JAMES L. MCGRATH, PHD
BIOMEDICAL ENGINEERING
DIRECTOR OF TRACE-bMPS

JOAN ADAMO, PHD CTSI
UNIVERSITY OF ROCHESTER
CO-DIRECTOR OF TRACE-bMPS

CTSI Grand Rounds
September 11, 2025

The Translational Center for Barrier Microphysiological Systems

Outline

1) Societal pressures driving the adoption of non-animal models
2) What is a microphysiological system ?
3) Overview of TraCe-bMPS
4) Example transformation: MPS -> DDT

WHERE THE PUCK IS GOING: NON-ANIMAL MODELS FOR DISEASE MODELING AND DRUG DEVELOPMENT

THE STORY OF TGN1412: A FIRST IN HUMAN TRIALS DISASTER

BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

Ganesh Suntharalingam, F.R.C.A., Meghan R. Perry, M.R.C.P., Stephen Ward, F.R.C.A., Stephen J. Brett, M.D., Andrew Castello-Cortes, F.R.C.A., Michael D. Brunner, F.R.C.A., and Nicki Panoskaltsis, M.D., Ph.D.

SUMMARY

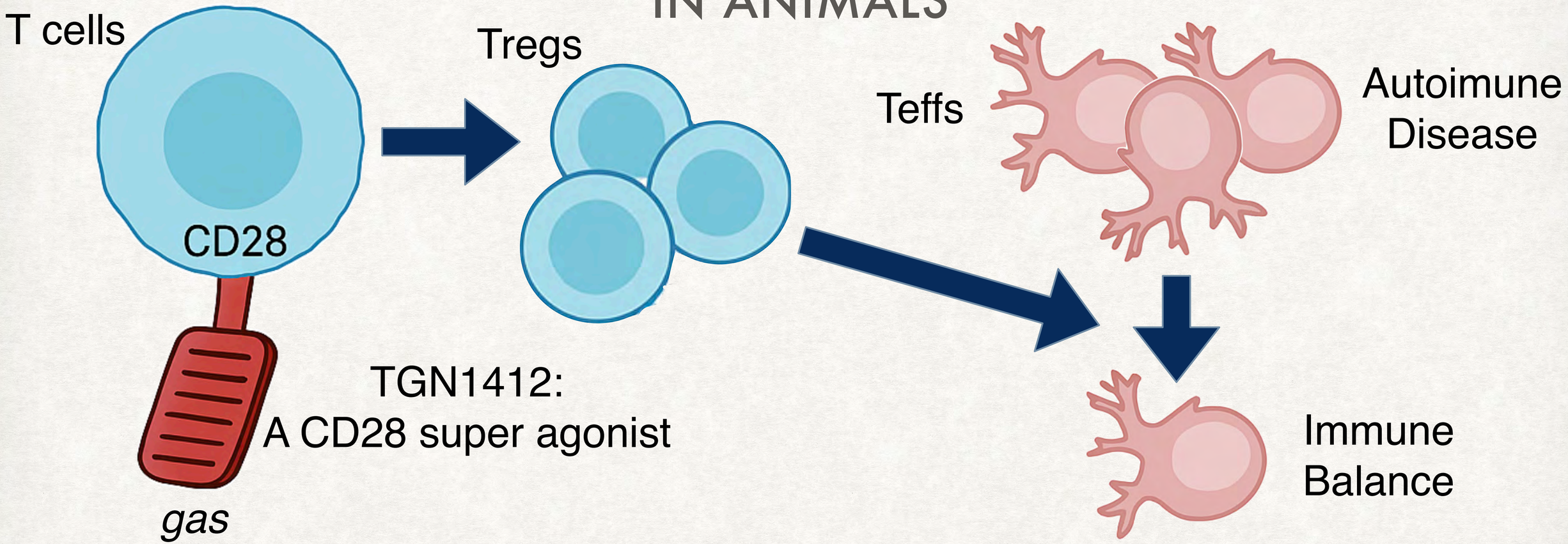
Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. All six patients were transferred to the care of the authors at an intensive care unit at a public hospital, where they received intensive cardiopulmonary support (including dialysis), high-dose methylprednisolone, and an anti–interleukin-2 receptor antagonist antibody. Prolonged cardiovascular shock and acute respiratory distress syndrome developed in two patients, who required intensive organ support for 8 and 16 days. Despite evidence of the multiple cytokine-release syndrome, all six patients survived. Documentation of the clinical course occurring over the 30 days after infusion offers insight into the systemic inflammatory response syndrome in the absence of contaminating pathogens, endotoxin, or underlying disease.

New England Journal of Medicine. 2006;355(10):1018-1028

Table 3. Common Features after Infusion of TGN1412.

System	Feature
Cardiovascular	Capillary leak Hemodynamic instability Lactic acidemia
Renal	Early acute renal impairment Urinary sediment 10–100 White cells <10 Red cells Granular casts (two patients)
Pulmonary	Acute pulmonary changes (six patients) Met criteria for acute lung injury (two patients)* Met criteria for acute respiratory distress syndrome (one patient)*
Hematologic and immunologic	Cytokine storm (TNF-α; interferon-γ; interleukin-10, 6, 2) Increased C-reactive protein level and erythrocyte sedimentation rate Lymphopenia Monocytopenia Thrombocytopenia Disseminated intravascular coagulation Normochromic, normocytic anemia Dysplastic neutrophils but preserved numbers
Hepatic	Increased alanine aminotransferase and alkaline phosphatase levels
Integumentary	Diffuse erythema Late desquamation
Neurologic	Delirium Partial amnesia Paresthesia or localized numbness Difficulty concentrating (late) Headaches (early and late)
Autonomic, gastrointestinal, or both	Bowel urgency or diarrhea Nausea or vomiting
Musculoskeletal	Myalgia in lower back (early) and calves (late)

THE STORY OF TGN1412: A HOPEFUL HYPOTHESIS, AFFIRMED IN ANIMALS



Hypothesis: Selective CD28 superagonist stimulation expands regulatory T cells and suppresses effector T cells, restoring immune balance and reducing autoimmune disease.

Preclinical Evidence Supporting IND Submission

- **Rats (Lewis; JJ316) — *Efficacy*: J Exp Med 2005;202:445–55 • Eur J Immunol 2003;33:626–38 • J Rheumatol 2006;33:110–18**
- **Non-human primates (cyno/rhesus; TGN1412) — *Safety only*: TeGenero, Investigator's Brochure (Dec 2005) • TeGenero, Investigational Medicinal Product Dossier(Apr 2006)**

THE STORY OF TGN1412: WHAT WENT WRONG?

March 2006

The Guardian

News Opinion Sport Culture Lifestyle

Six men in intensive care after drug trial goes wrong

· Volunteers were testing treatment for arthritis
· US company says adverse reaction is 'extremely rare'

FINANCIAL TIMES

Data for botched drugs trial show 'nothing' amiss

The paperwork for the disastrous drug trial that has left six men in intensive care, two of them still in a critical condition, did not contain anything to give cause for concern, the regulator for approving clinical trials has said.

April 2006

NATURE|Vol 440|13 April 2006 NEWS

Can super-antibody drugs be tamed?

As it becomes clear that the London clinical trial disaster was indeed the fault of the drug itself, **Michael Hopkin** looks at what went wrong, and whether there is any future for 'superagonist' antibody therapies.

September 2006

BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

Ganesh Suntharalingam, F.R.C.A., Meghan R. Perry, M.R.C.P., Stephen Ward, F.R.C.A., Stephen J. Brett, M.D., Andrew Castello-Cortes, F.R.C.A., Michael D. Brunner, F.R.C.A., and Nicki Panoskaltsis, M.D., Ph.D.

April 2009

Toxicologic Pathology
Volume 37, Issue 3, April 2009, Pages 372-383

https://doi.org/10.1177/0192623309332986

SAGE journals

Regulatory Affairs

The TeGenero Incident and the Duff Report Conclusions: A Series of Unfortunate Events or an Avoidable Event?

Christopher J. Horvath[1] and Mark N. Milton[2]

1. UK Secretary of State for Health (Duff Report)
2. Medicines and Healthcare Products Regulatory Agency

2007 - 2015

"Cytokine Storm" in the Phase I Trial of Monoclonal Antibody TGN1412: Better Understanding the Causes to Improve PreClinical Testing of Immunotherapeutics

Richard Stebbings, Lucy Findlay, Cherry Edwards,[1] David Eastwood, Chris Bird, David North, Yogesh Mistry, Paula Dilger, Emily Liefooghe, Isabelle Cludts, Bernard Fox, Gill Tarrant, Jane Robinson, Tony Meager, Carl Dolman, Susan J. Thorpe, Adrian Bristow, Meenu Wadhwa, Robin Thorpe, and Stephen Poole[2]

The CD28-specific mAb TGN1412 rapidly caused a life-threatening "cytokine storm" in all six healthy volunteers in the Phase I clinical trial of this superagonist, signaling a failure of preclinical safety testing. We report novel in vitro procedures in which TGN1412, immobilized in various ways, is presented to human white blood cells in a manner that stimulates the striking release of cytokines and profound lymphocyte proliferation that occurred in vivo in humans. The novel procedures would have predicted the toxicity of this superagonist and are now being applied to emerging immunotherapeutics and to other therapeutics that have the potential to act upon the immune system. Data from these novel procedures, along with data from in vitro and in vivo studies in nonhuman primates, suggest that the dose of TGN1412 given to human volunteers was close to the maximum immunostimulatory dose and that TGN1412 is not a superagonist in nonhuman primates. *The Journal of Immunology*, 2007, 179: 3325–3331.

BJP British Journal of Pharmacology

RESEARCH PAPER

Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells

D Eastwood, L Findlay, S Poole, C Bird, M Wadhwa, M Moore, C Burns, R Thorpe and R Stebbings

BJCP British Journal of Clinical Pharmacology

Severity of the TGN14 trial disaster cytokine s correlated with IL-2 re

David Eastwood,[1] Chris Bird,[1] Paula Dilger,[1] Jason Ho
Lucy Findlay,[1] Stephen Poole,[1] Susan J. Thorpe,[1] Mee
Robin Thorpe[1] & Richard Stebbings[1,3]

[1]Biotherapeutics Group, NIBSC, Potters Bar, Hertfordshire, [2]Biostatistics, NIBSC, P
Hertfordshire and [3]Department of Molecular and Clinical Pharmacology, Unive
Liverpool, UK

Rodent studies (mice/rats):

- Humans have CD28 on Teffs. Mice do not.
- So TGN1412 activated Teffs in humans, instead of Tregs. The opposite of what was intended.
- Autoimmune model improvements seen in rodents do not translate to humans.

Monkey studies:

- Monkey T cells have very little CD28, so no dangerous activation was seen even at high doses.
- Starting human dose was set too high for FIH because it relied on "no harm" dosing in monkeys.

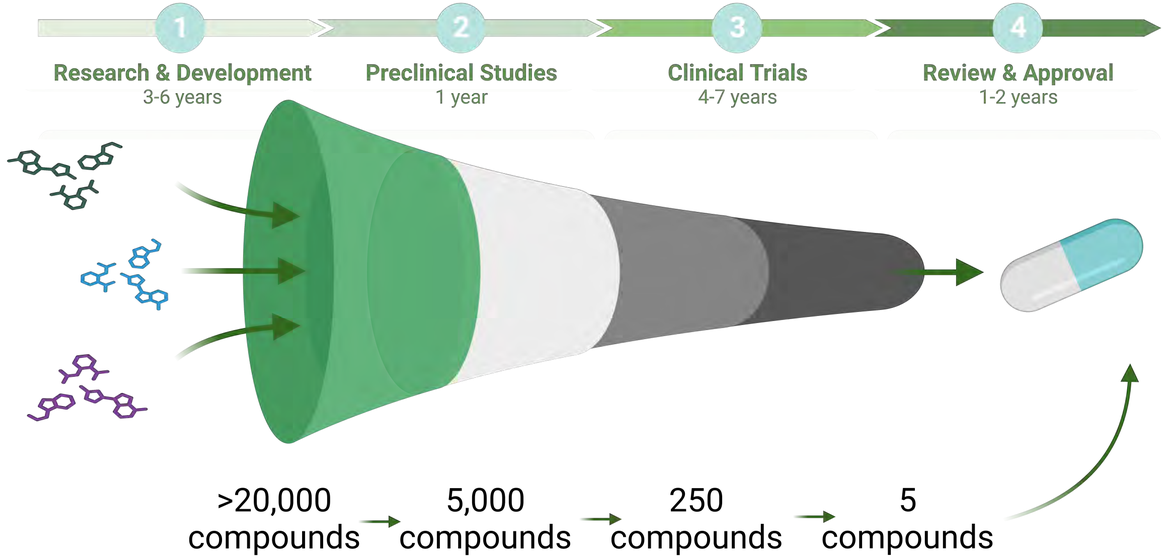
Take homes

- Animal studies missed the danger because rodents and monkeys have important differences from humans in their immune system.
- There are different reasons for the failure to predict trouble in the different species.

