MPS TRAINING COURSE

Quick-Reference Guide

MPS TRAINING COURSE OVERVIEW

IQ MPS
IQ Microphysiological Systems Affiliate

Objective

To provide a comprehensive **overview of complex in vitro models (CIVM), including microphysiological systems (MPS)**, and **best practices** from the perspective of pharmaceutical industry end-users for evaluating and applying these tools in drug discovery and development.

Scope

This course will cover the **current and anticipated future uses** of CIVMs, including applications in **efficacy**, **ADME**, and **toxicology** studies, with an emphasis on **preclinical assessments**.

Module I

Introduction to CIVM and MPS

- Definition of CIVM and MPS
- Overview of current uses in the pharmaceutical industry
- Technical Considerations for Experimental Design
- Use of PBPK/QSP approaches to integrate in-vitro model endpoints and facilitate risk assessment/clinical translation
- General CIVM/MPS qualification considerations and challenges

Module II

Established CIVM / MPS (Organ-Specific Sessions)

- Organ-specific qualification considerations
  - Phenotypic and functional characterization
  - Essential features
- Application-specific (Safety/ADME/ Pharmacology) qualification considerations
  - Test compounds and positive controls
  - Predictive endpoints
  - Comparability or superiority to standard approaches (in-vitro/ in-vivo)
- Case examples of use for internal decision making

Module III

Context of Use-Specific Applications of CIVM / MPS

- Overview of special considerations for other COUs
- Additional model qualification and characteristics required for use with non-small molecule modalities
- Focus on non-safety applications

Module IV

CIVM / MPS in Development

- Overview of newer models and applications for interrogating human relevant Safety/ADME/Pharmacology questions
- 'Must have' characteristics of newer models
- Case examples

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MPS TRAINING COURSE

Module I: Introduction and Qualification of CIVM and MPS

Quick Reference Guide

www.iqmps.org/course

ABOUT MODULE I

Over the past decade, Complex In Vitro Models (CIVM) and Microphysiological Systems (MPS) have become increasingly more diverse in design, capabilities, and sophistication, reaching a point where the industry can now begin to apply these tools across drug discovery and development. While the rigor of qualification required to apply these models is largely dependent upon the specific context of use, there are fundamental technical and methodological considerations for designing CIVM experiments. This module will provide an overview of CIVM and how they are currently used in drug discovery and development, as well as offer insight into how CIVM data may eventually be used in concert with in-silico modeling. This module will also outline key technical considerations, such as cell sources, statistical design, confounding device variables, and construction methodologies. The module will close with an overview of high-level qualifications considerations, such as recapitulation of known drug response and comparability to gold standards. See page 4 for a complete list of all sessions in Module I and the topics covered in each.

ABOUT THIS GUIDE

The Module 1 Quick Reference Guide is an overview of key lessons from Module I Sessions 1-5, specifically important considerations to take into account in the early stages of qualifying MPS for various contexts of use, including model and experimental design. The reference guide follows the roadwork for MPS/CIVM use as illustrated on page 5, with technical, biological, and context-of-use (COU) considerations specified for each stage of model qualification. While not exhaustive, this guide provides a high-level overview of the many factors to consider and the variables to control when qualifying MPS for use in pharmaceutical drug discovery and development.

