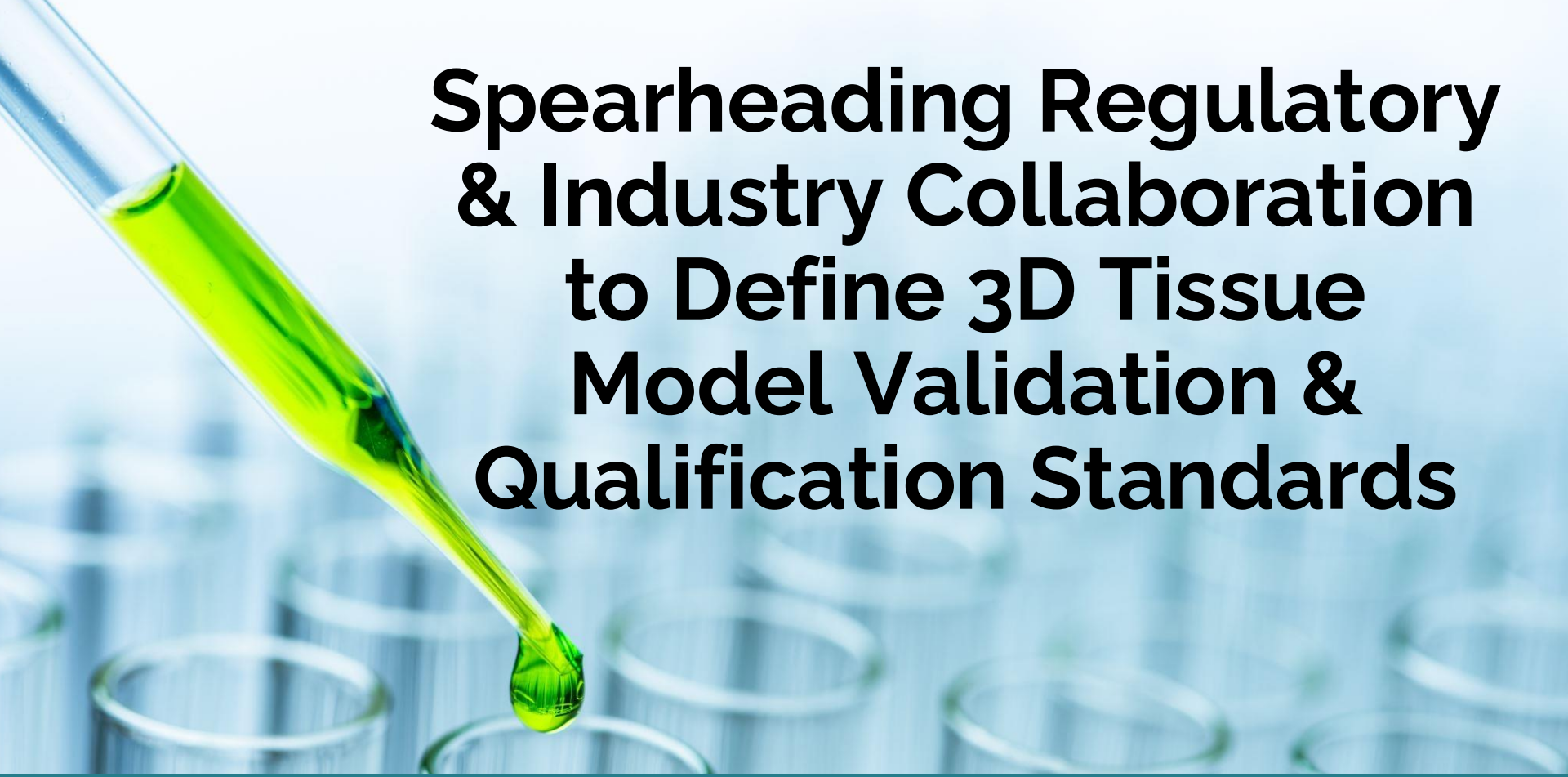
