From innovation to regulation:
Opportunities and limitations of non-animal evidence for efficacy

EMA Workshop: Non-clinical data for regulatory decision-making on the efficacy of medical countermeasures

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National Institute of Allergy and Infectious Diseases

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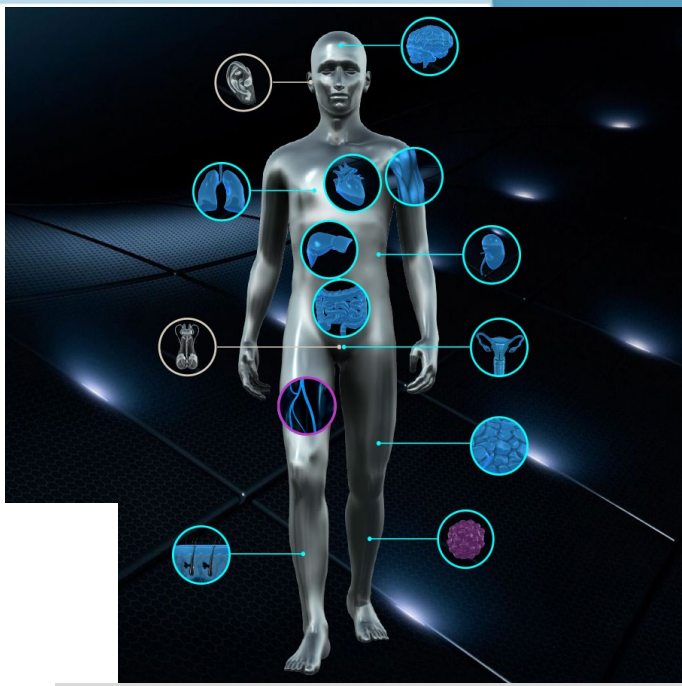
Why Microphysiologic Systems (MPS)?

Goal: To develop physiologic, pathophysiologic, 3D human-derived *in vitro* models, *to be used as*:

- Infectious disease models for basic research
- Product development tools to evaluate MCMs and predict activity/efficacy in humans



Rationale:

- Poor predictive quality of animal models for efficacy in humans
- Evaluate efficacy/toxicity of MCMs in relevant (human) cells/tissues
- Minimize requirement for animal-adapted pathogens and/or modified hosts
- Evaluate *host*-directed MCMs
- Identify human biomarkers of disease

and, importantly…

- Reduced use of and reliance upon experimental animals – especially important now with critical shortage of NHPs

Terminology:
MPS *= human* ***biomimetic****, and includes* ***Organoids****,* ***Organ on a Chip****, and complex in vitro models (****CIVMs****), New Approach Methodologies (****NAMs****), etc.*

Animal Rule, Animal Model Qualification & Pivotal Efficacy Studies

Final Approval !!! - DDT-AMQ-000006

Qualification of the Cynomolgus Macaque (Macaca fascicularis) Model of Pneumonic Tularemia
https://force-dsc.my.site.com/ddt/s/ddt-project?ddtprojectid=145

Involved in Generation of Data Supporting Regulatory Packages Submitted to FDA

1. **Tularemia -** Doxycycline and Ciprofloxacin
2. **Anthrax –** Amoxicillin, Augmentin, Anti-toxins
3. **Plague –** Doxycycline, Ciprofloxacin, Levofloxacin
4. **Orthopoxviruses -** Tecovirimat

What non-clinical data/models are needed to support therapeutic/vaccine development for current or future pathogens?

- Ebola, Marburg, Sudan, Nipah, Lassa, MERS, mPox, other EID?
- BEI Resources – obtain WCCM -– NextGen sequencing and CoA

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Moving from animal to MPS – what is needed?