Module I

MPS Introduction and Qualification of CIVM and MPS

Thursday, July 13 10:00 am – 11:30 am ET	**Session 1 • Introduction to CIVM and MPS** ***Nakissa Sadrieh,*** *CDER/FDA* ***Aaron Fullerton,*** *Genentech* ***Anna K. Kopec,*** *Pfizer, Inc.*	• Course introduction • Course outline • Overview of models • Industry use of CIVM/MPS
Thursday, July 20 10:00 am – 11:30 am ET	**Session 2 • Technical Considerations for Experimental Design (Part I)** ***Jason Ekert,*** *UCB Pharma* ***Rhiannon David,*** *AstraZeneca* ***Jonathan Cairns,*** *AstraZeneca*	• Importance of cell sources and related materials • Media and ECM compatibility • Qualification of critical analytical endpoints • Statistical design
Thursday, August 24 1:00 pm – 2:30 pm ET	**Session 3 • Technical Considerations for Experimental Design (Part II)** ***Tom Chan,*** *Boehringer Ingelheim Pharmaceuticals* ***James Gosset,*** *Pfizer* ***Rebecca Hsia,*** *Genentech* ***Ratnakar Potla,*** *Genentech*	• Methodologies for construction of models and implications • Confounding device variables • Test article pharmacokinetics within the model
Thursday, September 21 10:00 am – 11:30 am ET	**Session 4 • Leveraging MPS Data for In-Silico Modeling** ***Diane Ramsden,*** *AstraZeneca* ***David Stresser,*** *AbbVie* ***Maarten Huisman,*** *UCB Pharma* ***Carmen Pin,*** *AstraZeneca*	• Use of PBPK/QSP approaches to integrate in-vitro model endpoints and facilitate risk assessment/clinical translation
Thursday, September 28 10:00 am – 11:30 am ET	**Session 5 • CIVM/MPS Qualification Considerations and Challenges** ***Aaron Fullerton,*** *Genentech* ***Lindsay Tomlinson,*** *Pfizer* ***Christopher A. Hinckley,*** *Biogen* ***Leah Norona,*** *Genentech*	• Considerations around therapeutic modality • Appropriate biological verification • Recapitulation of known drug responses • Clinical translatability • Comparability to “gold-standard” in the paradigm
December 4th 1:00 pm – 2:30 pm ET	**Session 6 • Module I Summary** ***Aaron Fullerton,*** *Genentech* ***Deidre Dalmas,*** *GSK*	• Review of Module 1 key concepts and questions • Panel discussion • Introduce Course Module II

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Module I Session 1
Introduction to CIVM and MPS

Thursday, July 13
10:00 am – 11:30 am ET

This session will provide an overview of the objectives and content of the IQ MPS Affiliate Training course followed by an overview of MPS platforms and how the pharmaceutical industry is currently using these and other Complex In Vitro Models.

FDA U.S. FOOD & DRUG ADMINISTRATION

Nakissa Sadrieh
Senior Advisor for New Alternative Methods,
CDER/FDA

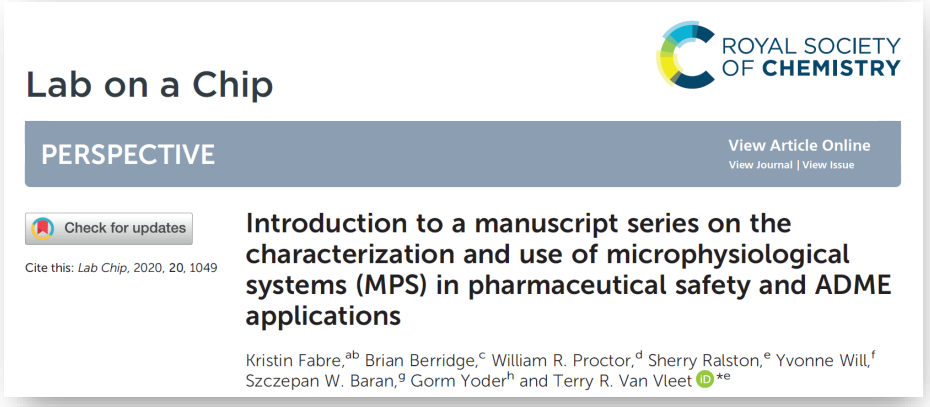
Aaron Fullerton, PhD, DABT
Director, Investigative Toxicology, Safety Assessment
Genentech

Anna K. Kopec, PhD
Director, Investigative Toxicology
Global Discovery, Investigative & Translational Sciences
Drug Safety R&D
Pfizer, Inc.

CIVM/MPS Definition within the Affiliate

Complex in vitro models (CIVM): Models going *beyond* traditional 2D culture and include several of the following design aspects:

- a multi-cellular environment within biopolymer or tissue-derived matrix
- a 3D structure
- mechanical factors such as stretch or perfusion/flow
- primary or stem cell derived cells
- immune system components



Fabre et al. *Lab on a Chip*, 2020, 20, 1049-57

Complex In Vitro Models (CIVM) typically denotes model types with a broad range of complexity

Microphysiological Systems (MPS) is used to specify models at the more complex end of the spectrum

