg/day/million cells)	24	37–105	Baudy *et al.* (2020)
Urea (μg/day/million cells)	170	56–159	
Production Rate	**Rat HEPATOPAC**	**Rat *In Vivo* Liver**	**Reference (*In Vivo* Liver)**
Albumin (μg/day/million cells)	250	~200	Bean and Atkinson (1984), Ruot *et al.* (2000), and Sohlenius-Sternbeck (2006)
Urea (μg/day/million cells)	1500	~500–1500	

Supplementary Slides: Miscellaneous