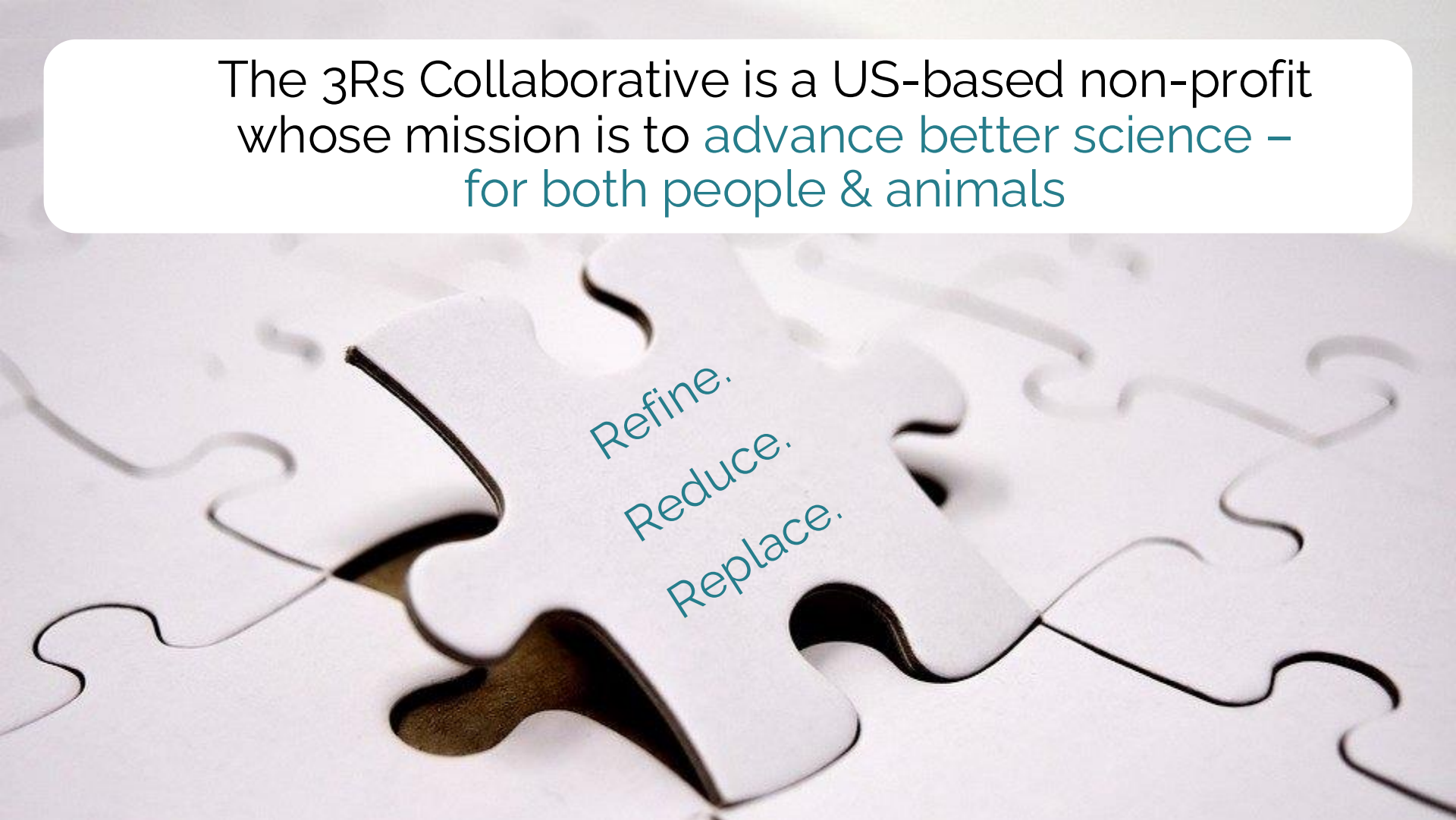


