Building Confidence in MPS Use in Regulatory Applications: The 3Rs Collaborative's & FDA-CDER's Cross-Platform DILI Project

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Purpose and Rationale

- **Drug-induced liver injury (DILI)** remains a major cause of late-stage drug attrition and regulatory action
  – Existing animal and conventional 2D in vitro models frequently fail to predict human risk.
- **Liver microphysiological systems (MPS)** offer more human-relevant biology but lack standardized evaluation and confidence in regulatory application
- The present work describes a **public–private collaboration between the 3Rs Collaborative and FDA-CDER** to systematically evaluate liver MPS for a **specific regulatory Context of Use (CoU)**

Objective

- To conduct a **cross-platform evaluation** of commercially available liver MPS platforms using harmonized principles and blinded compound testing, with a focus on **category-level performance evaluations** rather than ranking individual technologies

Project Timeline and Milestones

- **Dec 2023** – Consortium agreement between FDA-CDER & 3RsC-MPS
- **Jan – Aug 2024** – Project brainstorming of scope, stakeholders, & expectations
- **Sept – Dec 2024** – Finalize COU, protocol, & test compounds
- **Jan – May 2025** – Finalize chemistry request forms, sign RCAs, Draft & Submit LOI to ISTAND program
- **June – Dec 2025** – Conduct experiments & submit data. Answer LOI questions. Draft process paper
- **Jan – June 2026** – ISTAND LOI accepted. Submit process paper. Data analysis. Draft results paper.
- **July – Dec 2026** – Submit results paper & qualification plan. Follow-up transcriptomics.

Regulatory Application

Regulatory Context of Use (CoU)

Use of a liver MPS as a retrospective analytical tool to evaluate elevated liver enzymes (ALT/AST) observed in early-phase clinical trials, providing weight-of-evidence to inform continuation or modification of dosing.

- **Platform-agnostic**, aligning with FDA's ISTAND program emphasis on **category-level qualification**, not endorsement of individual products

Evidence of liver injury in clinical trials that has not met Hy's Law

- Elevated ALT/AST without meaningful bilirubin increases; may present with clinical symptoms
- Not explained by other causes

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Potential to cause severe DILI is suspected

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Assessment of DILI potential

- Investigate any class effects and mechanism of action
- Collect additional non-clinical data:
  - *In vitro*: metabolic pathways, mechanistic assays (e.g., reactive metabolite formation, mitochondrial toxicity, transporter inhibitions), **PROPOSED: human liver MPS (e.g., hepatocyte toxicity, DILI mechanistic endpoints)**
  - *In vivo:* histopathology and clinical pathology from animal toxicity studies
  - *In silico:* computational models
- Collect additional clinical data related to safety, ADME, patient-specific factors, etc.

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Weight of evidence to determine likelihood of causing severe DILI

- **Unlikely** → Continue clinical development with appropriate monitoring
- **Likely** → Discontinue clinical development or proceed with extra caution and careful monitoring if risk-benefit ration warrants to continuation of clinical development

Collaborative Framework

Stakeholder Type	Specific Organizations	Project Role
Independent Non-Profit	The 3Rs Collaborative	Project coordination and dissemination
Regulatory Agency	FDA Center for Drug Evaluation and Research	Project leadership and guidance
Independent Non-Profit	Critical Path Institute	Context of use and qualification expertise
End-Users	Merck & Other Consultants who formerly worked at other pharmaceutical companies	Technical leadership, context of use and qualification expertise, compound selection
Government Organization	NIH – NIEHS	Technical leadership, compound selection and blinded provision, independent data analysis, technical scientific support
Developers of MPS	BioIVT CN Bio Pixl Bio InSphero Lena Biosciences PredictCan TissUse Xellar Biosystems	Execute planned study using their commercial platforms and provide data

Platforms Evaluated

Eight commercially available liver MPS platforms representing a broad range of technical and biological designs:

- Plate-based and chip-based systems
- Static and flow-based configurations
- Primary human hepatocytes, iPSC-derived hepatocytes, and co-cultures with non-parenchymal cells

Platforms included:

BioIVT, CN Bio, InSphero, Lena Biosciences, Pixl Bio, PredictCan, TissUse, and Xellar Biosystems

Study Design and Governance

Eight commercial liver MPS platforms representing a wide range of biological complexity, throughput, and culture formats were included.