Human Microphysiological Systems (MPS) as Drug Development Tools (DDTs) for the Accelerated Development of Safe and Effective Drugs

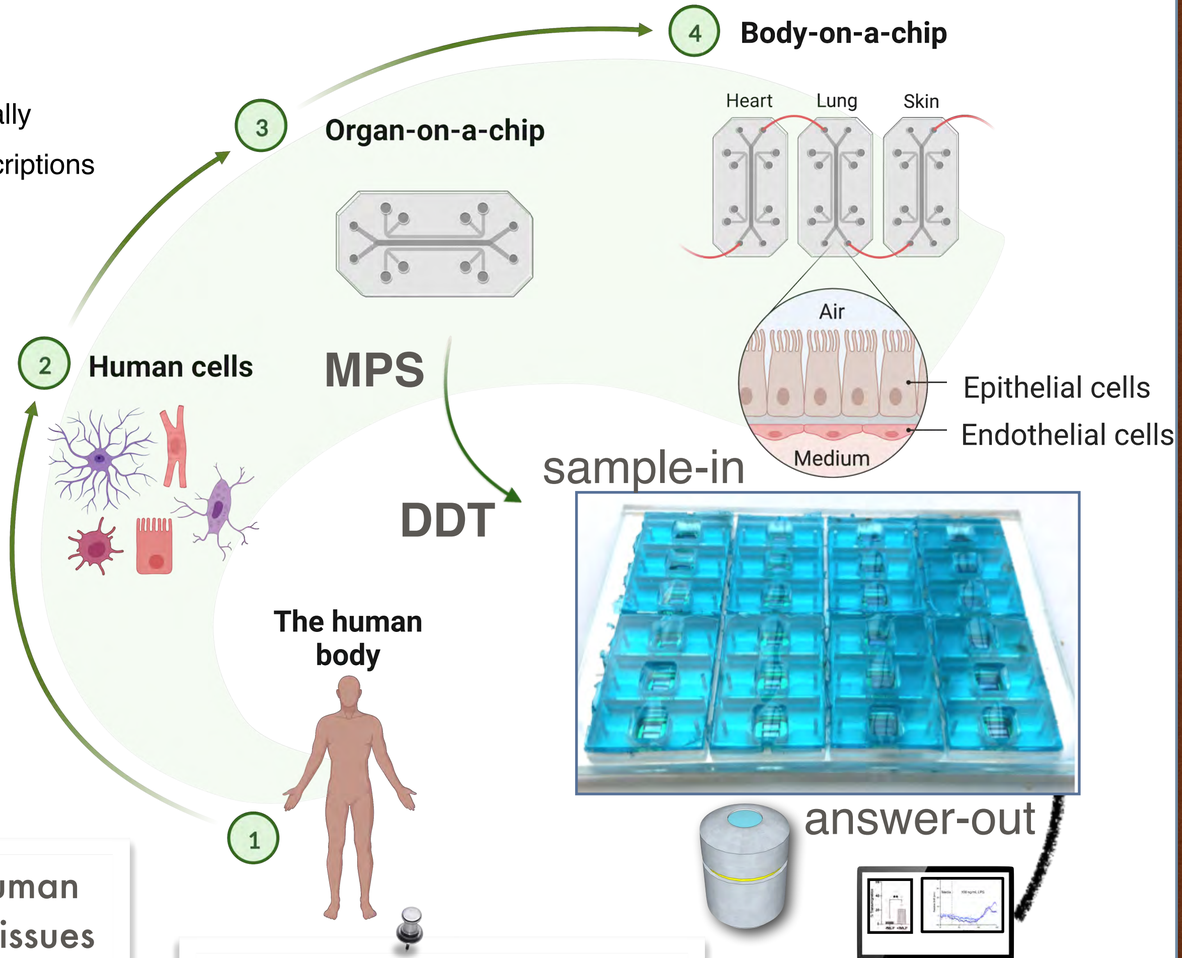
The Drug Development Bottleneck

- More than 90% of leading drug candidates fail to gain FDA approval for patient use
- The cost of failures late in the drug development pipeline is $2.6B per drug, adding to over $100B annually
- This bottleneck prolongs human suffering, burdens the health care system, and raises the costs of prescriptions



Drugs are currently selected for advancement to clinical trials based on safety and efficacy tests in mice and studies of cells in a dish.

MPS 'chips' feature human cells organized as 3D tissues to yield preclinical indications that are more predictive of success in clinical trials.



Unlike mice, MPS chips can be configured in high-throughput formats for rapid drug screening (DDT).

- Automated
- Reproducible
- Well-controlled
- Specific context of use

CONGRESSIONAL ACTION

In Fall '22 congress passed the FDA Modernization Act 2.0...

117TH CONGRESS
2D SESSION

S. 5002

To allow for alternatives to animal testing for purposes of drug and biological product applications.

IN THE SENATE OF THE UNITED STATES

SEPTEMBER 29, 2022

Mr. PAUL (for himself, Mr. BOOKER, Mr. BRAUN, Mr. CRAPO, Mr. MARSHALL, Ms. COLLINS, Mr. KING, Mr. PADILLA, Mr. SANDERS, Mr. TUBERVILLE, Mr. LUJÁN, and Mr. SCOTT of Florida) introduced the following bill; which was read twice, considered, read the third time, and passed

A BILL

To allow for alternatives to animal testing for purposes of drug and biological product applications.

Be it enacted by the Senate and House of Representatives of the United States of America in

SECTION 1. SHORT TITLE.

This Act may be cited as the "FDA Modernization Act 2.0".

SEC. 2. ALTERNATIVES TO ANIMAL TESTING.

(a) IN GENERAL.—Section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C.

(1) in subsection (i)—

(A) in paragraph (1)(A), by striking "preclinical tests (including tests on animals)"

(B) in paragraph (2)(B), by striking "animal" and inserting "nonclinical tests"; and

(2) after subsection (y), by inserting the following:

"(z) NONCLINICAL TEST DEFINED.—For purposes of this section, the term 'nonclinical test' means a test conducted in vitro, in silico, or in chemico, or a non-human in vivo test that occurs before or during the clinical trial phase of the investigation of the safety and effectiveness of a drug, and may include animal tests, or non-animal or human biology-based test methods such as cell-based assays, microphysiological systems, or bioprinted or computer models.".

(A)(i)(I) of the Public Health Service Act (42 U.S.C.

or studies described in item (aa) or (cc)); and".

CONGRESS.GOV

... to allow for alternatives to animal testing for purposes of drug and biological product applications.

... such as ... microphysiological systems ...

And now a new Bill the FDA Modernization Act 3.0...

... to replace or reduce animal testing and ... improve the predictive of nonclinical testing for safety and efficacy or reduce development time for a drug

A BILL

To amend the Federal Food, Drug, and Cosmetic Act to establish a pr
animals in nonclinical research, improve the predictivity of nonc
and for other purposes.

Be it enacted by the Senate and House of Representatives of the

SECTION 1. SHORT TITLE.

This Act may be cited as the "FDA Modernization Act 3.0".

SEC. 2. NONCLINICAL TESTING METHODS QUALIFICATI

(a) IN GENERAL.—Subchapter A of chapter V of the Federal
section 507 the following:

"SEC. 507A. NONCLINICAL TESTING METHODS QUALIFICATION PROCESS.

"(a) IN GENERAL.—

"(1) PROCESS DESCRIPTION.—The Secretary shall establish a process for the qualification of a nonclinical testing method, with respect to drugs, under which—

"(A) persons may request qualification of a nonclinical testing method for a particular context of use; and

"(B) the Secretary shall grant or deny such request in accordance with this section.

"(2) INITIATION.—The Secretary shall initiate the process under paragraph (1) not later than 1 year after the date of enactment of this section.

"(b) ELIGIBLE NONCLINICAL TESTING METHODS.—To be eligible for qualification under this section, a nonclinical testing method shall—

"(1) be intended to replace or reduce animal testing; and

"(2) either—

"(A) improve the predictivity of nonclinical testing for safety and efficacy; or

"(B) reduce development time for a drug (including any biological product)

... establish a process for the qualification of a nonclinical testing method, with respect to drugs ..

FDA ROADMAP SETS AN AGGRESSIVE PACE FOR REPLACING ANIMAL MODELS IN PRECLINICAL TESTING

Roadmap to Reducing Animal Testing in Preclinical Safety Studies

Executive Summary

April 10, 2025

This roadmap outlines a strategic, stepwise approach for FDA to reduce animal testing in preclinical safety studies with scientifically validated new approach methodologies (NAMs), such as organ-on-a-chip systems,

U.S. FOOD & DRUG ADMINISTRATION

Search Menu

Home / News & Events / FDA Newsroom / Press Announcements / FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

FDA NEWS RELEASE

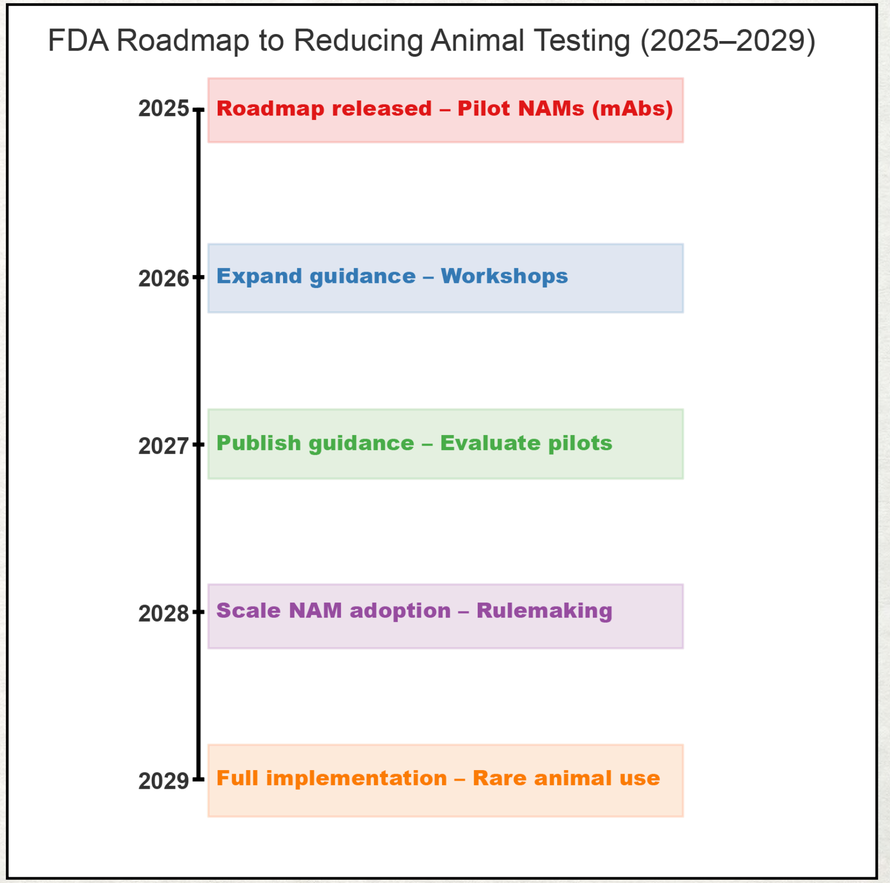
FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

More Press Announcements **For Immediate Release:** April 10, 2025

Content current as

disease susceptibility and progression.[5] Moreover, some safety risks may go undetected in animals – a notable example is the mAb TGN1412, which caused a life-threatening cytokine release syndrome in human volunteers despite appearing safe in preclinical monkey studies. That tragedy highlighted the limitations of animal models for certain immune-activating mAbs and spurred efforts to develop *in vitro* assays to better predict human-specific responses (7).

In the **long-term (3-5 years)**, FDA will aim to make animal studies the *exception* rather than the norm for pre-clinical safety/toxicity testing. By this stage, validated NAMs could cover all critical areas, and FDA requirements can shift to a NAM-based default. Animal tests might only be considered if a specific scientific question cannot yet be answered by NAM (and even then, only the minimal animal use necessary, with strong justification). Ultimately, the vision is that no conventional animal testing will be required for mAb safety, and eventually all drugs/therapeutics – instead, a comprehensive integrated NAM toolbox (human cell models + computational models) will be the new standard.



NON-ANIMAL MODELS ARE POISED FOR GROWTH, EVEN IN TROUBLING TIMES FOR BIOMEDICAL RESEARCH FUNDING

FDA

Roadmap to Reducing Animal Testing in Preclinical Safety Studies

Executive Summary

This roadmap outlines a strategic, stepwise approach for FDA to reduce animal test
studies with scientifically validated new approach methodologies (NAMs), such as c
computational modeling, and advanced *in vitro* assays. By partnering with federal a
VA through ICCVAM, FDA can accelerate the validation and adoption of these huma
improving predictive accuracy while reducing animal use. This transition will enhanc
streamlining drug development and ensuring safer therapies reach patients faster, w
global leader in modern regulatory science and innovation.

EPA New Approach Methods: Efforts to Reduce Use of Vertebrate Animals in Chemical Testing

The Environmental Protection Agency is prioritizing ongoing efforts to develop and use New Approach Methods (NAMs) to test chemicals for health effects. Using NAMs will help reduce the use of vertebrate animals in chemical testing while ensuring
nd the environment.

volved over time. Currently, it is broadly descriptive
ate animals technology, methodology, approach, or
be used to provide information on chemical hazard and

News & Updates

- Join EPA NAMs email list for updates

ives" to

NIH ENDS FUNDIN
ANIMAL-ONL
STUDIES

NIH National Institutes of Health
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NEWS RELEASES

Tuesday, April 29, 2025

NIH to prioritize human-based researc

New initiative aims to reduce use of animals in NIH-funded res

The National Institutes of Health (NIH) is adopting a new initiative to expand innovative, human-based science while reducing animal use in research. Developing and using cutting-edge alternative nonanimal research models aligns with the U.S. Food and Drug Administration's (FDA) recent initiative to reduce testing in animals. While traditional animal models continue to be vital to advancing scientific knowledge, using new and emerging technologies can offer unique strengths that, when utilized correctly or in combination, can expand the toolbox for researchers to answer previously difficult or unanswerable biomedical research questions.