Spearheading Regulatory & Industry Collaboration to Define 3D Tissue Model Validation & Qualification Standards

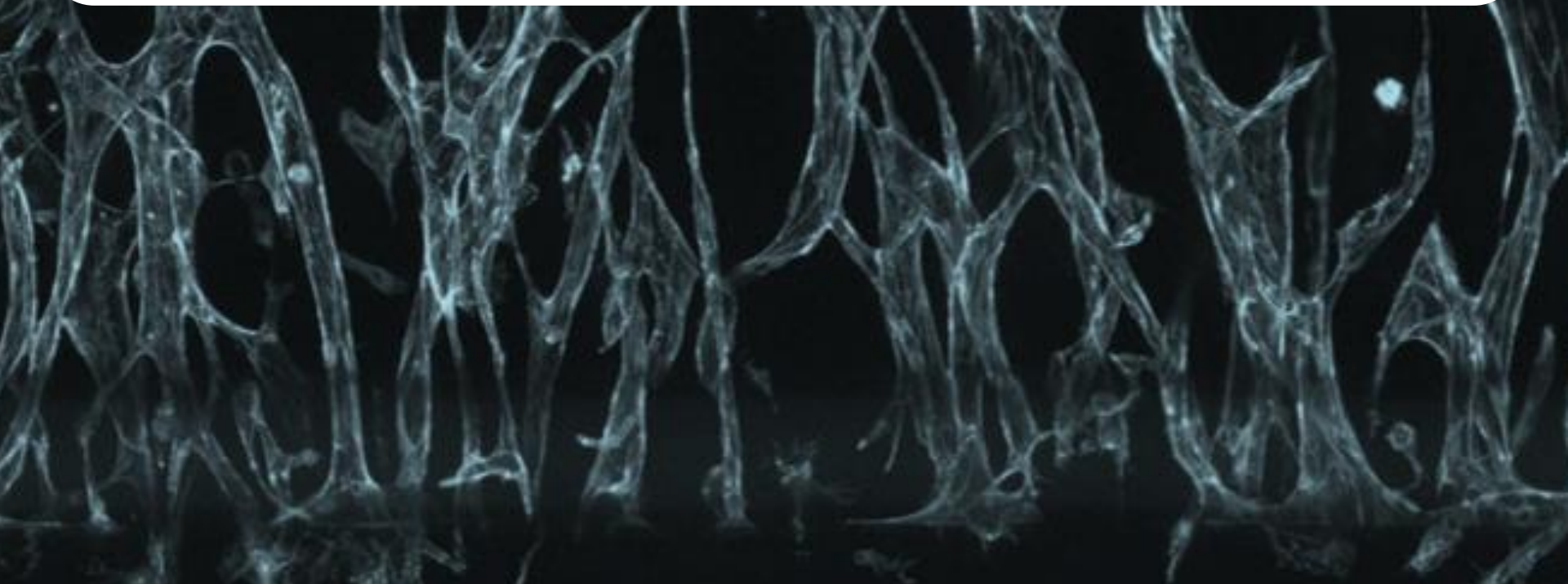
Megan LaFollette, The 3Rs Collaborative & Alican Ozkan, Abbvie

The 3Rs Collaborative is a US-based non-profit whose mission is to advance better science – for both people & animals