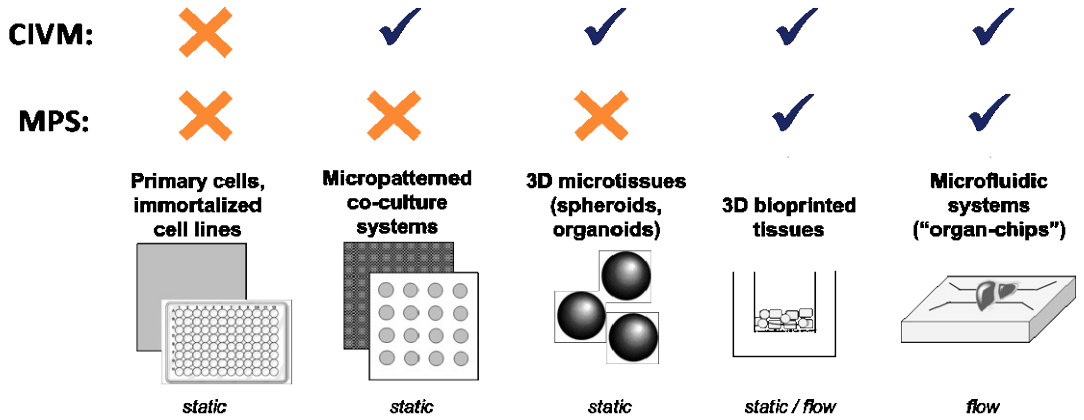


Image adapted from Dash and Proctor (2019)
Definition developed by IQ MPS Affiliate (Fabre, et al., 2019 & https://www.iqmps.org/)

Roadmap for MPS/CIVMs Use

Technical Considerations

Establish link between Biology ➡ Outcome

---- Organ-Specific ----

Context of Use

Establish Key Aspects of Biological Relevance:

- *Cellular composition*
  - *Multi-Cellular*
  - *Differentiation Status*
  - *Immune Competent*
- *Functional Readouts/Endpoints*
- *Model Format Selection*
  - *Single/Multiple Compartments*
- *Test Article PK/Exposure*
- *Calibration of Physical Cues:*
  - *Flow Rate, Stretch, etc…*

Alignment of model/assay endpoints with:

- *Molecular Initiating Events (MIEs)*
- *Adverse Outcome Pathways (AOPs)*
- *Toxicity/Disease Phenotypes*

- *Stability of Phenotype/Key Performance Indicators Over Experimental Time Course*

- *Characterization of Key Features*
- *Defined Reference Ranges*

Develop corresponding data analysis based on:

- Replicate Variability
- *Analytical Methods*

- *Experimental Design*

- *Normalization Strategies*
- *Positive Controls/Benchmarks*

Clearly defined objective(s) including:

- *Establish Criteria for Positive/Negative Outcomes*

- *Domain of Applicability*

- *Strengths and Limitations of the Model*

- *Comparison to Existing Performance Standards*

Assess sources of variability

- *Donor Variability*
- *Endpoint Linearity*
- *Model Differentiation/Maturation*
- *Sample Collection Protocols*
- *Assay Baseline (Signal/Noise Ratio)*
- *End Users*
- *Chip-to-Chip Variance*
- *Sample Stability*
- *Run-to-Run Batch Effects*

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Module I Session 2

Technical Considerations for Experimental Design (Part I)

Thursday, July 20, 2023
10:00 AM – 11:30 AM ET

This session will cover the key components that should be considered when developing a MPS model, such as the context of use, and physical and biological inputs (including cells, matrices and media). Furthermore, the concept of good experimental design for MPS will be presented using a case study example.

Jason Ekert
Head, US Discovery Translational Technology
UCB Pharma

Rhiannon David
Director of Microphysiological Systems (Safety Assessment)
AstraZeneca

Jonathan Cairns
Principal Biostatistician
AstraZeneca

- Rhiannon David (AZ)
- Jason Ekert (UCB)
- Jonathan Cairns (AZ)

Quick Reference:

Session 1.2 – Technical Considerations for Experimental Design (Part I)

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	Cell Source	Other Cell Variables	Media
Technical	• **Lifespan** concordant with required treatment duration • Stem cell **differentiation** – embryonic/iPSc -> multiple lineages, adult -> specific lineages • Confirm **cells express target of interest**	• **Donor number** and **demographics** (e.g. age, gender, ethnicity, heath status) • **Proliferation/Growth kinetics** - Passaging and time in culture	• Can all cells **access oxygen/nutrients** - can be challenging in larger cultures • If **serum** included, consider **source** (often animal derived) and **drug binding** potential (consider reporting free-drug concentration)
Organ-Specific		• **Disease cells** - source and method of inducing disease phenotype (if necessary) • **Phenotype maturity linked to functionality** (e.g. iPSC less mature but might be sufficient for organ functionality)	• Do the cells require **additional growth factors**? Can be added or supporting cells can secrete cytokines, ECM etc • **Multi-cell/multi-organ** cultures – if a **single medium** is used, ensure it supports all cell/organ types and maintains expected functionality.
COU-Specific	• **Cell types chosen to represent COU-specific needs/endpoints**	• **Donor number** and **demographics** • **HLA- matched vs autologous** (important for some COU e.g. immune responses) • **Disease cells** - source and method of inducing disease phenotype (if necessary) **Phenotype maturity linked to functionality** (e.g. iPSC less mature, but might be sufficient for COU)	• **Source of additional growth factors** (if needed)