Key features of the project:

- Harmonized but not identical protocols (recognizing platform diversity)
- Blinded testing of compounds
- Independent compound selection, blinding, data handling, and analysis by NIH-NIEHS/NICEATM
- Strong involvement of regulators, end users, and an independent coordinating body (3RsC)

The intent is NOT to provide head-to-head platform ranking and instead focuses on understanding variability and performance at the endpoint level for regulatory CoU

Disclaimer: The findings and conclusions in this poster have not been formally disseminated by the U.S. Food and Drug Administration (FDA) and should not be construed to represent any Agency determination or policy.

Experimental Strategy

- **8 drugs with known clinical truth:** 4 associated with high DILI risk and 4 structurally related low-risk controls
- Harmonized solvent conditions (0.2% DMSO)
- Exposure levels anchored to **multiples of human Cmax**
- Repeated dosing for **3–14 days**, depending on platform capabilities
- GLP-like documentation and quality control encouraged across all sites

Endpoints and Clinical Relevance

- **High DILI clinical relevance: ALT, AST (clinical liver injury markers); CYP3A4 activity, Phase I & II enzymes (drug metabolism predictor)**
- **Medium to high relevance: Albumin, urea, bile acid, cytokines (hepatocyte function markers); LDH (liver enzyme leakage)**
- **Medium relevance: ATP content (general cytotoxicity, bioenergetics)**
- **Emerging: Transcriptomics and LC-MS profiling (predictive, mechanistic insight)**

Summary of Assay Endpoints Provided Across Labs

	ATP	Albumin	Urea	LDH	ALT	AST	CYP3A4	Bile acids	Viability	IL6	Other Proprietary
PredictCan	Y	N	N	N	N	N	N	N	N	N	N
InSphero	Y	N	N	N	N	N	N	N	N	N	N
Xellar	Y	Y	Y	N	Y	N	N	N	Y	N	N
Lena	Y	Y	Y	Y	N	N	Y	N	N	N	Y
DefiniGEN	Y	Y	N	N	Y	Y	Y	N	N	N	N
CN Bio	N	Y	N	Y	Y	Y	Y	Y	N	Y	N
BioIVT	Y	Y	Y	Y	N	N	N	N	N	N	N
TissUse	Y	Y	Y	Y	Y	Y	N	N	N	N	N
Total	7	6	4	4	4	3	3	1	1	1	1

Acknowledgments

Supported in part by NIH-NIEHS & in-kind contributions from participating organizations.

The authors gratefully acknowledge individuals who contributed to this project including Nicole Kleinstreuer (National Institute of Health), Samantha Wilkins (Critical Path Institute), Suramya Waidyanatha (NIH/NIEHS), and those from participating commercial model developers: BioIVT, CN Bio, InSphero, Leno Biosciences, Pixl Bio, PredictCan, TissUse, Xellar Biosystems.

Data Analysis Approach

- Standardized NIH-NIEHS data collection templates
- Independent data management and statistical evaluation by NICEATM
- Cross-platform analysis focused on **endpoint behavior within the defined CoU**, not direct head-to-head platform comparisons

Preliminary Results Summary

- Most labs **successfully distinguished the safer compound** for the 4 drug analogue pairs evaluated (8 blinded drugs); some platforms were less effective
  – 4 drugs of high DILI concern
  – 4 drugs of lower DILI concern
- Most technologies **correctly identified high vs. low DILI concern**; with some platforms revealing more nuanced categorization of DILI concern (i.e., high, moderate, low, none)
- Analysis of potency and liver injury severity estimates for DILI in progress
- Analysis of variation within and across technologies in progress
  – Liver enzyme leakage biomarkers
  – Hepatocellular function indicators

Key Insights and Lessons Learned

- A **narrow, clinically anchored CoU** was critical to feasibility and regulatory relevance
- Platform diversity reflects real-world use and harmonization of **principles** (not identical protocols) enabled inclusion of diverse platforms
- **Independent coordination and strong project management** were essential to managing complexity across many stakeholders
- Legal and operational barriers (eg, MTAs and RCAs) were a major time constraint, highlighting the need for **early agreement templates and negotiations**

Limitations

- Limited number of compounds (only four DILI-positive) restricts mechanistic breadth.
- Technology platform variations do **not** enable direct head-to-head platform comparison or causal variable attributions to differences
- Experiments were run by expert developers, so **transferability to external labs** and reproducibility were not assessed
- Despite these constraints, the study is positioned as a **proof-of-concept** for regulatory qualification pathways

Overall Significance

- This work demonstrates how **cross-platform, blinded, and CoU-driven evidence generation** can build regulatory confidence in liver MPS without requiring full standardization
- It provides a **practical blueprint** for how MPS and other New Approach Methodologies (NAMs) can be evaluated for regulatory use
- The project supports the 3Rs and strengthens **human relevance in safety assessment**