One of the 3RsC's key efforts is our MPS-focused initiative

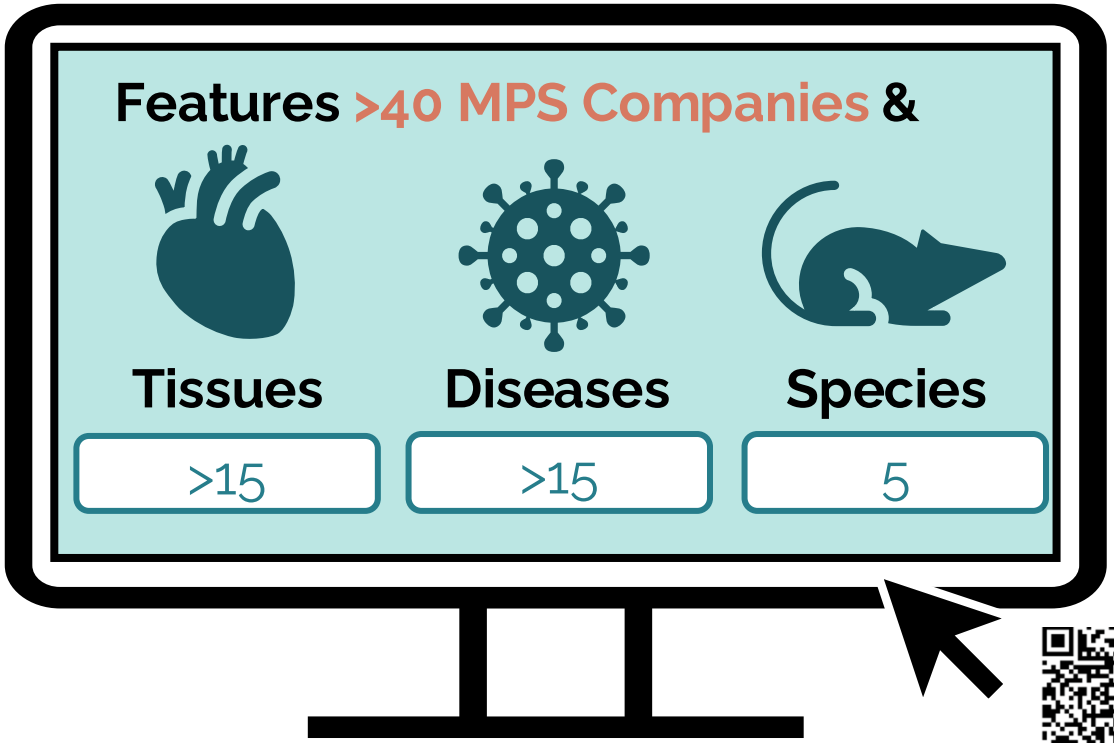
This initiative's core goal is to increase adoption & regulatory use of MPS where scientifically justified.



Specifically, the 3RsC-MPS Initiative aims to:

1. Provide thought leadership and expert consensus from developers combined with other stakeholders on implementation of MPS.
2. Facilitate appropriate discussion, collaboration, and sharing between commercial developers of MPS.
3. Create direct engagement between developers, end-users and regulatory agencies on the use of MPS.
4. Develop external partnerships and collaborations
5. Create resources to facilitate increased engagement with MPS.

Our tech hub connects end-users with relevant commercial tech providers.



New!

Publication Hub: explore publications from commercially available companies using the same filters!

In 2025, the 3RsC-MPS Initiative published a paper on "The use of MPS in 3Rs & Regulatory Applications"

End-Users, Regulators, & Developers Should Work Together On…

Develop & validate MPS for specific contexts of use

Considerations for standards and outcome measures for specific contexts of use

Showing the advantages of MPS in regulatory submissions

The US-FDA is also committed to
advancing alternative methods
for regulatory use to advance science & the 3Rs.

One way the FDA achieves this goal is through its **partnerships** to facilitate communication & collaboration.

In Dec of 2023, the 3RsC & FDA-CDER entered into a formal **public private partnership agreement**.

Disclaimers

The larger 3RsC-DILI project is being conducted under a Private Public Partnership (PPP) agreement with the FDA-CDER. While this presentation represents some of the work done under that PPP, it does not constitute FDA policy/determination nor is it being disseminated by FDA. Furthermore, this presentation has not been reviewed in advance by the FDA.

Independent analysis of the 3RsC-DILI study results is ongoing and will be available in future publications/presentations

Together, we decided to focus on **liver MPS**.

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.

Objective: To support **building confidence** in NAMs through a **cross-platform evaluation** of commercially available liver MPS platforms using **harmonized principles** grounded in a common study protocol which recognizes **platform diversity**.