"For decades, our biomedical research system has relied heavily on animal models. With this initiative, NIH is ushering in a new era of innovation," said NIH Director Dr. Jay Bhattacharya. "By integrating advances in data science and technology with our growing understanding of human biology, we can fundamentally reimagine the way research is conducted—from clinical development to real-world application. This human-based approach will accelerate innovation, improve healthcare outcomes, and deliver life-

Combining microfabrication techniques with modern tissue engineering, the lung-on-a-chip, designed by the Wyss Institute at Harvard University, offers a new in vitro approach to drug screening by mimicking the complicated mechanical and biochemical behaviors of a human lung. The lung-on-a-chip work was supported by NIH Common Fund and FDA. *Wyss Institute, Harvard University*

May 2025

United States Gove
Report to Congress

TECHNOLO
Human
Technologies
but Challenge

NIH GRANTS & FUNDING

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Home > News & Events > NIH Extramural Nexus (News) > NIH Funding Announcements to Align with NIH Initiative to Prioritize Human-based Research

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NIH Funding Announcements to Align with NIH Initiative to Prioritize Human-based Research

July 10, 2025

On April 29, NIH announced it is prioritizing human-focused research and reducing animal use in research. To further this initiative, all new Notices of Funding Opportunity that relate to animal model systems (NOFOs) must now also support human-focused approaches such as clinical trials, real world data, or new approach methods (NAMs). Examples of NAMs include ex vivo human-based approaches, including perfused human organs and precision-cut tissue slices; in vitro methods, including microphysiological systems and organoids; computational and artificial intelligence-based approaches; and combinations thereof.

Importantly, NIH will no longer issue NOFOs exclusively supporting animal models or limit/specify the types of models that must be used. The intent of this effort is to ensure investigators consider the models most appropriate for understanding human states of health and disease and are not constrained by the NOFO. NIH Institutes, Centers and Program may issue NOFOs that exclude proposals for animal use entirely.

This new emphasis on human-based research will accelerate medical advances, save animals and help NIH achieve its crucial mission of improving human health.

Categories: Top Stories
Topics: Animal Welfare

Animal Welfare Organizations

*Have funds for MPS research

IFER
INTERNATIONAL FOUNDATION FOR ETHICAL RESEARCH *

ECEAE
THE EUROPEAN COALITION TO END ANIMAL EXPERIMENTS *

Replacing Animal Research *

Doctors Against Animal Experiments

RE-Place
ALTERNATIVE METHODS TO ANIMAL TESTING

ESTIV *
European Society of Toxicology In Vitro

PETA SCIENCE CONSORTIUM INTERNATIONAL e.V. *
Advancing 21st Century Toxicology

Humane World for Animals™ *

ANIMAL AID

CAAT *

The Humane Research Trust CIO *
Helping people, saving animals

The Marty Project

Animal-Free Science Advocacy

JUSTIFY

Physicians Committee for Responsible Medicine *

COLAAB
Coalition to Illuminate and Address Animal Methods Bias

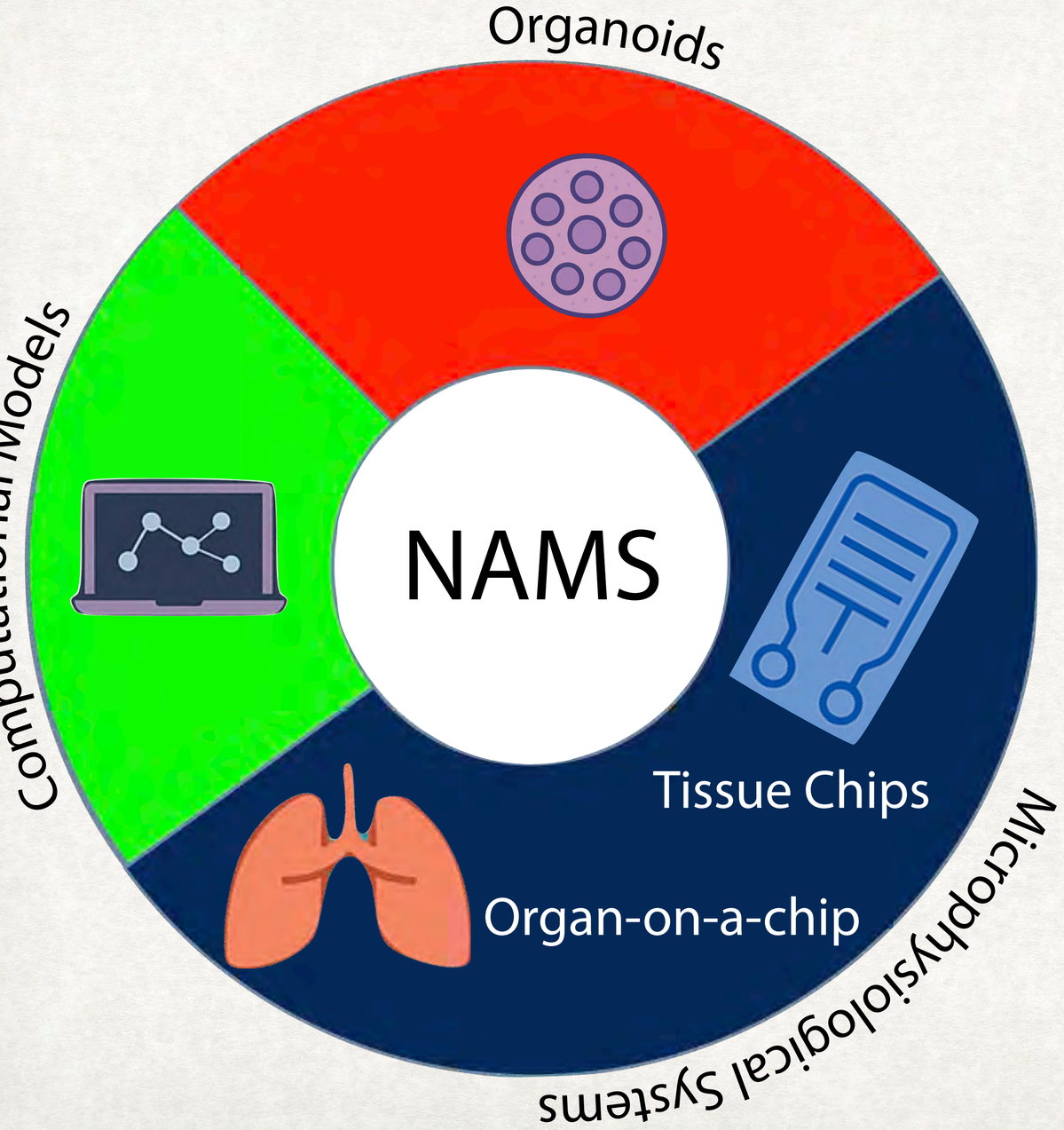
ALTERNATIVES RESEARCH & DEVELOPMENT FOUNDATION *

STOP TAXPAYER FUNDED ANIMAL EXPERIMENTS
WHITE COAT WASTE PROJECT

LUSH PRIZE *

SAO *
Science Advancement & Outreach
A DIVISION OF PETA

WHEEL OF NAMS



NAMS = **N**on-**a**nimal **m**odels?
New **A**pproach **M**ethodologie**s**

- **Microphysiological Systems (MPS):** An umbrella term encompassing OoCs and tissue chips, but also including multi-organ “body-on-a-chip” and other engineered culture models that recapitulate human physiology.
  - **Organ-on-a-Chip (OoC)**: The earliest and most widely recognized term, usually referring to microfluidic devices engineered to mimic the structure and function of a single organ.
  - **Tissue Chips:** A term popularized by NIH/NCATS (through the Tissue Chip for Drug Screening program) and the FDA, referring to OoC/MPS platforms explicitly aimed at regulatory science and drug development.
- **Organoids** – 3D self-organized stem-cell-derived mini-tissues, not necessarily on chips.
- **Computational Models** – in silico methods: AI, machine learning, PK/PD models, systems biology.

Drug Development Tool (DDT)

DDTs are methods, materials, or measures that can potentially facilitate drug development.

Animal model
Biomarker
Clinical outcome assessment

FDA DDT Qualification Program

- To encourage development of DDTs for unmet need contexts of use (COU)
- To encourage collaborative groups to undertake DDT development programs
- To encourage innovation in drug development
- To create a shared learning environment for exchanging information on DDT development

Regulatory Process for DDTs


GUIDANCE DOCUMENT

Qualification Process for Drug Development Tools Guidance for Industry and FDA Staff

NOVEMBER 2020

Download the Final Guidance Document | Read the Federal Register Notice

Final | Level 1 Guidance

Share | Post | Linkedin | Email | Print

ISTAND

Qualification Process Stages

- Letter of Intent — 3 months
- Qualification Plan — 6 months
- Full Qualification Package — 10 months
  1. Is it complete and reviewable?
  2. Comprehensive review
  3. Analysis of review and determination letter

Letter of Intent (LOI)

1. Submission Title
2. Requesting Organization
3. Drug Development Need Statement
4. ISTAND Applicability Statement
5. Context of Use Statement
6. Drug Development Use
7. Technical Description
8. Previous Regulatory Interactions

Context of Use Statement

A statement that fully and clearly describes the way the drug development tool will be used and the drug development-related purpose of the use.

. . . COUs that do not address a specified drug development use are outside the scope of the program.

Context of Use:
The µSiM-CA is an *in vitro* assay that quantifies the capacity of *S. aureus* to enter submicron spaces that mimic the physical restrictions of the OLCN.

Qualification

A conclusion from the FDA, that within this very precise Context of Use, the DDT can be relied upon to have a specific interpretation and application in drug development and regulatory review

TRANSLATIONAL CENTER FOR BARRIER MPS

AN OFFICIAL CENTER OF THE UNIVERSITY OF ROCHESTER

Center Missions

1. Advance barrier-based microphysiological systems for use as drug development tools by Pharma and the FDA
2. Support global access and reliable use of University of Rochester technologies for MPS applications
3. Support the creation of MPS models for translational biomedical research in Rochester's academic community

Global Impact of UR MPS Technologies

Industrial Partnerships

Improve Clinical Trials

1. Patient safety
2. Patient stratification
3. Rare disease

'Discovery chips'

Pharmaceutical Drug Development

- B&L
- Abbvie
- UBC (UK)
- Apoglyx (Sweden)
- Pfizer

'Regulatory chips'

Regulatory Drug Development

Academic Partnerships

Local Support for Translational MPS

Translational Center for Barrier Microphysiological Systems

Home Protocols µSiM hToC Membranes Fludics Simulations

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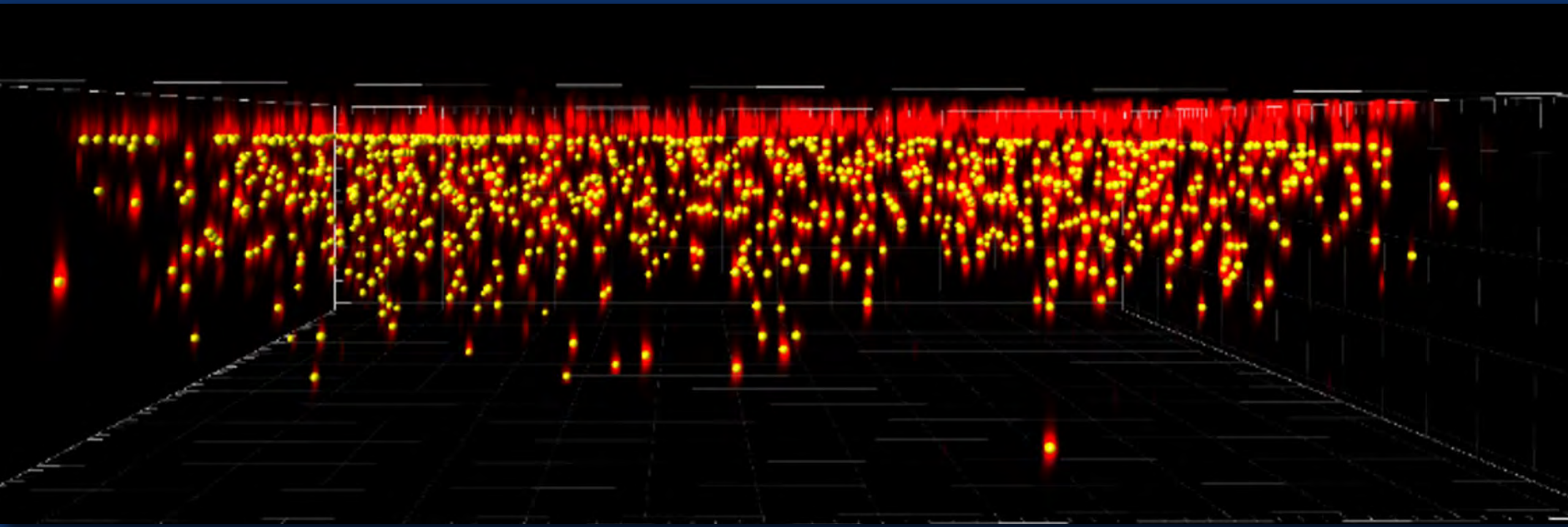


Image by Isabelle Linares

Translational Center for Barrier Microphysiological Systems - TraCe-bMPS

Joan Adamo, PhD
Director, Office of Regulatory Support
Clinical Translational Science Institute (CTSI)

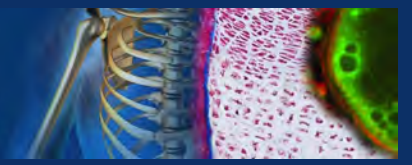
Hani Awad, PhD
Donald and Mary Clark Distinguished Professor of Orthopaedics
Associate Director of Center for Musculoskeletal Research
Professor of Biomedical Engineering