- Characterization/Standardization of cells - population/differentiation
- Primary cells, iPSC and/or Organoid banks?
- Well characterized challenge material (WCCM)
- Humanize and/or standardize exposure to both pathogens and countermeasures
- Endpoints/biomarkers – primary and secondary endpoints, validated assays
- Powering studies – replicates, statistical analyses

Implementing Animal Alternative Models for Preclinical Testing

Tissue	Example Pathogen(s)
Liver	Toxicology, EBOV, NIV, HepB
Lung/Airway	Influenza, MERS, MTB
Brain/BBB	EEVs, WNV, RVFV
Intestine	Norovirus, C. difficile
Reproductive Tract	Chlamydia, Gonorrhoeae
Blood Vessel	CCHFV, EBOV
Skin	S. aureus, Candidiasis, DENV

NIH National Institute of Allergy and Infectious Diseases

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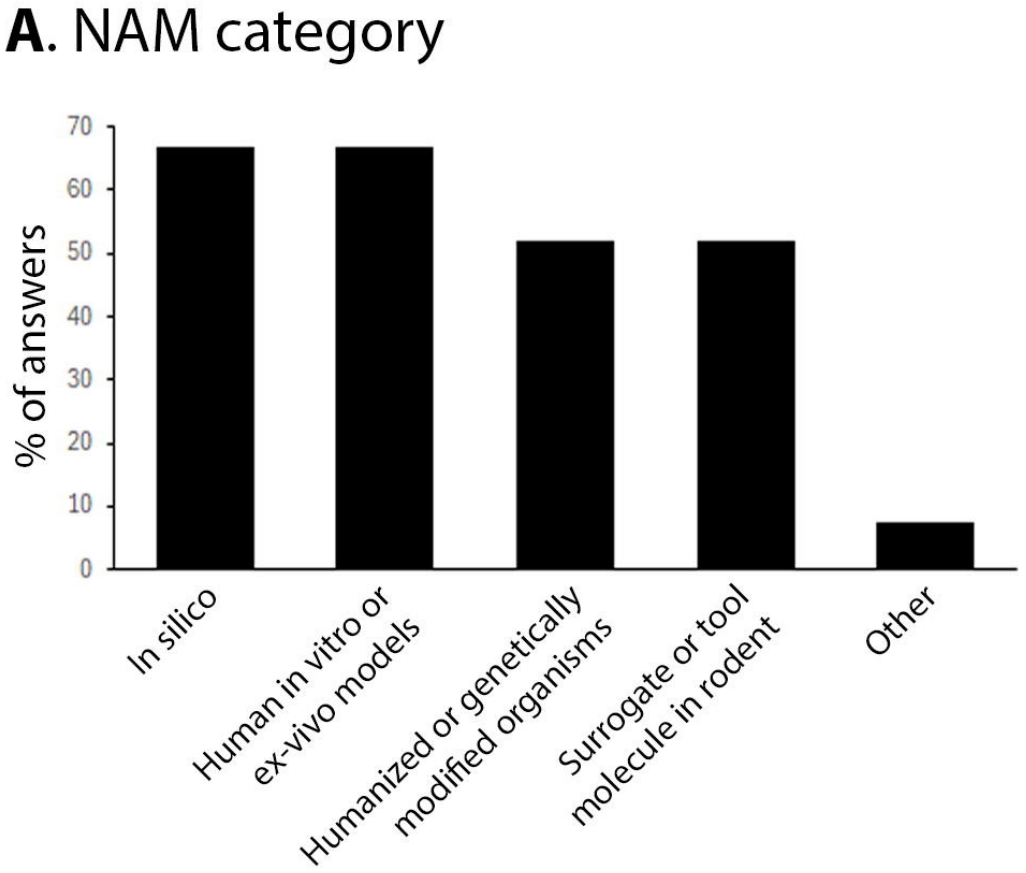
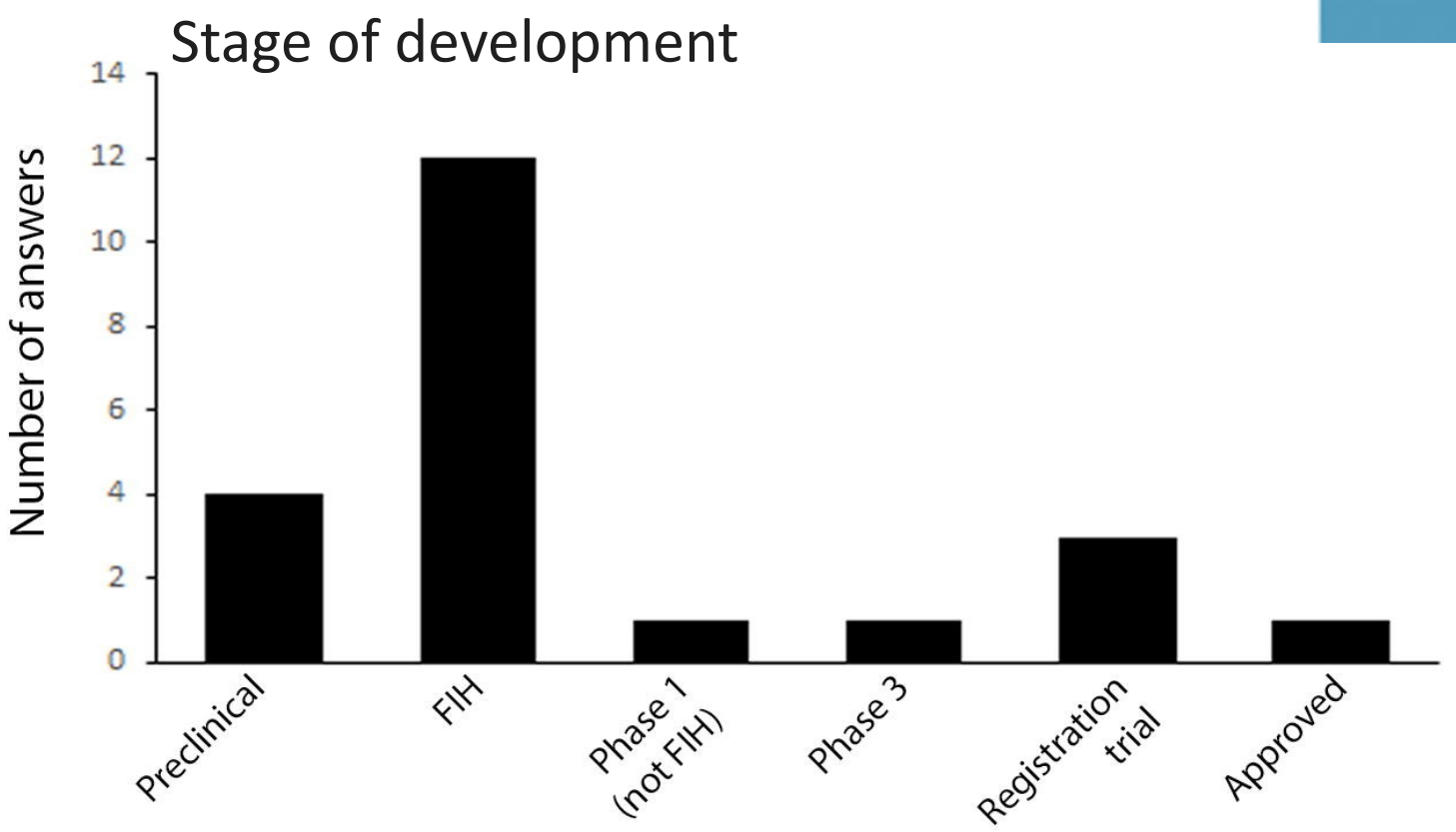
Survey of Pharma Use of NAMs to Support Non-clinical PK or Safety Assessments - Biotechnology Innovation Organization (BIO) NAMs Task Force





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https://doi.org/10.1016/j.drudis.2025.104328