Our project includes **all major stakeholders** in MPS drug development

STAKEHOLDER	ORGANIZATION(S)	PROJECT ROLE
Independent Non-Profit	The 3Rs Collaborative	Project coordination and dissemination
Regulatory Agency	FDA Center for Drug Evaluation & Research	Project leadership and guidance
Independent Non-Profit	Critical Path Institute	Context of use and qualification expertise
End-Users	Merck & Other Pharma Consultants	Technical leadership, context of use and qualification expertise, compound selection
Government Organization	NIH – NIEHS	Technical leadership, compound selection & 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

A huge thanks to the 3RsC DILI Leadership Team

Megan R LaFollette[1], Wen Kang[2], Stephen Ferguson[3], Laura Word[3] Graham Marsh[4], Deidre Dalmas[5], Ksenia Blinova[6], and Nakissa Sadrieh[6]

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[3]NIH/NIEHS, Durham, NC

[4]Critical Path Institute, Tucson, AZ

[5]Independent, Collegeville, PA

[6]FDA, Sliver Spring, MD

Key Project Deliverables

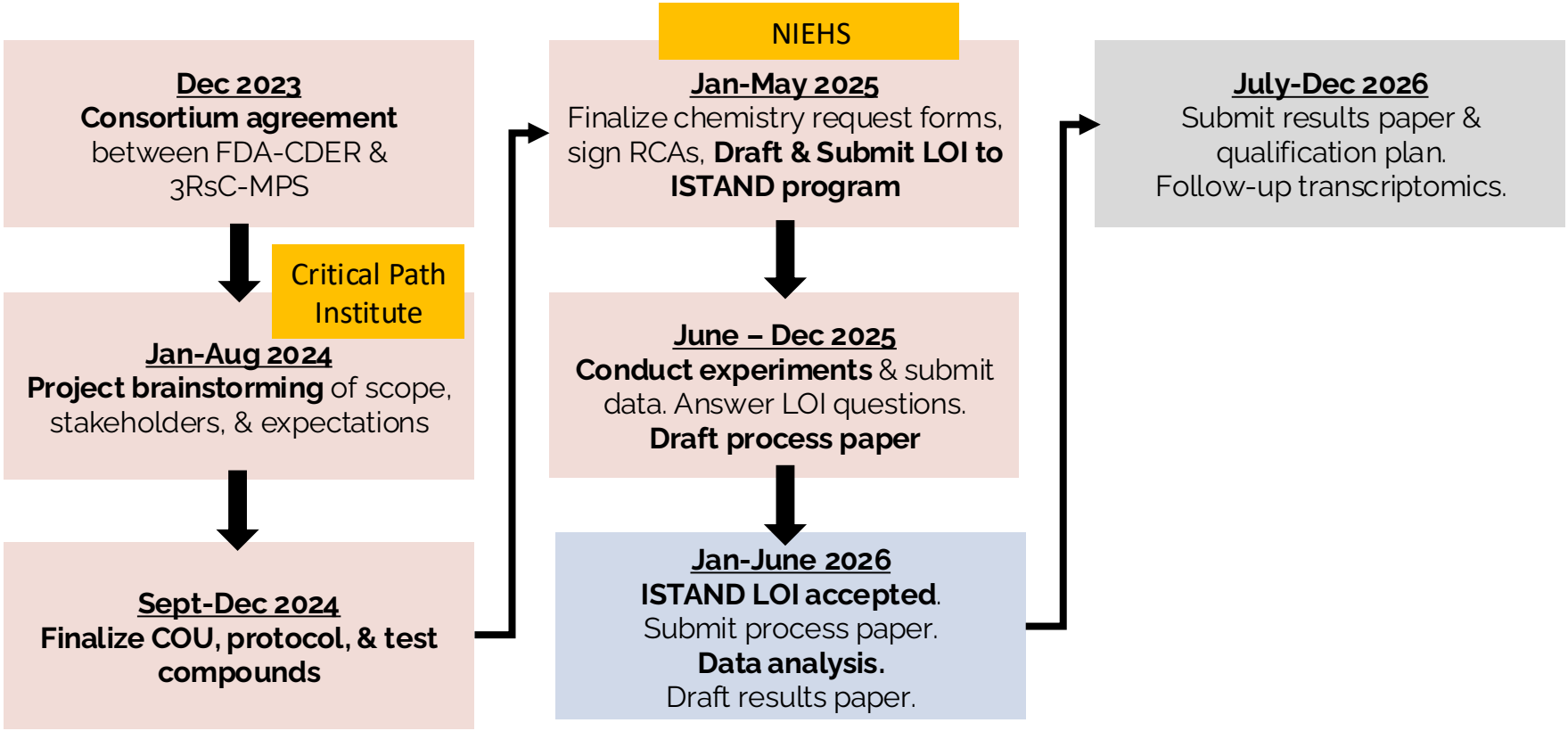
FDA ISTAND Program Qualification
(LOI Accepted in Jan 2026
QP In Progress)

Process Paper
(Submitted)

Cross-Platform Results Paper
(In Progress)

Best Practices & Guidelines
(In Progress)

We've made major progress in just over 2 years, although there is more work to go.