James McGrath, PhD
William R. Kenan Professor of Biomedical Engineering
Director of TraCe-bMPS

Ben Miller, PhD
Dean's Professor of Dermatology
Professor of Biochemistry and Biophysics, Biomedical Engineering, Optics
Academic Lead for Photonic Sensors in AIM Photonics

George Truskey, PhD
R. Eugene and Susie E. Goodson
Professor of Biomedical Engineering
Past Associate Vice President for Research at Duke University

Center for MusculoSkeletal Research



RIT

Duke Clinical & Translational Science Institute

CTSI
University of Rochester Medical Center

University of Rochester BME Biomedical Engineering

Jonathan Flax, PhD, Urology
Harris Gelbard, MD, PhD, Neuroscience
Jeanne Holden-Wiltse, MPH, MBA, CTSI Informatics
Minsoo Kim, PhD, Dept. of Micro. and Immunology
Richard Waugh, PhD, BME (Emeritus)

Duke University

Niccolo Terrando, PhD, Dept. of Anesthesiology

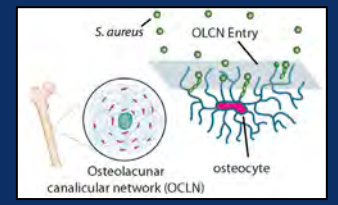
RIT

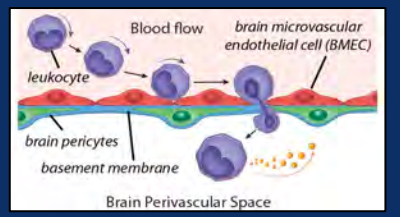
Vinay Abhyankar, PhD, BME
Tom Gaborski, PhD, BME

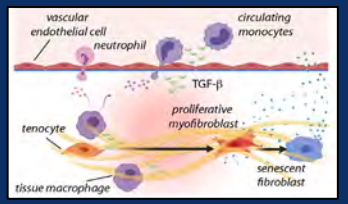
Original Year 1 plans

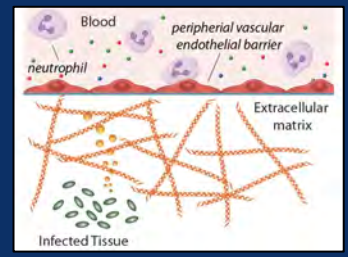
Focused on discovery, mechanism of action, lead compound optimization

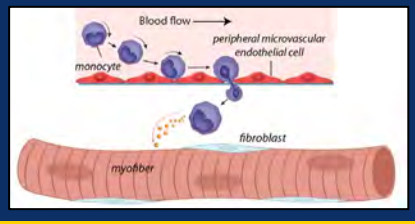
Disease	DDT	COU
Osteomyelitis - A persistent bone infection occurring in 1-5% of elective orthopedic surgeries caused most often by *S. aureus*.	µSiM-CA	Drugs that block *S. aureus* invasion of the osteocyte canalicular network
Multiple Sclerosis - Autoimmune disease where T-cells destroy myelin and injure nerves. Affects ~1M Americans.	µSiM-CVB	Drugs that limit the ability of lymphocytes to cross the blood-brain barrier
Tendon Injury - Healing via a fibrotic scar risks re-injury and morbidity. Significant socioeconomic burden and diminished of life.	hToC	Anti-inflammatory treatments that limit scar formation
Sepsis - Dysregulated immune response to infection that can lead to multi-organ failure. Largest cause of deaths in US hospitals.	µSiM-MVM	Drugs that restore ability of neutrophils to home to source of infection
Juvenile Dermatomyositis - An autoimmune myopathy affecting 2-4 million children each year. Immunosuppressive therapies associated with significant toxicity.	µSiM-hMoC	Drugs that reduce the infiltration of circulating immune cells into muscle or the inflammation it causes





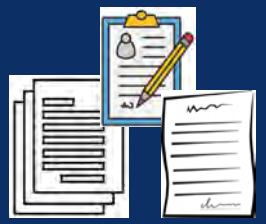






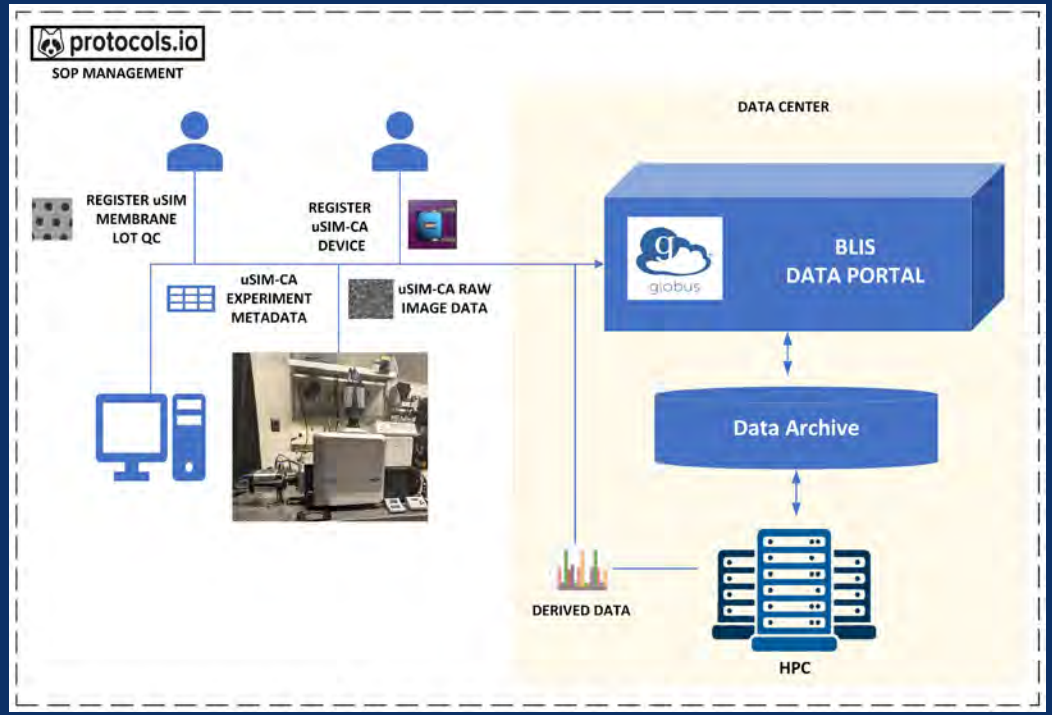
Quality System – Design and Implementation

Collect and formalize all research steps into Standard Operating Procedures (SOPs)



protocols.io

- CA-20_Ver 01 - Making TSB media
- CA-21_Ver 01 - Starting a Bacterial Culture from a Frozen Glycerol Stock
- CA-22_Ver 01 - Starting a Bacterial Culture from a Streak Plate
- CA-23_Ver 01 - Quantifying Growth of *Staphylococcus aureus* on a Spectrophotometer
- CA-24_Ver 01 - Subculturing *Staphylococcus aureus* Bacteria
- CA-30_Ver 01 - Loading Control, Reference and Test Samples into Devices
- CA-31_Ver 01 - Acquiring Data Sets on a Spinning Disc Confocal Microscope

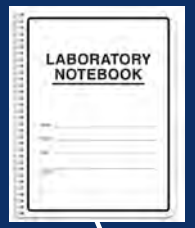


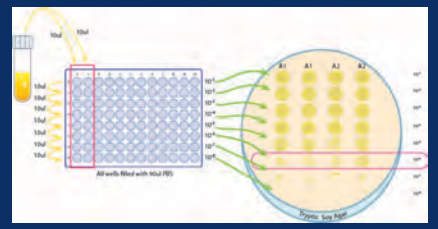
Over 50 SOPs have been written

~18 laying the overall foundation for future studies

~35 which are project-specific

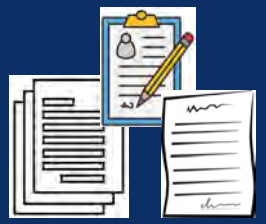
Quality System – Design and Implementation





NANOMEMBRANE RESEARCH GROUP

Collect and formalize all research steps into Standard Operating Procedures (SOPs)



protocols.io

Technology Transfer

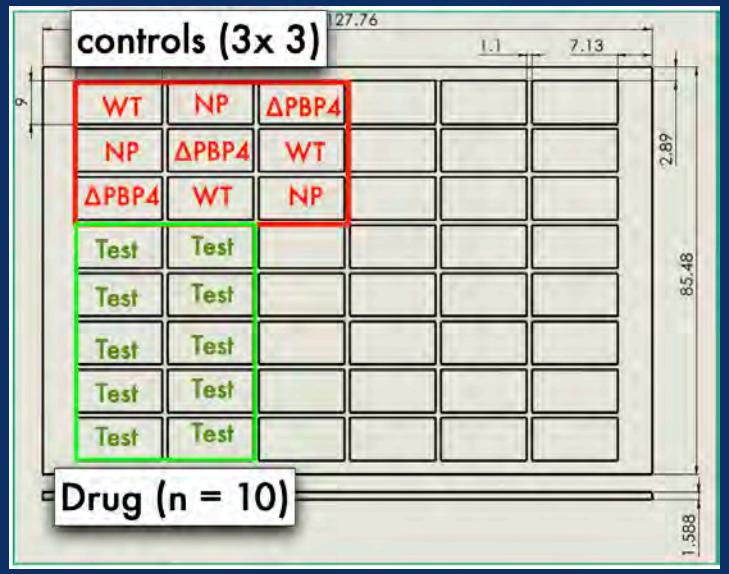
Internal labs

External, independent labs

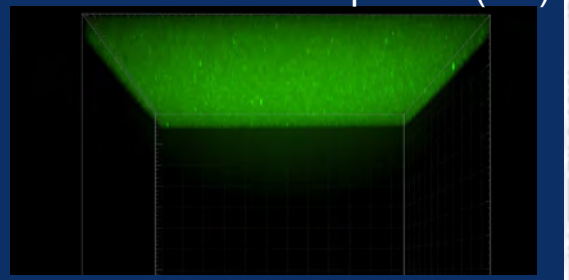
Reproducibility testing of µSiM-CA DDT

Using Two Tailed T-test	Effect Size (d)	B = 0.8	B=0.95
Wild Type vs Nonporous	7.008367	n=2 each	n=3 each
Wild Type vs ΔPBP4	6.761747	n=2 each	n=3 each

Condition	Sample
physical control	WT USA 300 non-porous (NP)
molecular control	ΔPBP4 USA300
active sample	WT USA 300

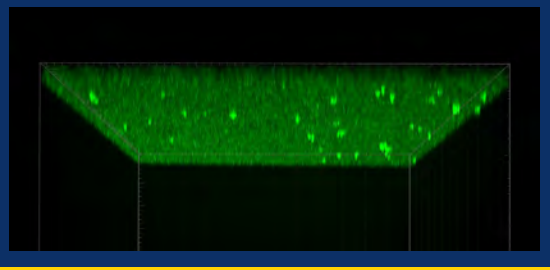


WT USA300 non-porous (NP)

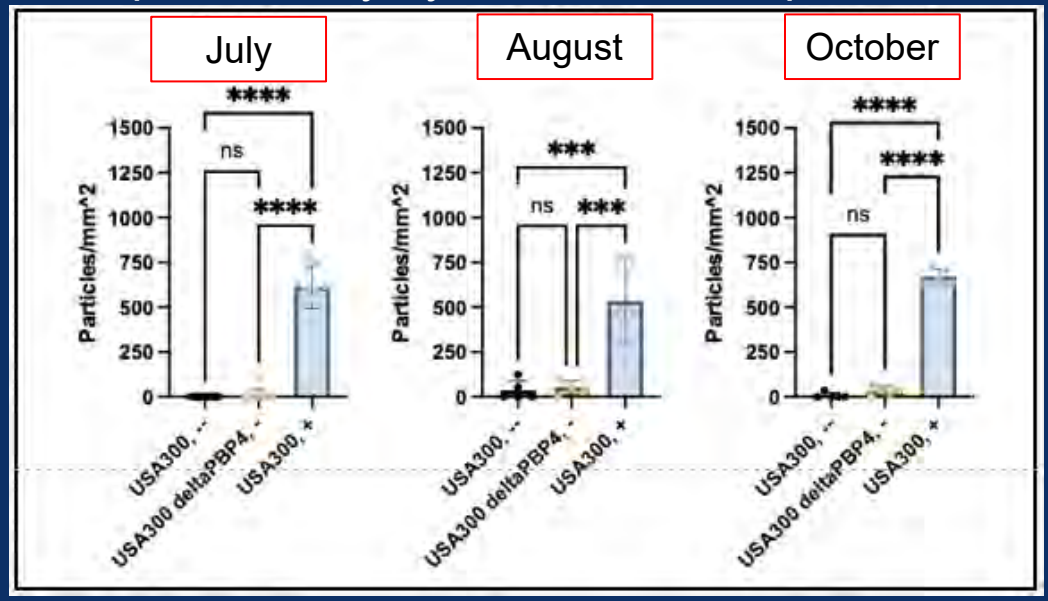


ΔPBP4 USA300

WT USA300



Reproducibility by Internal Development Team



Assay was repeated three times over the course of several months and results were reproducible

School of Medicine and Dentistry
Edmund A. Hajim School of Engineering and Applied Sciences
Department of Biomedical Engineering

6 September 202

ISTAND Pilot P
ISTAND@fda.h

Dear Dr. Tomita

Pursuant to the 2
and Technology
we wish to reque
format of a Lette
team as the first
Researchers fron
assaying the tran
we understand th
resources availab

The purpose of t

1. To discus
2. To reviev
process

As requested by
supporting conte

FDA U.S. FOOD & DRUG ADMINISTRATION

September 25, 2024

University of Rochester Medical Center
Attn: Joan E. Adamo, Ph.D.
Director, Office of Regulatory Support Clinical
& Translational Science Institute
265 Crittenden Blvd
Box # CU 420708
Rochester, NY 14642-0708

Dear Dr. Adamo:

This communication is in reference to the Pre-Letter of Inten
submitted on behalf of University of Rochester Medical Cent
Innovative Science and Technology Approaches for New Dr
September 6, 2024. You are proposing an assay that will de
bacteria can change their shape to accommodate the narrow
osteomyelitis infection. This assay would be used to assess
antibiotics that prevents this shape change, resulting in the s
survive in the bone.