Examples of Alternative/non-Animal Evidence that has been Advanced to Support Regulatory Decision-Making

https://c-path.org/impact/

C-PATH REGULATORY SUCCESSES

ALZHEIMER'S DISEASE

- EMA qualification opinion and FDA letter of support for AD imaging biomarker
- FDA letter of support for cerebrospinal fluid biomarker
- EMA letter of support for pre-dementia clinical trial simulation tool
- FDA Fit for Purpose & EMA qualification opinion for clinical trial simulation tool

CLINICAL OUTCOME ASSESSMENTS

- Four qualification decisions for five PRO measures

DUCHENNE MUSCULAR DYSTROPHY

- EMA letter of support for clinical trial simulation platform

MULTIPLE SCLEROSIS

- EMA qualification opinion for test battery of four PerfO measures

PARKINSON'S DISEASE

- EMA qualification opinion for model-based PD imaging biomarker
- FDA & EMA letters of support for PD imaging biomarker
- EMA letter of support for clinical trial simulation platform
- FDA letter of support for use of alpha-synuclein (α-syn) seed amplification assay (SAA) in clinical trials

POLYCYSTIC KIDNEY DISEASE

- EMA & FDA qualified Total Kidney Volume (TKV) imaging biomarker
- FDA letter of support for TKV imaging biomarker
- FDA designated reasonably likely surrogate marker for PKD trials (TKV)

PREDICTIVE SAFETY TESTING

- EMA, FDA & PMDA qualified non-clinical kidney safety biomarkers
- FDA qualified clinical kidney safety markers
- Six FDA & five EMA letters of support

TRANSPLANT THERAPEUTICS

- EMA qualification opinion for iBox Scoring System

TUBERCULOSIS

- EMA qualification opinion for translational drug development platform

TYPE 1 DIABETES

- EMA letter of support and subsequent qualification opinion for model-based islet autoantibodies biomarker for trial enrichment

FDA

- 8 Qualification Decisions
- 11 Letters of Support
- 1 Fit-for-Purpose Endorsement

EMA

- 9 Qualification Opinions
- 10 Letters of Support

PMDA

- 1 Qualification Decisions

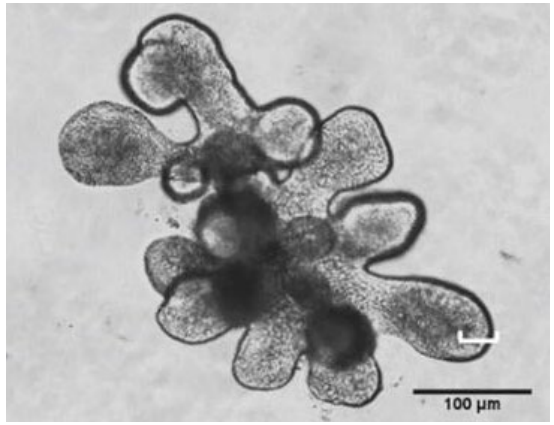
Selected iSTAND Drug Development Tools Submission/Status

https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-program

Project Number	Project Name	Latest Submission Stage	Organization Name
DDT-IST-000016	Human Liver-Chip for Prediction of Drug-Induced Liver Injury	QP - Qualification Plan	Emulate
DDT-IST-000019	In-Vitro 3D Human Model for Screening and Prioritization of NASH Clinical Candidates	LOI - Letter of Intent	InSphero AG
DDT-IST-000034	Liver acinus microphysiological system (LAMPS) for determining drug candidate dosing in clinical trials of liver disease.	LOI - Letter of Intent	Organ Pathobiology and Therapeutics Institute
DDT-IST-000041	An in-vitro, high throughput, 3D primary human spheroid model to assess Drug Induced Liver Injury (DILI)	LOI - Letter of Intent	InSphero AG
DDT-IST-000045	Human Kidney Chip for Assessment of Relative Nephrotoxicity	LOI - Letter of Intent	Icahn School of Medicine at Mt. Sinai
DDT-IST-000047	Human chorio-decidual interface organ on chip for derisking positive rodent DART studies for new modality investigational new drug candidates	LOI - Letter of Intent	Texas A&M University