Our project is framed with a **single context of use (COU)** in mind

- Took learnings from the 2023 Critical Path Institute's Predictive Safety Testing Consortium workshop that included stakeholders from the FDA, academia, developers, and end-users.

Core Collaborator:  CRITICAL PATH INSTITUTE **& End-Users**

Regulatory Context of Use: 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 of dosing

Why this COU?
Regulatory-focused (e.g., data that regulators would see versus discovery data)
Specific & targeted
Gap for animal models
MPS already developed.

Study Design: 8 Commercially Available Liver MPS Developers were represented.

Represent a broad range of technical & biological complexity:

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

BIOIVT ELEVATING SCIENCE®

CN-BIO

insphero

LENA BIOSCIENCES

pixlbio

PredictCan Biotechnologies

TISSUSE Emulating Human Biology

Xellar biosystems

NOTE: 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.

Study Design: Companies ran versions of a **harmonized protocol** that were optimized for their platforms.

(e.g., not identical across diverse platforms)

Set-up	Seeding	Maturation	Dosing	End
Set-up components & characterize	Seed cells into platform & characterize	Allow cells to mature & assess baseline	Dose compounds for 3-14 days	Conduct final assessment of toxicity

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.

8 matched drugs with known clinical truth were selected, prepared, blinded and shipped to project participants by NIH-NIEHS/NICEATM.

Test Compound	Compound Type	Indication	Compound Class	DILI – Human Clinical Truth
Tolcapone	Positive	Parkinson's	COMT Inhibitors	⚠️ High risk: boxed warning for hepatotoxicity; requires frequent liver enzyme monitoring
Entacapone	Control	—	COMT Inhibitors	✅ Low risk
Troglitazone	Positive	Type 2 Diabetes	PPARγ Activators	⚠️ High risk: withdrawn from market due to severe DILI cases
Pioglitazone	Control	—	PPARγ Activators	✅ Low risk
Trovafloxacin	Positive	Broad-spectrum bacteriocidal	Bacterial Topoisomerase Inhibitors	⚠️ High risk: withdrawn from market due to severe DILI cases
Levofloxacin	Control	—	Bacterial Topoisomerase Inhibitors	✅ Low risk
Lumiracoxib	Positive	Inflammation	COX-2 Inhibitors (NSAIDs)	⚠️ High risk: withdrawn from market due to severe liver toxicity
Celecoxib	Control	—	COX-2 Inhibitors (NSAIDs)	✅ Low risk

Important Note: The drugs were blinded as a group of 8, NOT as structural analogues

Why Chosen? To represent our chosen COU across 4 distinct indications & drug classes within the small molecule designation.

**Thank you to Steve Ferguson & Wen Kang for this work!

Study Design: Companies ran versions of a **harmonized protocol** that were optimized for their platforms.

(e.g., not identical across diverse platforms)

Company	Technical Replicates	Dosing Day 1	# of Dosing Days	# of concentrations per compound
Range	3 - 8	0 - 8	1 - 14	4 - 9
Median	3	5.5	6.5	7.5

Assessment of Hepatocyte Function at End of Maturation	Albumin	Urea	P450-Glo	ALT	AST
# of Companies	6	6	5	2	1

Takeaway: Variety

4 companies used Albumin, Urea, & P450-Glo together
2 companies used 3-4 different assays
2 companies used only 1 assay

Study Design: Additional Experimental Strategies

- Independent compound selection, **blinding**, data handling, and analysis by NIH-NIEHS/NICEATM
- 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