The FDA has made a determination and your request for a P
FDA is declining your meeting request for the following reas

The primary utility of this assay seems to be in the preclinica
candidates, and as such does not address a specified drug development need that fits within
our regulatory framework. If you believe that this biomarker would be useful for circumstances
that fit within the scope of FDA regulation, please clarify what those circumstances are.

School of Medicine and Dentistry
Edmund A. Hajim School of Engineering and Applied Sciences
Department of Biomedical Engineering

UNIVERSITY of ROCHESTER

Take 2

25 October 2024

ISTAND@fda.hhs.gov

Dear Ms. Borges-Roman,

In response to your reply, sent on 25
providing further clarification regar
regulatory framework. We are provi
of use (COU).

- The COU makes clear that w
- The COU makes clear that w
decision-making.
- The COU makes clear that t
safety and specificity data to
the regulatory framework of

In addition to the COU, have provid
our DDT.

Pursuant to the 21st Century Cures A
and Technology Approaches for Ne
we again request a request a Type B
the Context of Use to be included in

FDA U.S. FOOD & DRUG ADMINISTRATION

DRUG DEVELOPMENT TOOL
PRE-LETTER OF INTENT
MEETING REQUEST-3

March 18, 2025

University of Rochester Medical Center
Attention: Joan E. Adamo, Ph.D.
Director, Office of Regulatory Support
Clinical & Translational Science Institute
University of Rochester Medical Center
265 Crittenden Blvd, Box # CU 420708
Rochester, NY 14642-0708

Dear Dr. Adamo:

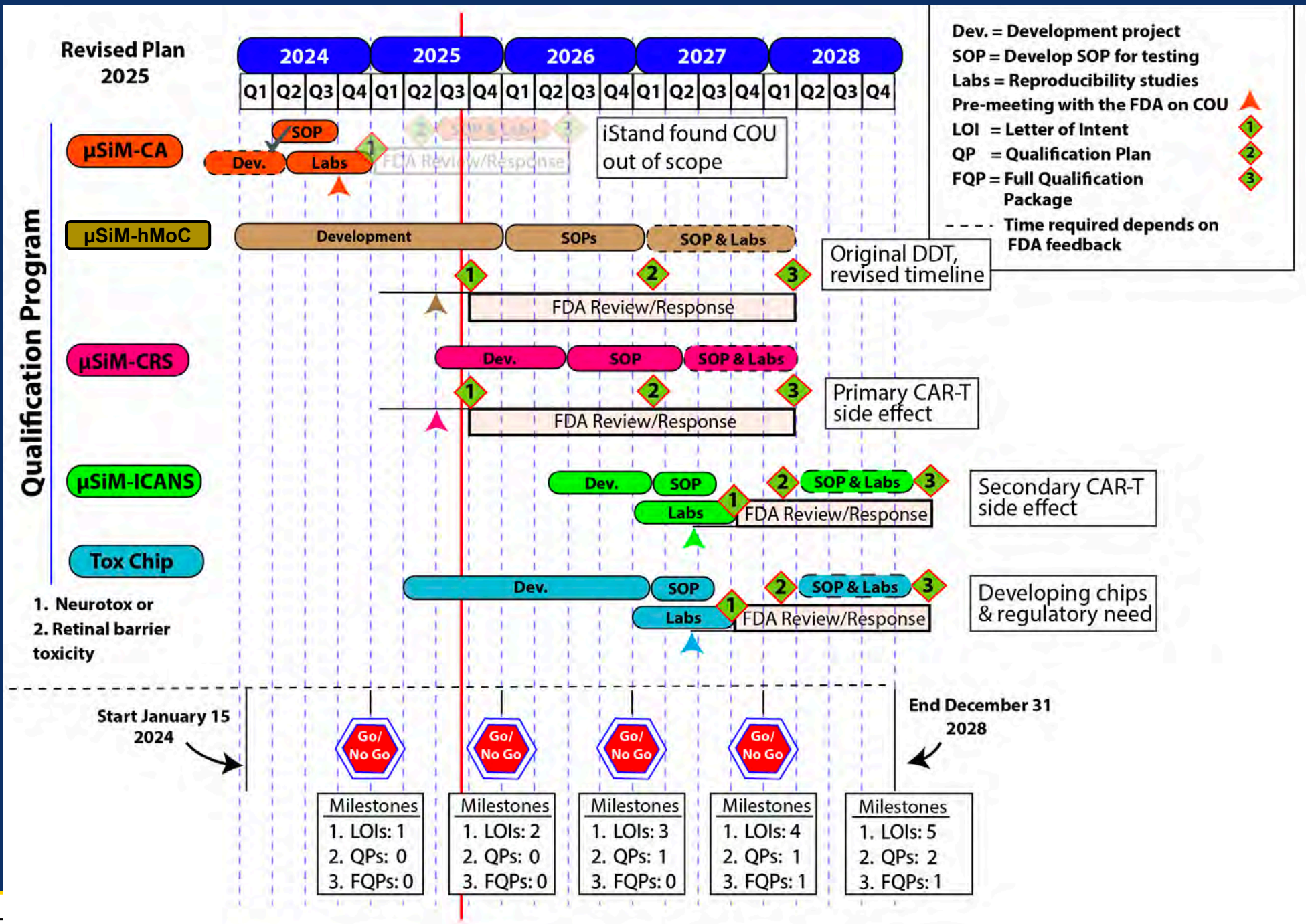
This communication is in reference to the Pre-Letter of Intent (LOI) inquiry that you submitted on behalf of University of Rochester Medical Center, received by the CDER Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program on February 19, 2025. In your pre-LOI meeting request dated October 26, 2024, you provide the following Context of Use (COU): "The µSiM-CA is an in vitro assay that quantifies the capacity of *S. aureus* to enter submicron spaces that mimic the physical restrictions of the OLCN. In this way the assay is used as a tool to demonstrate that a drug that does not kill *S. aureus*, is effective in targeting the specific pathways *S. aureus* uses for deep bone infection in chronic osteomyelitis."

The FDA has made a determination and your request for a Pre-LOI meeting is denied for the following reason:

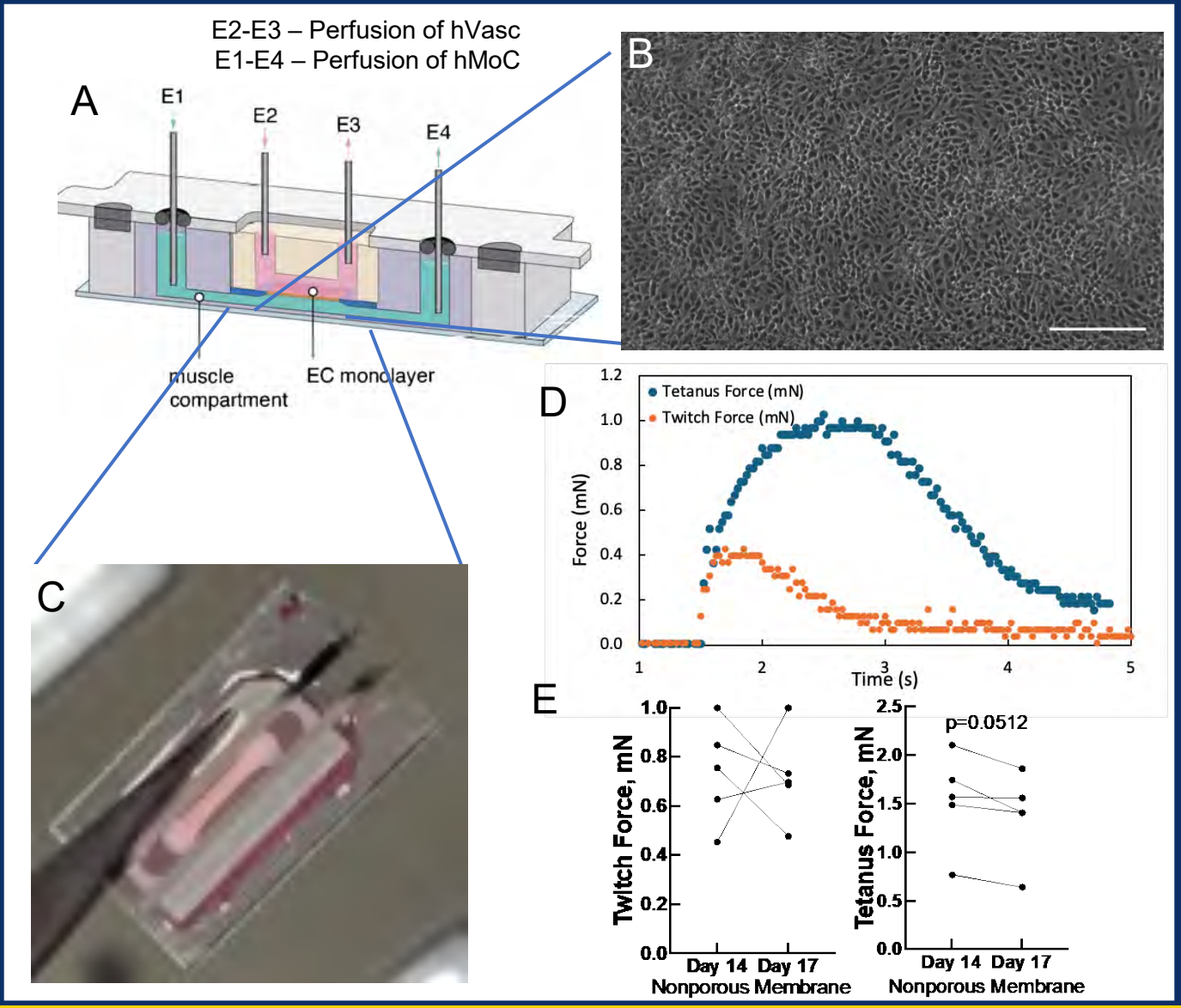
You have not provided a COU that addresses an existing drug development need within the regulatory framework of the FDA. If you believe the tool would assist in identifying drugs that target the pathways *S. aureus* uses for deep bone infection in chronic osteomyelitis, then we recommend you consider working with a pharmaceutical company that is developing a drug targeting that pathway to see if this tool could help them as they develop evidence for proof of concept or proof of mechanism.

This response closes out the above meeting request. If you have any questions regarding this communication, email the ISTAND Pilot Program staff at ISTAND@fda.hhs.gov.

Milestones


Revised Plan 2025
2024
2025
2026
2027
2028
Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4
Dev. = Development project
SOP = Develop SOP for testing
Labs = Reproducibility studies
Pre-meeting with the FDA on COU
LOI = Letter of Intent
QP = Qualification Plan
FQP = Full Qualification Package
Time required depends on FDA feedback
Qualification Program
µSiM-CA
SOP
Dev.
Labs
FDA Review/Response
iStand found COU out of scope
µSiM-hMoC
Development
SOPs
SOP & Labs
FDA Review/Response
Original DDT, revised timeline
µSiM-CRS
Dev.
SOP
SOP & Labs
FDA Review/Response
Primary CAR-T side effect
µSiM-ICANS
Dev.
SOP
Labs
SOP & Labs
FDA Review/Response
Secondary CAR-T side effect
Tox Chip
Dev.
SOP
Labs
SOP & Labs
FDA Review/Response
Developing chips & regulatory need
1. Neurotox or
2. Retinal barrier toxicity
Start January 15 2024
End December 31 2028
Go/ No Go
Milestones
1. LOIs: 1
2. QPs: 0
3. FQPs: 0
Milestones
1. LOIs: 2
2. QPs: 0
3. FQPs: 0
Milestones
1. LOIs: 3
2. QPs: 1
3. FQPs: 0
Milestones
1. LOIs: 4
2. QPs: 1
3. FQPs: 1
Milestones
1. LOIs: 5
2. QPs: 2
3. FQPs: 1

μSiM-hMoC to Better Replicate Juvenile dermatomyositis (JDM)



Established human Myobundle on Chip (hMoC)

Testing IFN-β treatment on endothelial cells and myobundles

Context of Use

Human skeletal myobundles treated with interferon-β (IFNβ) are a drug development tool used to assess the range of efficacy of candidate therapeutics to inform dosing for clinical trials to treat Juvenile Dermatomyositis. Results from this μSiM-hMoC system will provide a more efficacious dosage range for regulatory decision-making to enable Phase 1 clinical trials.

FDA NEWS RELEASE

FDA Advances Rare Disease Drug Development with New Evidence Principles

For Immediate Release: September 03, 2025

The U.S. Food and Drug Administration today introduced the Rare Disease Evidence Principles (RDEP) to provide greater speed and predictability in the review of therapies intended to treat rare diseases with very small patient populations with significant unmet medical need and that are driven by a known genetic defect. Through the RDEP process, sponsors will receive clearer guidance on the types of evidence that can be used to demonstrate substantial evidence of effectiveness.