Animal Alternative Models in DMID Preclinical Services

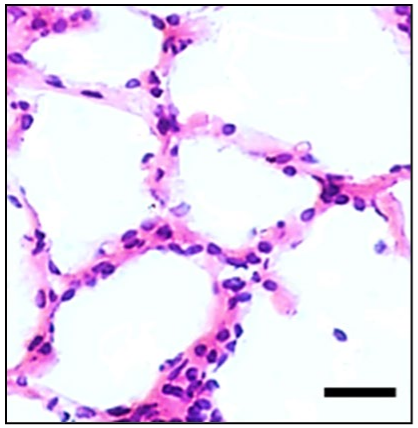
Human Intestinal Enteroids - Baylor

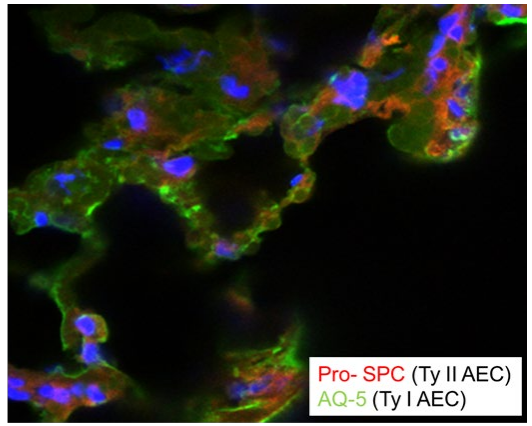


Model for Norovirus

a. Growth
b. Efficacy testing

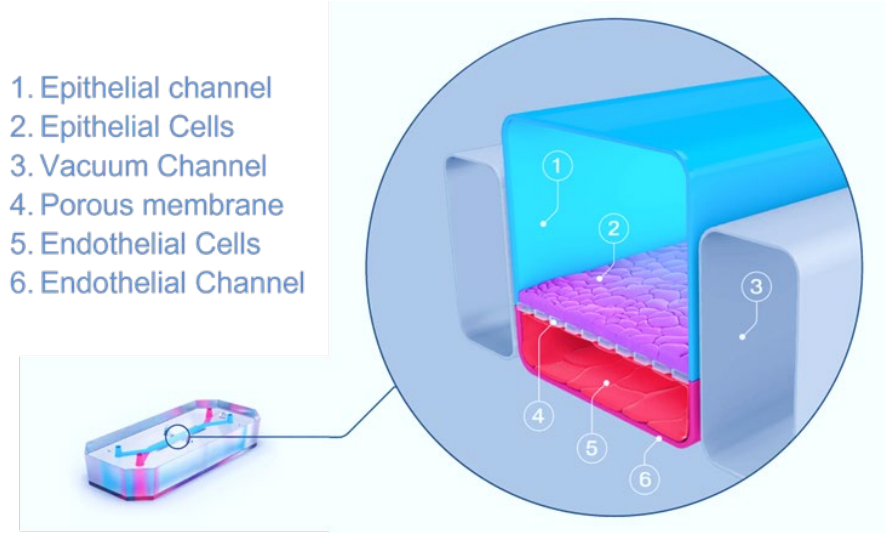
Human Lung Microphysiologic System - UTMB





1) H1N1, H7N9
2) SARS-CoV-2
3) F. tularensis

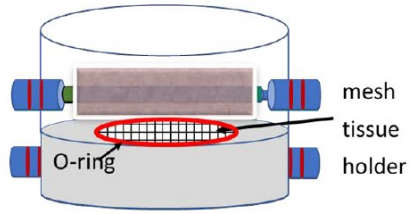
Lung and Lymphoid Chips - Wyss Institute (IAA w/FDA)