Companies used a **variety** of assay endpoints

Assay	ATP	Albumin	Urea	LDH	ALT	AST	CYP3A4	Bile acids	Viability	IL6	Other Proprietary
# of Companies	7	6	4	4	4	3	3	1	1	1	1
Clinical Relevance	General Cytotoxicity & Bioenergetics	Hepatocyte Function Markers		Liver Enzyme Leakage	Clinical Liver Injury Markers		Drug Metabolism Predictor	Hepatocyte Function Markers			
Clinical Relevance Ranking	Medium	Med-High			High			Med-High			

	Total # of Assays
Range	1 - 6
Median	5

Takeaway: Variety. There was not a single assay run by EVERY company

Data is being **analyzed independently** by NIH-NIEHS.

Study Reports

Companies were asked to prepare the standard study reports they would send to clients after running such as study. Most classified DILI yes/no.

From these, we will calculate sensitivity, specificity, & accuracy. *And their correlation with # of assays run.*

Raw Data

Companies sent raw data in a standard template.

Assay results variability will be compared across platforms

NIEHS will determine results independently & which assays were most predictive.

To mimic what FDA might see, companies were asked to provide a "**study report**" to NIEHS with their conclusions evaluating all 8 drugs together

Little guidance was given to companies at this time as the project team wanted to see what tech providers might conclude based on their internal analysis and to determine if there was any sort of natural consensus on how these reports might be written.

Company created study reports were **hugely variable** in format and context.

- Conclusions ranged from “inconclusive” and simply listing assay results to “High DILI”, “Maximal DILI”, “Most DILI”, “Medium DILI”, “Moderate DILI”, etc.
- Some companies recommended further testing with additional donors, concentrations, or assays (e.g., RNA seq or transcriptomics)
- Some companies commented on differences based on dosing
- **Takeaway = Variety**

Preliminary Results: Some compounds were correctly identified more often than others.

		% Accuracy per Chemical Across All 8 Drugs and All 8 Companies
High incidence DILI drugs	A*	63%
	B	81%
	C	88%
	D	88%
Low incidence DILI drugs	A	69%
	B	81%
	C	63%
	D	75%
	#Assays	Range = 1 - 7

Letters indicate structurally matched pairs. Red letter = lowest accuracy. Darker green = higher accuracy.

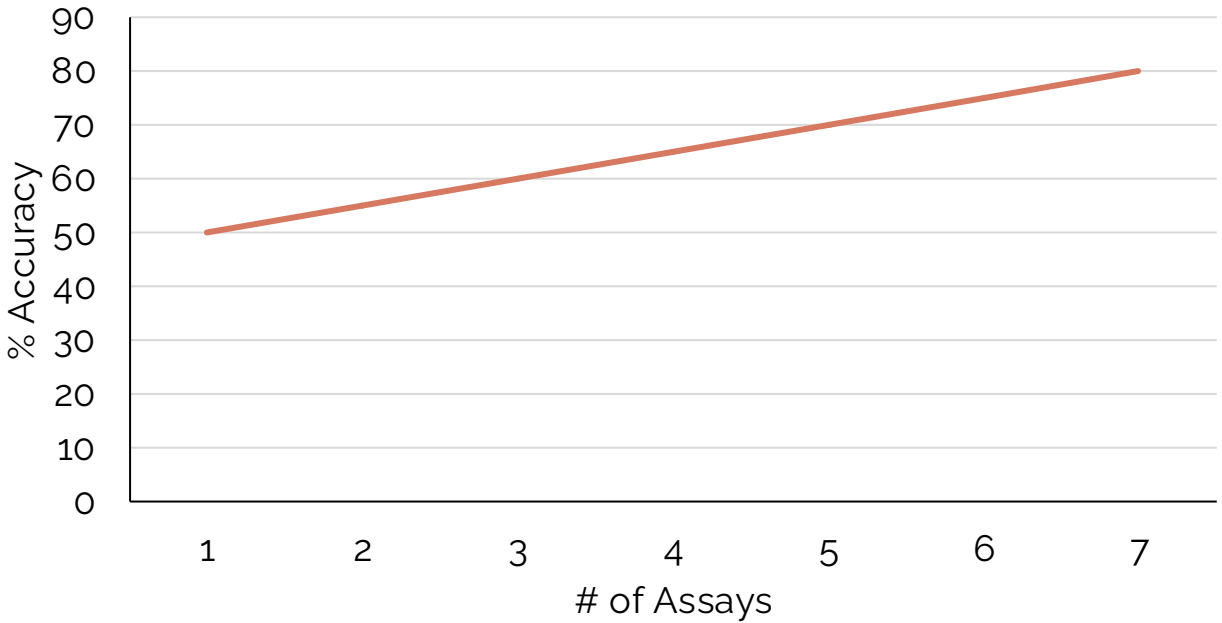
**This high DILI drug appeared to have a more challenging MOA for certain platforms*