"Drug developers – and the patients they hope to treat – deserve clear, consistent information from the FDA," said **FDA Commissioner Marty Makary, M.D., M.P.H.** "These principles ensure that FDA and sponsors are aligned on a flexible, common-sense approach within our existing authorities, and that we incorporate confirmatory evidence to give sponsors a clear, rigorous path to bring safe and effective treatments to those who need them most."

It is well understood that developing drugs for rare diseases can make it difficult or even impossible to generate substantial evidence of safety and efficacy — as required by statute — using multiple traditional clinical trials. Instead, rare disease drug developers and the FDA must work together to identify alternative methods for meeting the statutory standard that are both rigorous and viable for rare disease populations.

The RDEP – developed and implemented jointly by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) – addresses the inherent uncertainties of rare disease drug development by assuring sponsors that reviews will encompass additional supportive data. Approval under the process may be based on one adequate and well-controlled study plus robust confirmatory evidence, which may include:

- Strong mechanistic or biomarker evidence
- Evidence from relevant non-clinical models
- Clinical pharmacodynamic data
- Case reports, expanded access data, or natural history studies

Rare Disease Focus

r use as drug development tools by Pharma and the FDA
f Rochester technologies for MPS applications
biomedical research in Rochester's academic community

Industrial Partnerships

TraCe-bMPS

FDA

Regulatory Drug Development

UR MEDICINE

Local Support for Translational MPS

Improve Clinical Trials

1. Patient safety
2. Patient stratification
3. Rare disease

'Regulatory chips'

µSiM-hMoC for Juvenile Dermatomyositis - Letter of Intent

Duke

GEORGE A. TRUSKEY, P
DEPARTMENT OF BIOM
101 SCIENCE DRIVE, BO
DURHAM, NC 27708-028

15 Aug 2025

Food and Drug Ad
Center for Drug Ev
ISTAND Pilot Prog
Central Document
5901-B Ammenda
Beltsville, MD 207

RE: ISTAND Letter
(JDM)

Dear ISTAND team

Please accept this
development tool
Innovative Science
Submission Proces
first in the multi-step Drug Development Too
muscle myobundle model that has been deve
disease, Juvenile Dermatomyositis (JDM) to i

3. Drug Development Need Statement: Describe the drug development need that submission is intended to address, including (if applicable) the proposed benefit over currently used tools in similar contexts of uses (COUs). (200 words maximum target)

Juvenile Dermatomyositis (JDM) is a highly morbid systemic disease with unknown cause or cure. JDM is the most common pediatric inflammatory myopathy, yet US annual incidence is only 2 to 4 cases per million[1,2]. Approximately 60% of patients develop a chronic course. Standard treatments frequently cause significant toxicity, increase infection risk, and are not always effective[3]. Treatments include methotrexate, often causing nausea and hepatotoxicity[4], and prolonged high-dose
growth, and bone health.
diminished quality of life

JDM pathogenesis involv
pathway and increases in
perpetuate inflammation
pathogenesis[13,14].

Animal models replicate
weakness onset at a your
treatment or reduced co
have been underpowered
need to develop new app
to inform regulatory deci

**5. Context of Use Statement: A statement that fully and clearly describes the way the drug development tool will be used and the drug development-related purpose of the use. Examples of COU statements are available on the Biomarker Qualification Submissions and the Qualified Biomarkers web pages. Pl
qualified as tools to aid in drug developmen
purposes (e.g., to aid in clinical decision mal
drug development use are outside the scope
included in your LOI. (250 words)**

Human skeletal myobundles treated with inte
to assess the range of efficacy of candidate th
treat Juvenile Dermatomyositis. Results from
efficacious dosage range for regulatory decisi

Conditions for use:

1. Drug candidate addresses key features of
2. Standardized protocols are applied to asse
   enzymes.
3. Results from the assay are included in an

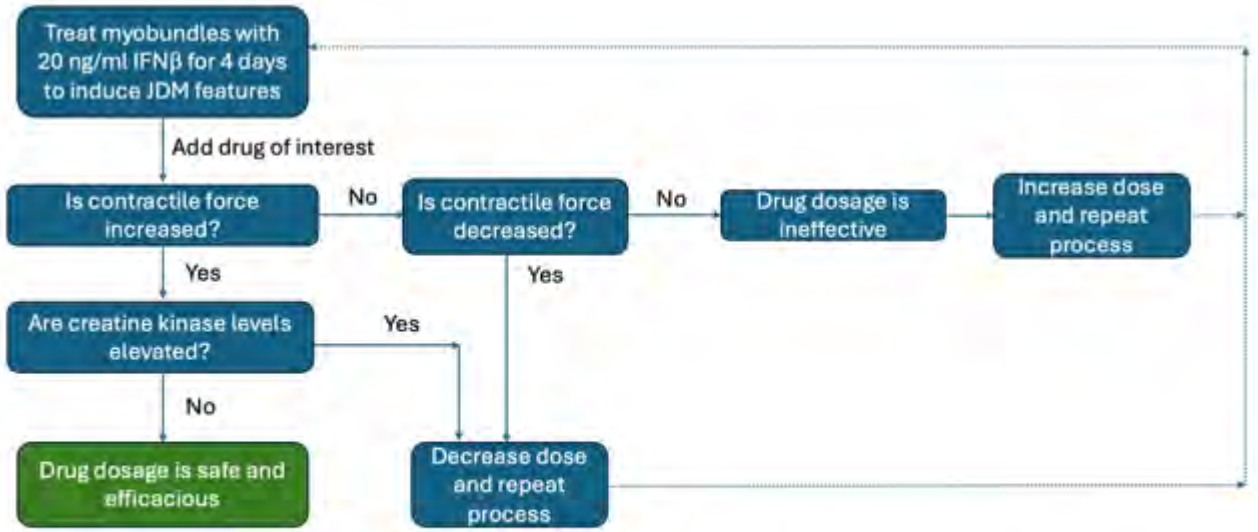


Figure 2. Flow diagram showing how the µSiM-hMoC drug development tool can aid in evaluating a safe and efficacious dosage range.

Year 2 and Beyond

Disease	DDT	COU	Status
Juvenile Dermatomyositis - An autoimmune myopathy affecting children. Immunosuppressive therapies associated with significant toxicity.	μSiM-hMoC	Human skeletal myobundles treated with Interferon β are drug development tools to assess the safety range of candidate therapeutics that inform doses for clinical trials to treat Juvenile Dermatomyositis	Developing LOI with C-Path
ICANS – Neurotoxicity from Immunotherapy (ex. CAR T)	μSiM-ICANS	Evaluating capacity of immunotherapy-generated CRS to trigger blood brain barrier breakdown	Developing LOI with C-Path
Rheumatoid arthritis – platform for drug efficacy in the treatment or RA and other Rheumatoid disorders	hJoC	Discovery Chip	Phase I project sponsored by Abbvie
Macular Degeneration – Platform for retinal permeability and mechanism of action/toxicity discovery	hBRB	Discovery Chip	Phase I project sponsored by B&L

BUILD YOUR OWN TISSUE CHIP

⚕ **1. Define Disease Context**

Identify the relevant tissue unit (e.g., BBB, alveolus) and pathophysiological hallmarks (e.g., barrier leak, edema), and clinical benchmarks (biomarkers, imaging, PK/PD) to emulate.

💡 **2. Formulate the Hypothesis and/or Drug MOA**

Pose a mechanistic hypothesis (dysregulation of protein Y amplifies pathway Z; Drug X reduces pathology via Mechanism Y). Clarify causal pathways and required perturbations (e.g., cytokines, shear). Define quantitative success criteria.

🫓 **3. Engineer the Microenvironment**

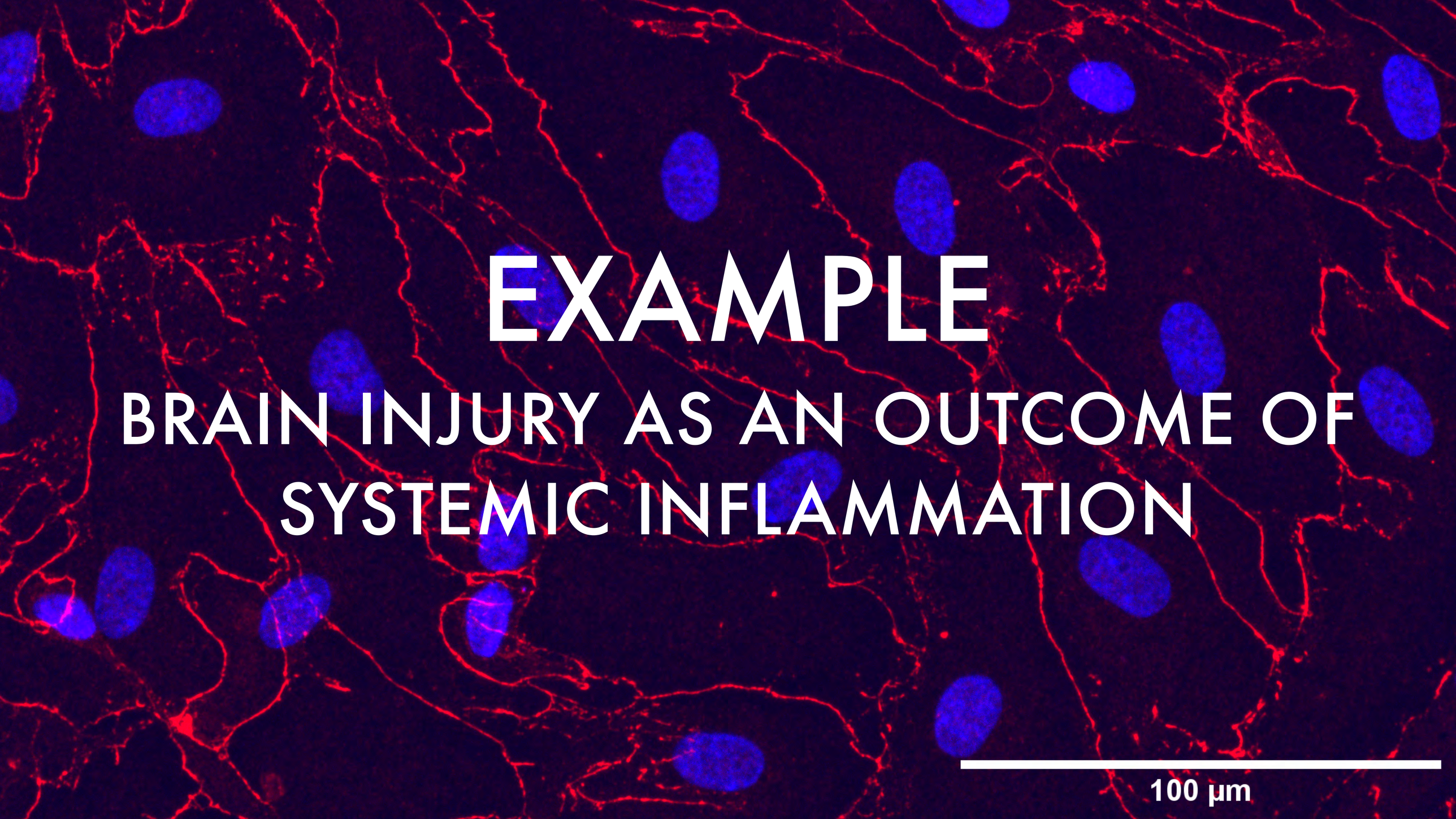
What cells are needed and how will you source them (primary or iPSC). Start with a minimum. What matrix materials (eg. collagen IV, laminin) are required? Key biophysical inputs (eg. shear, stretch) or biochemical (eg. cytokines, oxygen)? . Include disease triggers (e.g., LPS) or systemic contributors (eg., immune system) for fidelity. START WITH A CARTOON.

📊 **4. Specify Functional Readouts**

Select phenotypic (TEER, permeability), molecular (cytokines, RNAseq), and structural (tight junctions) metrics. Align each with clinical analogues and define quantitative acceptance thresholds.