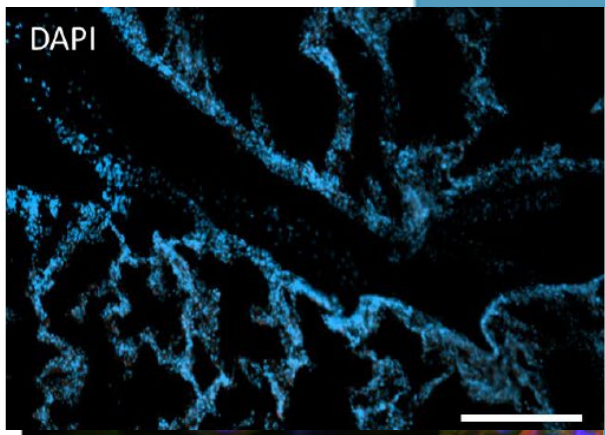


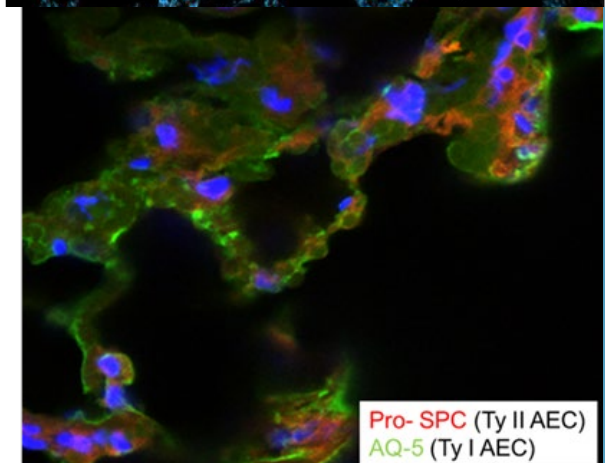
1) H1N1, H3N2
2) SARS-CoV-2
3) Lymphoid w/vaccines

Human Lung MPS System
(contractor - UTMB)

1. Primary human lung cells with a vascular channel to add MCM and collect outflow
   - Type I and Type II alveolar epithelial cells, fibroblasts, smooth muscle cells, and vascular endothelial cells (all from same donor)
     - Use functional and expression (RNAseq) markers to define/characterize cells
     - Test multiple donors for reproducibility
   - Endpoints: viral burden, cytokine secretion, viability/apoptosis
2. Immune cell addition - **"Fit for Purpose"**
   - Macrophages - resident tissue or alveolar
   - PMNs - added to vascular channel and extravasate into tissue







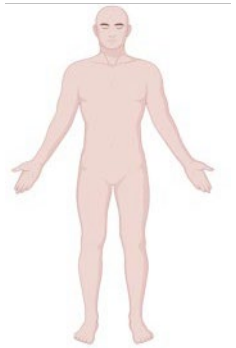
<u>Infection Models used to date (with positive control test drug)</u>

- Low-Path Avian Influenza (H7N9) - oseltamivir
- *Francisella tularensis* - levofloxacin
- Seasonal flu (H1N1, H3N2) – oseltamivir, molnupiravir
- SARS-CoV-2 – molnupiravir, nirmatrelvir

Characterization of NHP Chip Systems and Comparison with Human Responses (Contractor: Wyss Institute)

Rhesus macaques

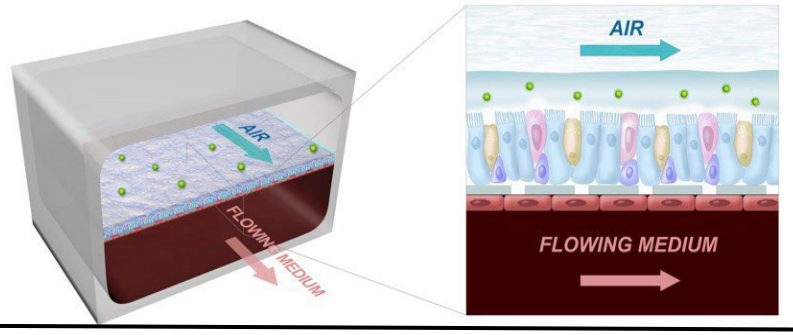
vs.