Preliminary Results: Accurate identification of all 8 blinded compounds in company study reports varied greatly in verbiage & "accuracy".

Statistics	Range	Median
Sensitivity	25-100%	94%
Specificity	0-100%	82%
Accuracy	50-100%	85%

**Note: sensitivity, specificity, & accuracy could be much higher with a larger compounds sample size, follow-up analysis, and knowledge of the chemical structure.*

Preliminary Results: There was a trend towards **more assays** leading to **more accurate conclusions** in company generated study reports when all 8 compounds were grouped together.



Note: This is a composite, representative graph with the specific values removed to preserve company blinding. Outliers were seen to this general trend.

**Follow-up analysis is needed to determine which assays were most impactful as sheer number was not 100% predictive.*

Preliminary Results Summary

- Platform diversity in protocol, assay, and reporting reflects **real-world use even when evaluating the same CoU.**
- **Harmonization of principles** (not identical protocols) enabled inclusion of diverse platforms
- There was a preliminary trend showing that the **more endpoints** were analyzed, that the **more accurate** were the DILI predictions
- **Independent coordination and strong project management** were essential to managing complexity across many stakeholders.
- Legal and operational barriers (e.g., MTAs and RCAs) were a major time constraint, highlighting the need for **early agreement templates and negotiations.**
- **Additional analyses are ongoing and needed to more fully interpret the findings**

Limitations

- **Limited number of compounds** restricts mechanistic breadth.
- **Technology variations** do NOT enable direct head-to-head platform comparison or causal attributions of findings.
- Initial study reports **did not group drugs in structural analogues** due to blinding.
- **Interpretations** across multiple assays to conclude whether a drug causes DILI or not may be different for each company
- Some companies tested at **higher concentrations than 10X Cmax**, which may have impacted their DILI call conclusions

Despite these constraints, the study is positioned as a **proof-of-concept for regulatory qualification pathways**.

This work demonstrates how cross-platform, blinded, & 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.

Acknowledgments

- Supported in part by NIH-NIEHS & in-kind contributions from participating organizations
- The authors gratefully acknowledge all the additional 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.

You're invited to share your experiences with using MPS in drug discovery & development.

The 3Rs Collaborative

~10 minute survey
Confidential
Goal is to understand:
- Real work practices
- Barriers
- Opportunities

Led by researchers at the 3Rs Collaborative's MPS Initiative
(with input from the IQ-MPS & US-government reps)
Survey closes April 30.

Upcoming 3RsC-MPS Events

- Find the 3RsC & many of our members at the **MPS World Summit**
  - 3RsC-DILI Project (with MORE results)
  - **Thursday Afternoon:** 3RsC-MPS Poster
  - **Thursday Evening, 6:30-7:30pm Roundtable:** Advancing Global Alignment on NAMs: Regulatory Roadmaps, Challenges, and Opportunities

Upcoming 3RsC-MPS Event: Liver Webinars

- Join us for a 3RsC liver webinar: **June 8 & 9** (data-driven presentations about commercially available liver platforms). Scan the QR codes below to sign-up

Liver MPS Webinar - Day 1

Liver MPS Webinar - Day 2

Sign-up for our newsletter to connect with the 3RsC

Check out the tech hub & website: 3rc.org/mps

Email the 3RsC's Executive Director to connect: meglafollette@3rc.org