🔬 **5. Design the Chip Layout**

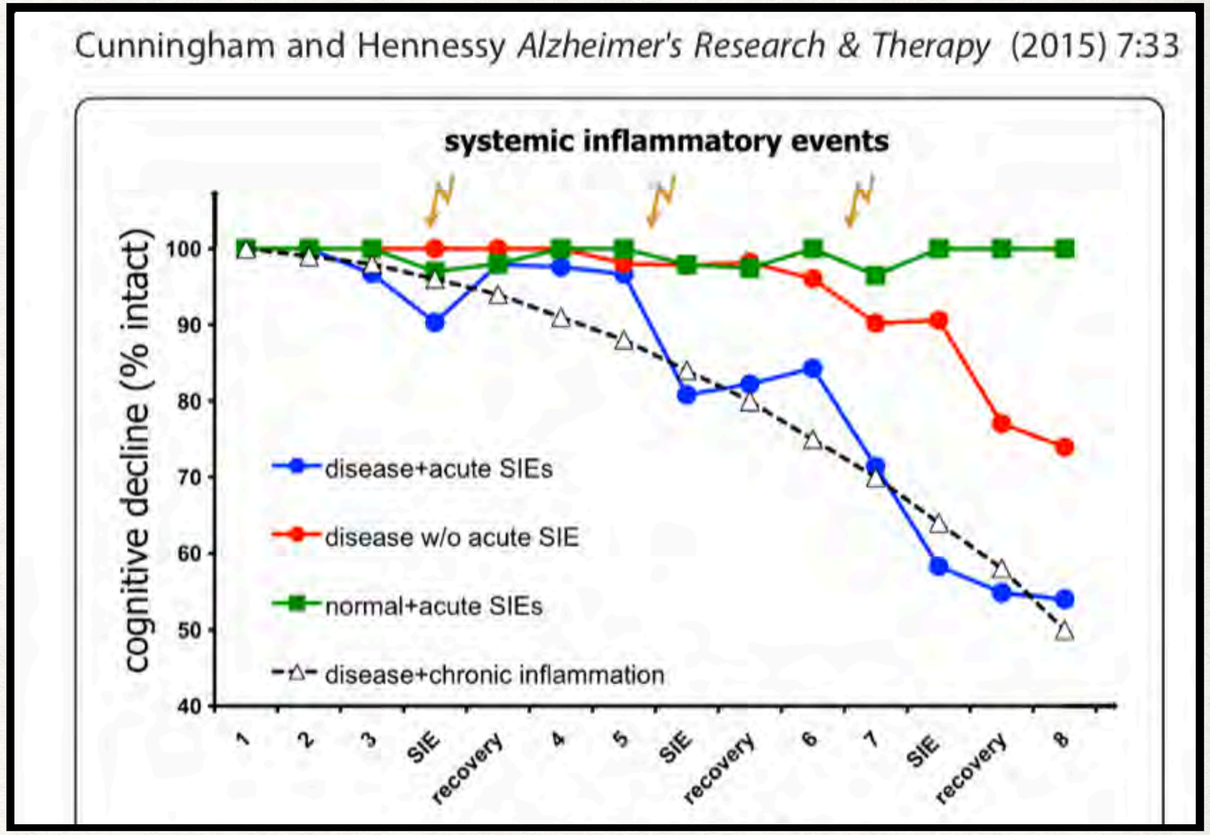
Choose appropriate architecture (e.g., dual-channel, stacked membranes), with input/output ports, sensors (TEER, imaging), and multiplexable sampling. Enable automation and scaling.


EXAMPLE
BRAIN INJURY AS AN OUTCOME OF SYSTEMIC INFLAMMATION
100 µm

MODELING SYSTEMIC INFLAMMATION LEADING TO BRAIN INJURY

GUIDEPOSTS AND INSPIRATIONS

- "In AD, patients who may have been previously resilient to their underlying neuropathology, commonly experience an abrupt worsening of cognitive impairment after hospitalization for critical illness." - Ben Singer, MD (U. Michigan)
- Many scenarios where 'cytokine storm' advances neurodegenerative disease and causes cognitive decline: 1) Sepsis (A. Pietropaoli); 2) Surgery in older adults (N. Terrando); 3) Acute lung injury (H. Gelbard); 4) Immunotherapies (CAR T; bispecific mAbs - M. Kim).

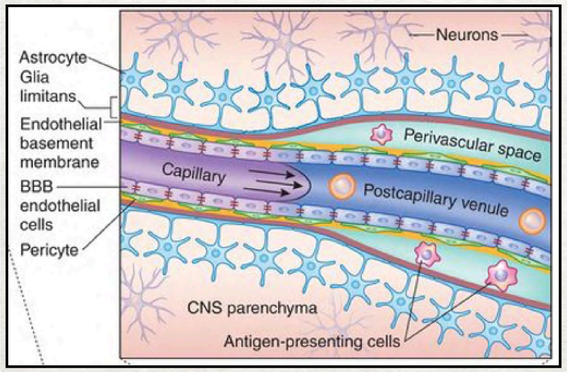


Leukocytes as an agent of brain injury …

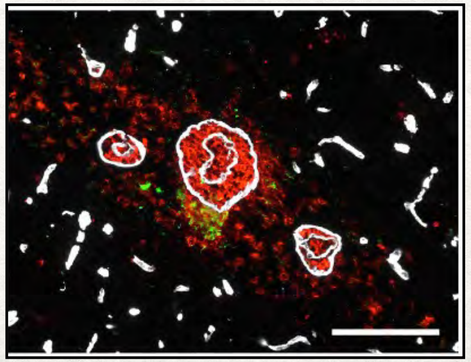
Trzeciak, et al. J Immunol, 2019. 203(11):2979-2989.

Andonegui, et al. (2018). JCI Insight 3(9): e99364.

Leukocytes enter the brain at the post-capillary venule … which is two barriers in series



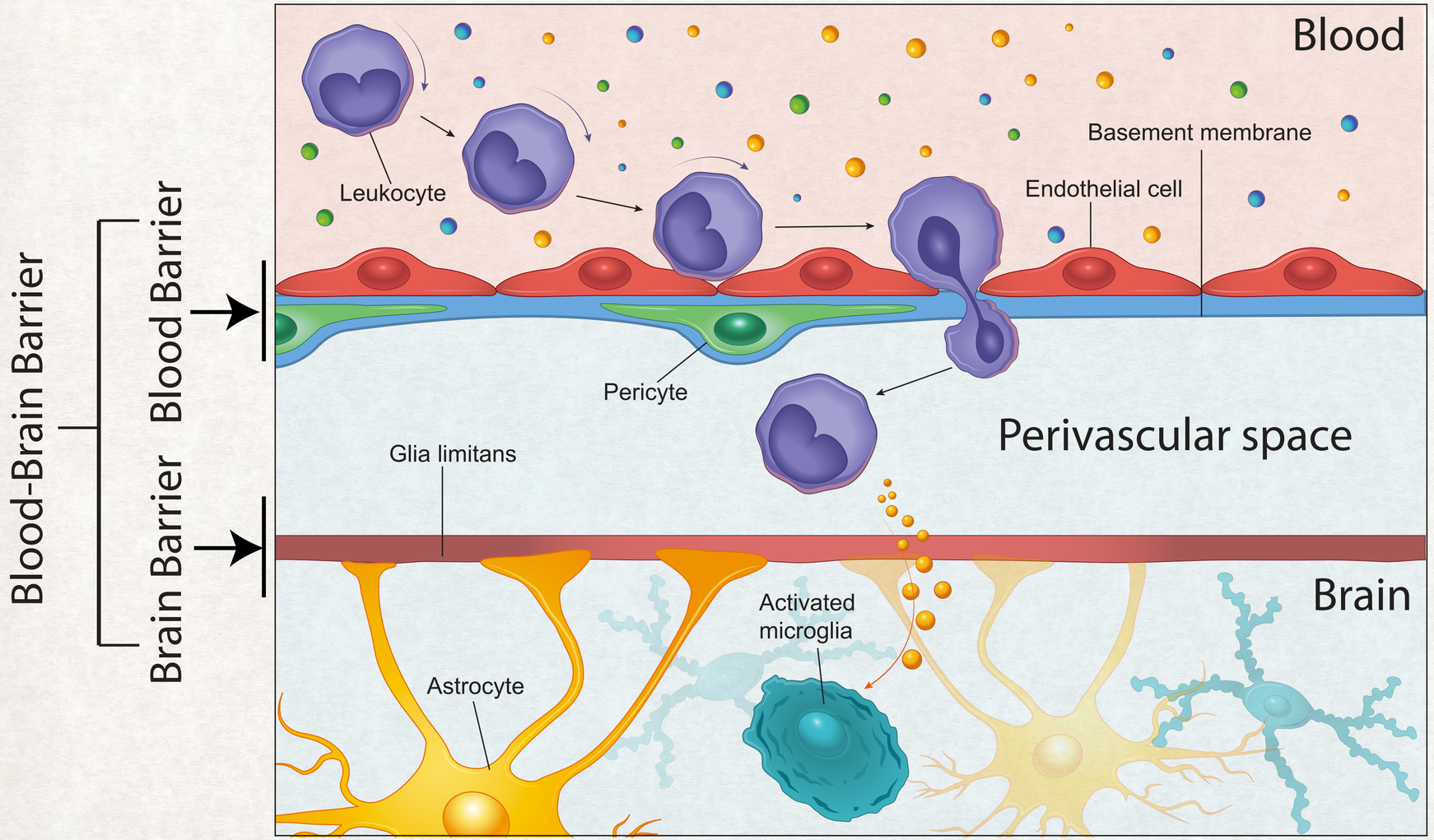
Engelhardt, B., et al., 2017. Nat Immunol, 18: 123-31.

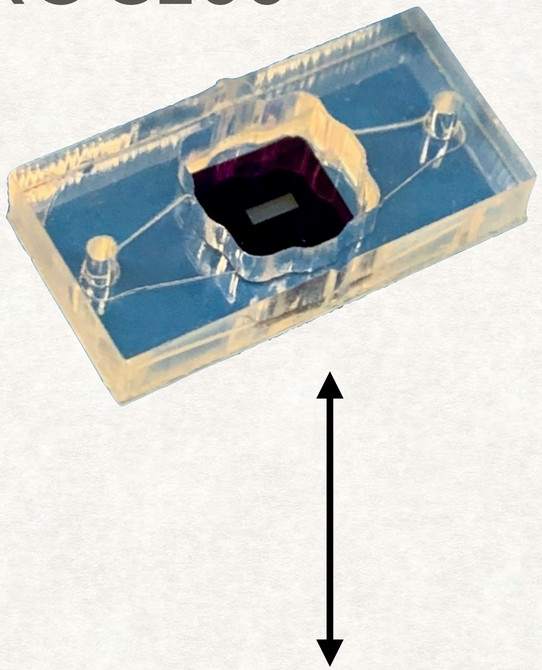


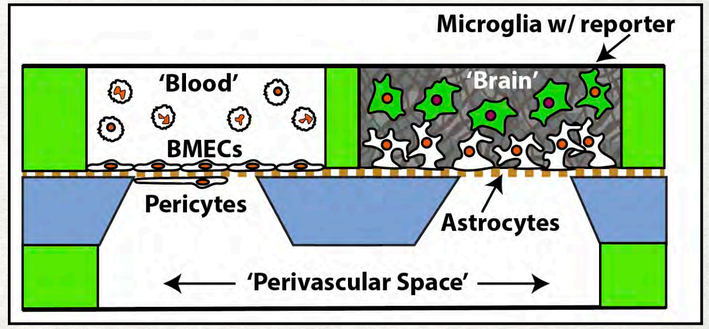
Song, C. et al., Cell Rep 10(7) (2015) 1040-54.

So our lab is building 'healthy' and 'vulnerable' models of the blood brain barrier at the post-capillary venule

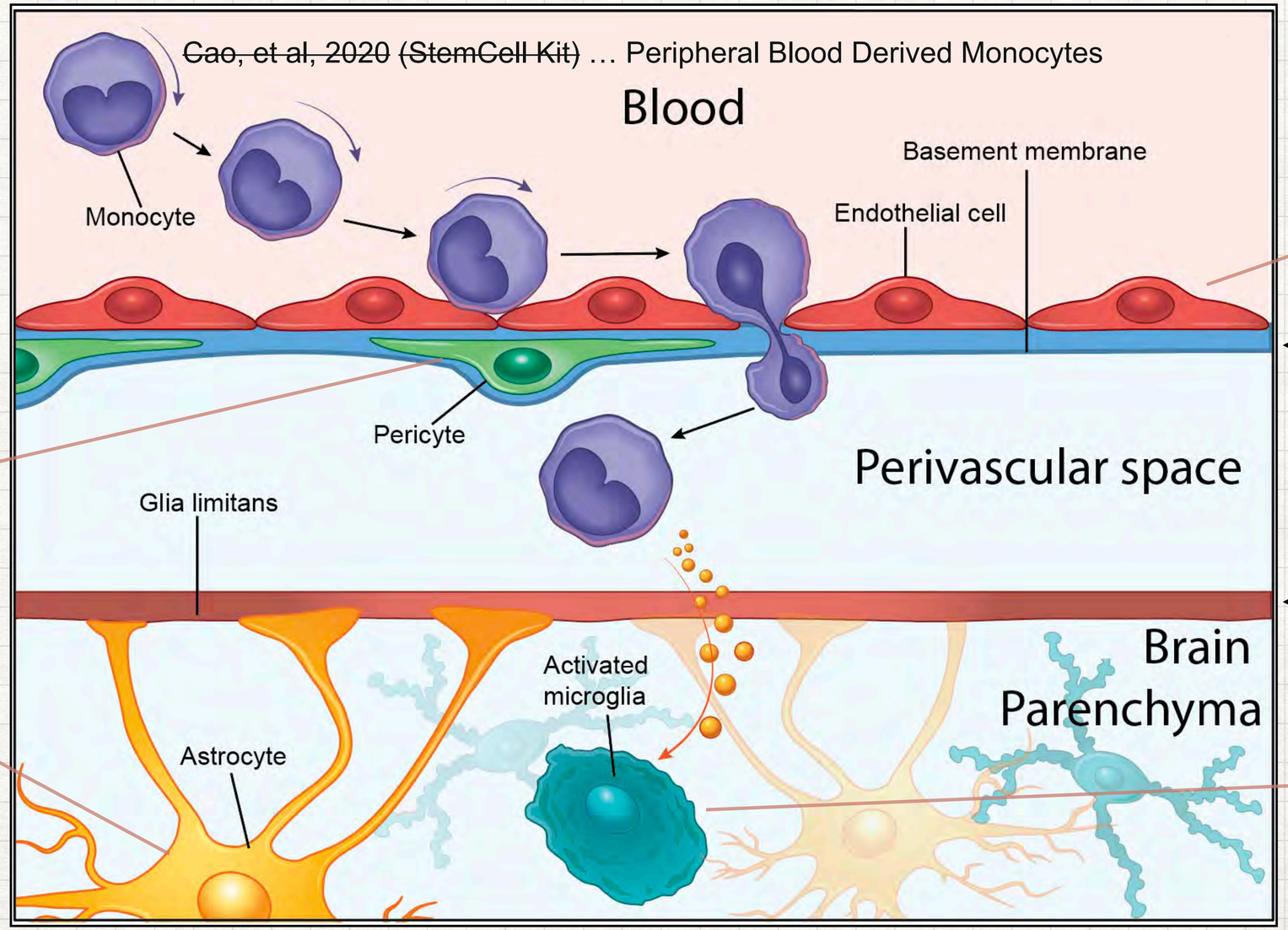
MODELING THE POST CAPILLARY NEUROVASCULAR UNIT ON THE μSIM: A STEP BY STEP PROCESS