Does an NHP MPS 'mimic' *in vivo* NHP data? Or the Human MPS?

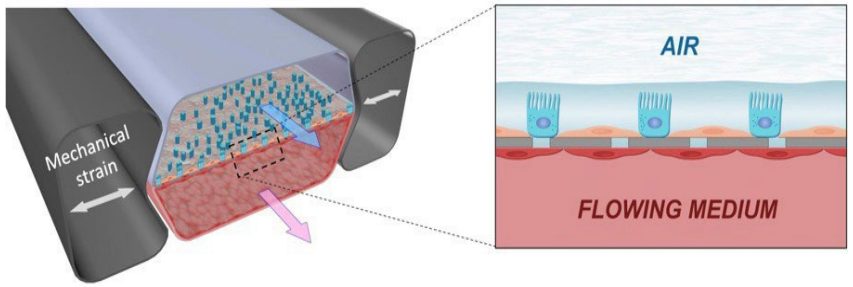
- Establish iPSC/cell banks
- challenge with H3N2 and SARS-CoV2
- immune responses to influenza vaccine

➢ *Helps buy confidence in the human MPS and allows comparison to historical animal data*



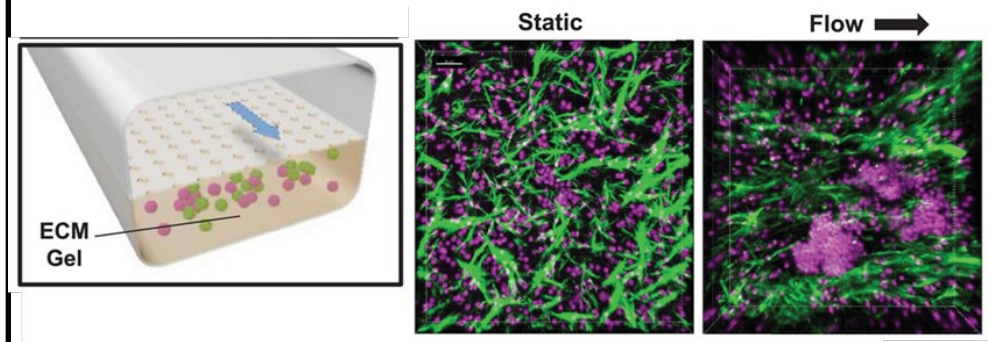
Lung Airway

- Primary airway/trachea cells & iPSC generation
- Human MPS - *Nat Biomed Engin. v.5, pp815–829 (2021)*



Lung Alveolar space

- Primary lung cells & iPSC generation
- Human MPS - *Nat Commun. 2022 v.13(1):1928*



ECM fibrils (green) lymphocytes nuclei (magenta)

Lymphoid Follicle

- Negatively selected PBMC (T-, B-cell, monocyte) and DC
- Human MPS - *J Exp Med 2024 v.221(10):e20240289*

MPS Data in Lieu or Support of Animal Data to Advance Regulatory Submissions for Product Development . . .

- Complexity to MPS - must be ‘fit for purpose’ – there is no ‘one size fits all’ (**Context of Use**)
  - Tissue(s) should reflect pathogen and/or MCM target
    - Humanized exposure and MCM dosing
  - Selected and validated disease endpoints
  - Can toxicology concerns be addressed concurrently?
- Well characterized cell source(s) (abundant, reliable)
  - Markers of differentiation, function
  - Establish cell/organoid banks to standardize/harmonize?
- Must be transferable and accessible – must meet **Rigor & Reproducibility**
  - Powering studies – replicates, statistical analyses
- Throughput and scalability; Costs can’t be prohibitive