- Rhiannon David (AZ)
- Jason Ekert (UCB)
- Jonathan Cairns (AZ)

Quick Reference:

Session 1.2 – Technical Considerations for Experimental Design (Part I)

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	Cell Characterization (prior to treatment)	Matrices & Scaffolds	Statistical Experimental Design
Technical	• **Cell health/viability**	• **Source**- Natural (Human/Animal/Tumor/Plants) or Synthetic and potential interaction with cells • **Reproducibility** (natural-derived typically have more batch-to-batch variability compared to synthetic) may need to be balanced with **ease of access/use** (e.g. Matrigel more available than decellularized matrix)	• Aim - a **clear, testable aim** matching design & analysis • **Blocking of confounding factors** to manage known technical variables • **Randomisation** used to defend against unknown factors • **Feasibility** – there is always a trade-off between an ideal design and the practical limitations.
Organ-Specific	• **Cell marker expression** and **localization/polarity** • Organ/cell-specific **function** to demonstrate physiological relevance	• Choice of scaffold or matrix should be **appropriate for the cell/organ type**	• **Generalisable** to the population of interest
COU-Specific	• Organ/cell-specific **function** to demonstrate relevance for COU	• Choice of scaffold or matrix should be **appropriate for the COU**	• Aim – a **clear, testable aim** matching design & analysis • Study **has appropriate sample size & power** (appropriate powering provides a good balance between reproducibility (avoiding "flukes") and conditions explored) • **Appropriate controls**

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Module I Session 3

Technical Considerations for Experimental Design (Part II)

Thursday, August 24, 2023
1:00 PM – 2:30 AM ET

Basic 2D monolayer cultures can fail to predict in vivo pharmaceutical candidate properties such as efficacy, ADME, or toxicity as they lack the necessary physical and biochemical cues found in vivo. MPS aim to fill this gap by exploiting advances in material sciences, microfabrication processes, and engineering to recreate the in vivo environment.

In this session, technical design considerations of MPS are discussed with respect to:

- Material considerations
- Fluid dynamics in systems with flow
- Mechanical stimuli were applicable
- Device qualification and operation
- Body-on-a-chip configuration
- Immune cell incorporation

Tom Chan
Senior Principal Scientist
Boehringer Ingelheim
Pharmaceuticals Inc.

James Gosset
Associate Research Fellow
Pfizer, Inc.

Rebecca Hsia
Scientist
Genentech

Ratnakar Potla
Principal Scientist
Genentech-Roche

- James Gosset (Pfizer)
- Tom Chan (Boehringer Ingelheim)
- Rebecca Hsia (Genentech)
- Ratnakar Potla (Genentech)

Quick Reference:

Session 3 – Technical Considerations for Experimental Design (Part II)

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	Materials	Physical Cues

Technical	• Accurate measurement of **drug(s) or metabolite(s) exposure** where primary endpoint is observed.
	• Account for **non-specific binding** or **ad/absorption** into MPS apparatus material.
	• Account for potential impact of **residual reactants** of the MPS on the viability and endpoint readout.
	• Assess the impact of **bioprinting shear** on:
	  • Viability
	  • Function
	  • Pluripotency
		• For **mechanical stretch** MPS models:
		  • Ensure that the chip material is stretch-compatible
		  • Ensure that cells are healthy and or exhibiting expected phenotype

Organ-Specific		• When mucus is washed periodically on chip, it is often necessary to introduce **recovery periods** after ALI and washes.
		• For **air liquid interface (ALI)** MPS models:
		  • Ensure that MPS material can withstand ALI
		  • If intermittent washing is required, ensure that barrier functions are maintained after washing

COU-Specific	• Impact of MPS material on **cell phenotype(s)** (e.g. level of differentiation applicable to CoU). • If **permeable barriers** are used, ensure that the pore sizes are suitable for the CoU (e.g., is size large enough for cell migration).	• Ensure that **physical cues** such as the flow directionality, flow rate and stretch dimensions are relevant to the CoU. • Account for **inhomogeneity** of vascular localization in vascularized MPS models via applying appropriate normalization measures.

- James Gosset (Pfizer)
- Tom Chan (Boehringer Ingelheim)
- Rebecca Hsia (Genentech)
- Ratnakar Potla (Genentech)

Quick Reference:

Session 3 – Technical Considerations for Experimental Design (Part II)

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	Operation, Setup, Fabrication

Technical	• Assess **consistency** of the setup and operation between repetitions or across different operators.
	• Account for impacts from **dead volume** or **evaporation** on endpoints, particularly when sampling repeatedly or in long-term incubations..
	• Custom MPS:
	  • Is the **fabrication consistency** of the MPS proven?
	  • Are the **materials** and **operating machinery** suitable for the CoU?
	  • How do the custom assays compare to established ones?

Organ-Specific	
COU-Specific	

- James Gosset (Pfizer)
- Tom Chan (Boehringer Ingelheim)
- Rebecca Hsia (Genentech)
- Ratnakar Potla (Genentech)

Quick Reference:

Session 3 – Technical Considerations for Experimental Design (Part II)

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	MPS Linking	Live Cell Assays

Technical	**PK and PD studies required**
	• Technical challenges:
	  • **PDMS absorption** of test article and/or metabolites
	  • Maintain **metabolite stability**
		• Several new methods are uniquely used with MPS – Need to ensure standard **SOPs** before reporting results.
		• **Variability**
		  • **Chip-Chip** – need for establishing values within each MPS platform
		  • **Assay-Assay** – SOPs have to be normalized to establish acceptable ranges for each assay
		  • **Donor-Donor** – can't be studied unless above two are well defined

Organ-Specific	• Necessity of **universal medium** required for organ specific endothelium to emulate the kinetics of clinical biomarkers	

COU-Specific	**PK and PD studies**
	• Biological challenges:
	  • Organ specific endos
	  • Cell lot/donor qualification	

- James Gosset (Pfizer)
- Tom Chan (Boehringer Ingelheim)
- Rebecca Hsia (Genentech)
- Ratnakar Potla (Genentech)

Quick Reference:

Session 3 – Technical Considerations for Experimental Design (Part II)

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	Immune Cells	Endpoint assays

Technical	• **Cell- device issues**
	  • Appropriate controls to factor immune cell-device material, ECM cross talk
	• **Cell secreted products**
	  • Factoring in loss of secreted product to device material, ECM.
	  • Establishing baseline levels and defining variability (See endpoint assays)
		• MPS devices enable measurements at a single cell level of multiple analytes.
		  • Need for **standardizing analyses** at such complex data intense scales.
		  • Achieving **statistical significance** is not possible without limiting variability at the level of single analyte omics.
		  • Need detailed 'omics' data to describe model features.

Organ-Specific		
COU-Specific		• **Baselines** and **acceptable ranges** need to be established for standard endpoint assays for CoU assays.

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Module I Session 4

Leveraging MPS Data for in-Silico Modeling

Thursday, September 21, 2023
10:00 AM –11:30 AM ET

With new approach methodologies (NAM) evolving in tandem and at a rapid pace, there is an opportunity to integrate them in ways previously thought impossible. This session aims to initiate discussion on how the diverse and complex datasets derived from CIVM and MPS models may be analyzed using quantitative systems biology (QSP), machine learning, and in silico modeling approaches. Examples will be provided through case studies, and a realistic view of the current gaps as well as future outlooks and opportunities will be discussed.

Diane Ramsden
Director, Oncology DMPK
AstraZeneca

David Stresser, PhD
Senior Principal Research Scientist
AbbVie

Maarten Huisman
Director, Quantitative Pharmacology and DMPK
UCB Pharma

Carmen Pin
Senior Director
AstraZeneca

- David Stresser (AbbVie)
- Diane Ramsden (AZ)
- Maarten Huismann (UCB)
- Carmen Pin (AZ)

Quick Reference:

Session 4 – Leveraging MPS data for in-silico modeling

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	PK properties	Integration	Future Efforts
Technical	• **QSP** Quantitative Systems Pharmacology • **PBPK** Physiologically Based Pharmacokinetic Modeling	• **Computational/Systems Biology** • Optimize in vitro and in vivo studies • Mechanism of action • **Statistics** • Understanding design requirement	
Organ-Specific	• **Modeling is organ-independent** • Can be applied anywhere collected data describes a response, trend, etc • MPS specific considerations may include, drug diffusion and/or partitioning cells or device, flow rates, peristaltic motion, strain cycle frequency, organ size, fluid dynamics, oxygen transport, biosensors/imaging techniques, evaporative loss, environmental cues		
COU-Specific	• **MPS models have the potential to bridge the gap between in vitro and in vivo studies**	• **Starts by defining the question** • Biology (Data generation, omics, in vitro/vivo) • Target and biomarker selection • Clinical Pharmacology/ Pharmacometrics • Build/integrate CP knowledge • Select relevant patient population • Clinical trial design	• **MPS has the potential for higher-throughput and in vivo-like complexity and predictivity** • Potential to be exception to the traditional tradeoff between throughput & predictivity

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Module I Session 5

CIVM/MPS Qualification Considerations and Challenges

Thursday, September 28, 2023
10:00 AM –11:30 AM ET

This session will provide an overview of qualification efforts for the use of CIVMs in various contexts of use and will include both a high-level overview of the current qualification landscape as well as examples of internal fit-for-purpose qualification efforts within Pharma companies for different contexts of use and discuss how these complex models present unique challenges to using traditional assay validation framework.

Aaron Fullerton, PhD, DABT
Director, Investigative Toxicology, Safety Assessment
Genentech

Lindsay Tomlinson, DVM, DVSc
Global Pathologist Resource Lead
Pfizer, Inc.

Christopher A. Hinckley, PhD
Associate Director
Biogen

Leah Norona, PhD, DABT
Senior Scientist
Genentech

- Aaron Fullerton (GNE)
- Lindsay Tomlinson (Pfizer)
- Christopher Hinckley (Biogen)
- Leah Norona (GNE)

Quick Reference:

Session 5 – CIVM/MPS Qualification Considerations and Challenges

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COU: Pharmacology – Skeletal Muscle	Cellular Composition	Biological Validation	Impact on candidate selection
Technical	• **Are cell line differences disease dependent:** How are donor to donor differences minimized? • **Are support cells are used and do they impact disease phenotypes?**	• **Does CIVM/MPS reach a functional steady state?** Candidate molecules are ideally tested at steady state unless required by COU.	• **Do CIVM/MPS have properties allowing replacement of traditional in vitro and/or in vivo approaches?**
Organ-Specific	• **Do cell types used recapitulate gene/protein/pathway expression targeted by therapeutic approach?**	• **Do CIVM/MPS recapitulate basic muscle contractile properties?**	
COU-Specific	• **Do candidate therapies impact disease relevant cell type and/or support cells?**	• **Are expected disease relevant phenotypes observed?** What is the similarity / translatability of model disease phenotype to clinical endpoints.	• **Is the CIVM/MPS necessary to demonstrate preclinical efficacy**? Does candidate not cross react with rodent and/or are *in vivo* efficacy models lacking? • **How does CIVM/MPS inform preclinical development?** Does data influence dose selection for IND enabling tox?

- Aaron Fullerton (GNE)
- Lindsay Tomlinson (Pfizer)
- Christopher Hinckley (Biogen)
- Leah Norona (GNE)

Quick Reference:

Session 5 – CIVM/MPS Qualification Considerations and Challenges

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COU: Safety - Liver	Biological Relevance	Internal Qualification	Integration with existing Approaches
Technical	• **Has a baseline been established/does the model maintain stable functionality over time?** • **Have sources of variability been identified?** Use of technical performance controls to understand key sources of variability across time and experiments	• **Has model performance and stability been spot-checked?** Particularly relevant in the context of commercially available platforms • **Have reference compounds been identified/evaluated to establish confidence in the approach?** Positive and negative compounds with known clinical outcome.	• **What additional mechanistic insights does the CIVM/MPS enable over existing alternative methods?**
Organ-Specific	• **Does CIVM/MPS maintain basic liver features and functionality?** Albumin production, metabolic competence, etc.	• **Are expected responses to clinically relevant compounds recapitulated?**	• **What DILI-relevant mechanisms are covered by existing approaches?**
COU-Specific	• **Are the correct cell types represented in the model to recapitulate the apical outcome/intended COU?** Inclusion of relevant cell types should be anchored to relevant biological processes (e.g., stellate cell activation and deposition of collagen for fibrotic injury)	• **Are additional features relevant to a particular COU characterized?** (e.g., bile acid synthesis/functional characterization of transporters for intrahepatic cholestatic DILI • **Are the reference compounds relevant to the adverse outcome being evaluated?**	• **Does the CIVM/MPS fill a gap in the current strategy?**