DIFFERENTIATION OF iPSCS for the μSiM - pcNVU



Nishihara et al., 2020

Blood Barrier

Gastfriend et al., 2021

Parenchymal Barrier

Perroit et al, 2021

McQuade et al., 2018

[1] A. McQuade, Mol Neurodegener 13(1) (2018) 67

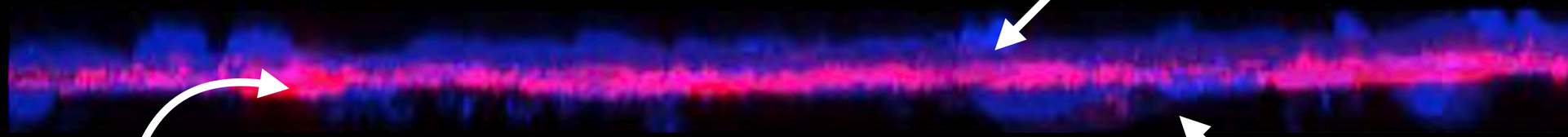
[2] B.D. Gastfriend, Curr Protoc 1(1) (2021) e21

[3] H. Nishihara, B.D. Faseb. J. 34(12) (2020) 16693-16715

BMECS / PERICYTES CO-CULTURES ON EITHER SIDE OF AN 'INVISIBLE' NANOMEMBRANE

DAPI COL4

Continuous Endothelial Cells



Collagen 4 is a shared layer in contact with both cell types

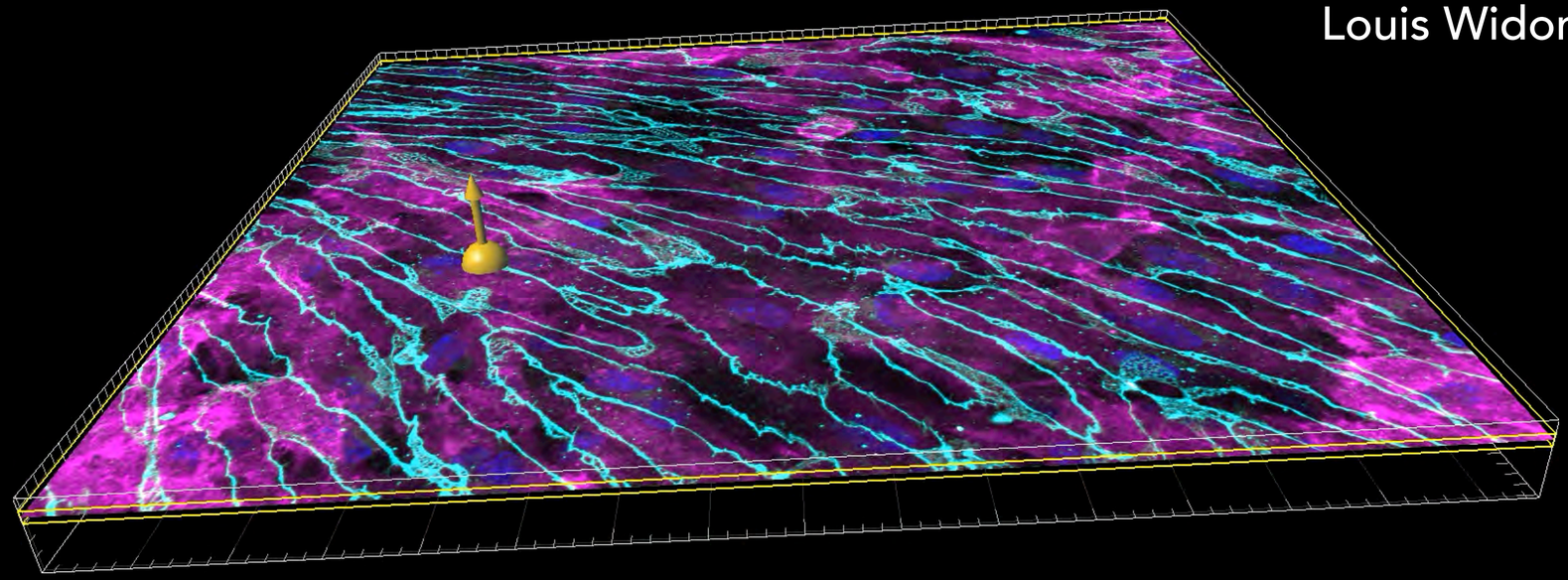
Sparse pericyte

w/ Tom Gaborski and Louis Widom @ RIT

BMECs (VE-cadherin)

↓

Pericytes (PDGFRb)



COMPOSITION OF THE BASEMENT MEMBRANE

BMECs

Laminin

Fibronectin

Col IV

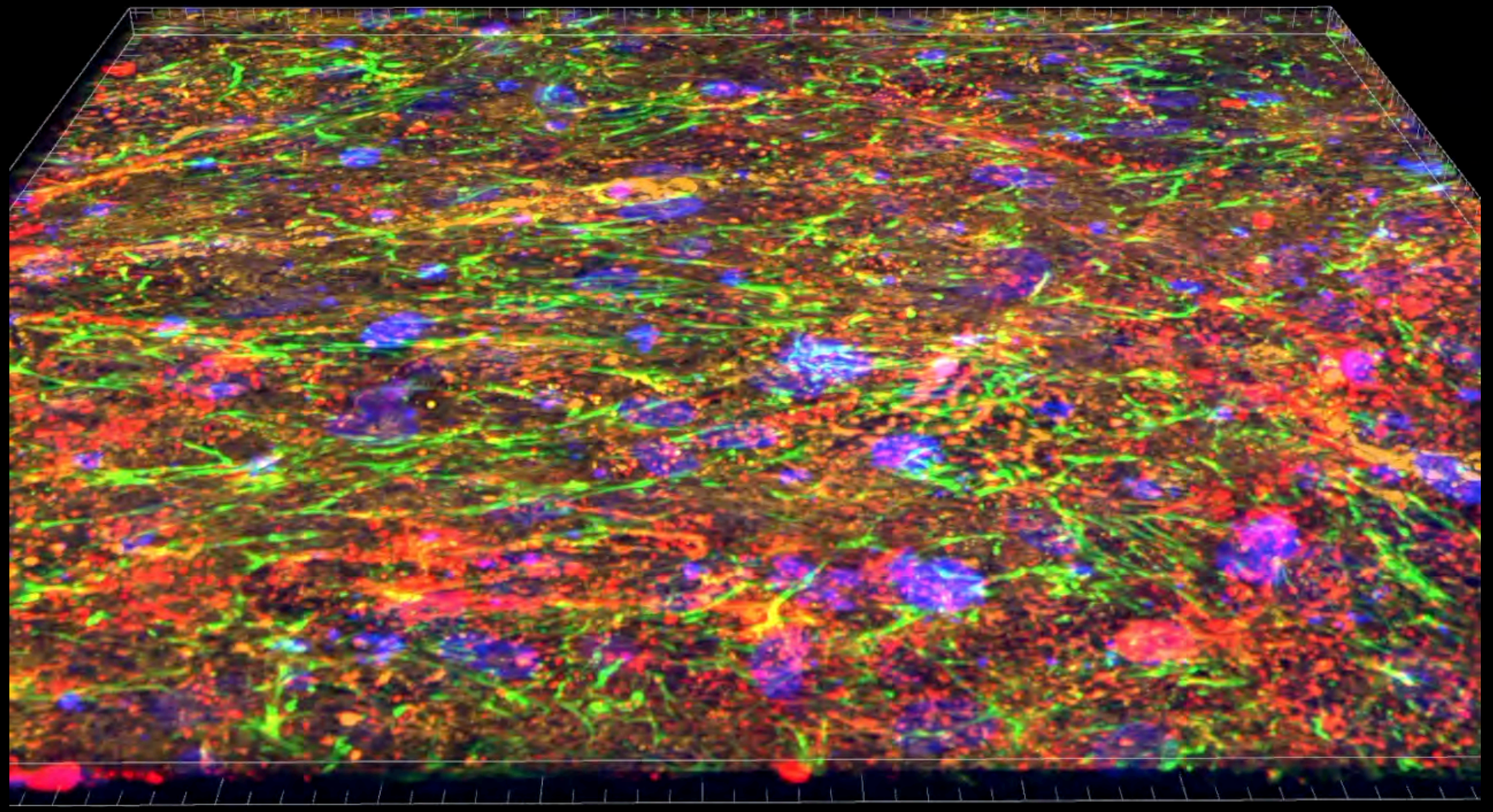
Pericytes

Hoechst

Laminin is diffuse and planar

Fibronectin is fibrous with more toward the membrane

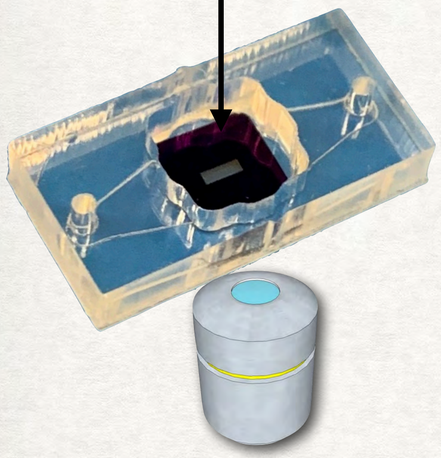
Col IV is fibrous far furthest from the membrane and globular elsewhere

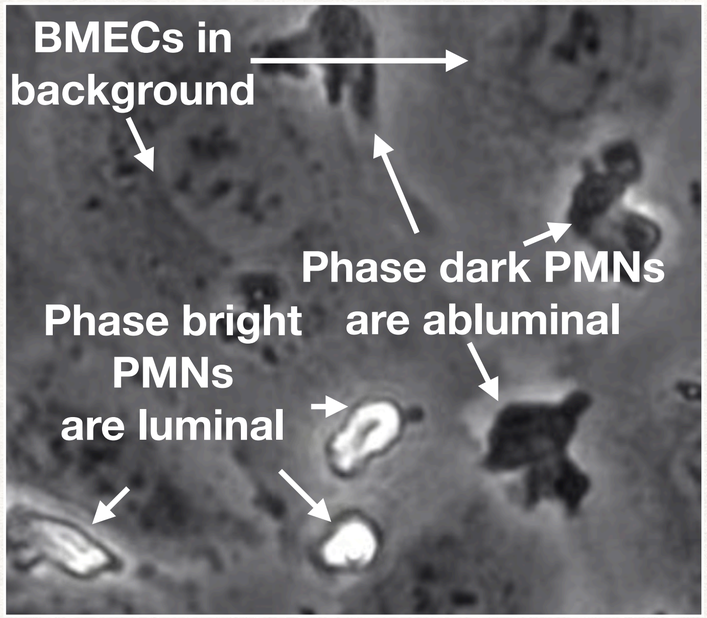


Michelle Trempel

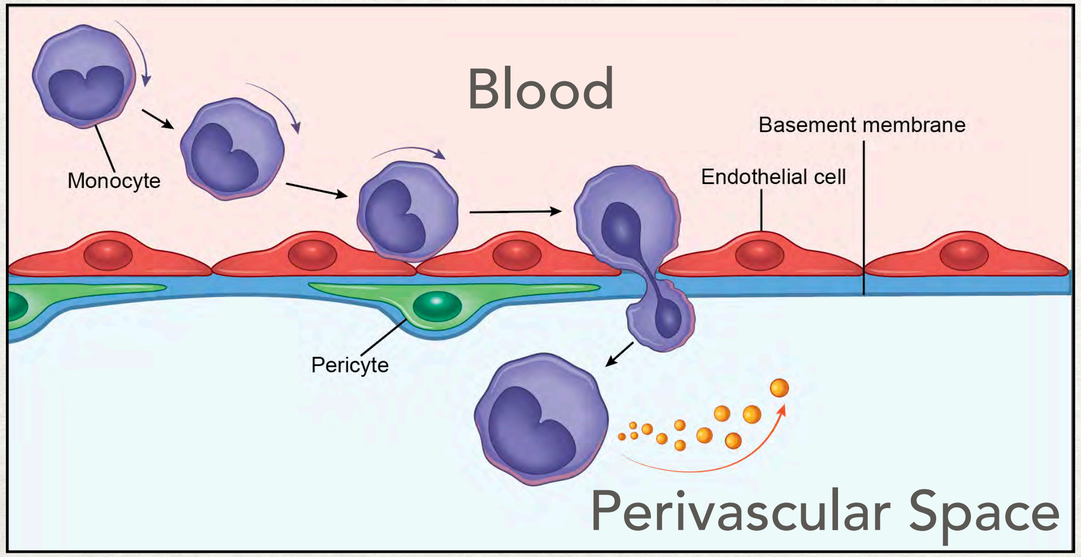
Neutrophils Trafficking Across an Isogenic BBB Model

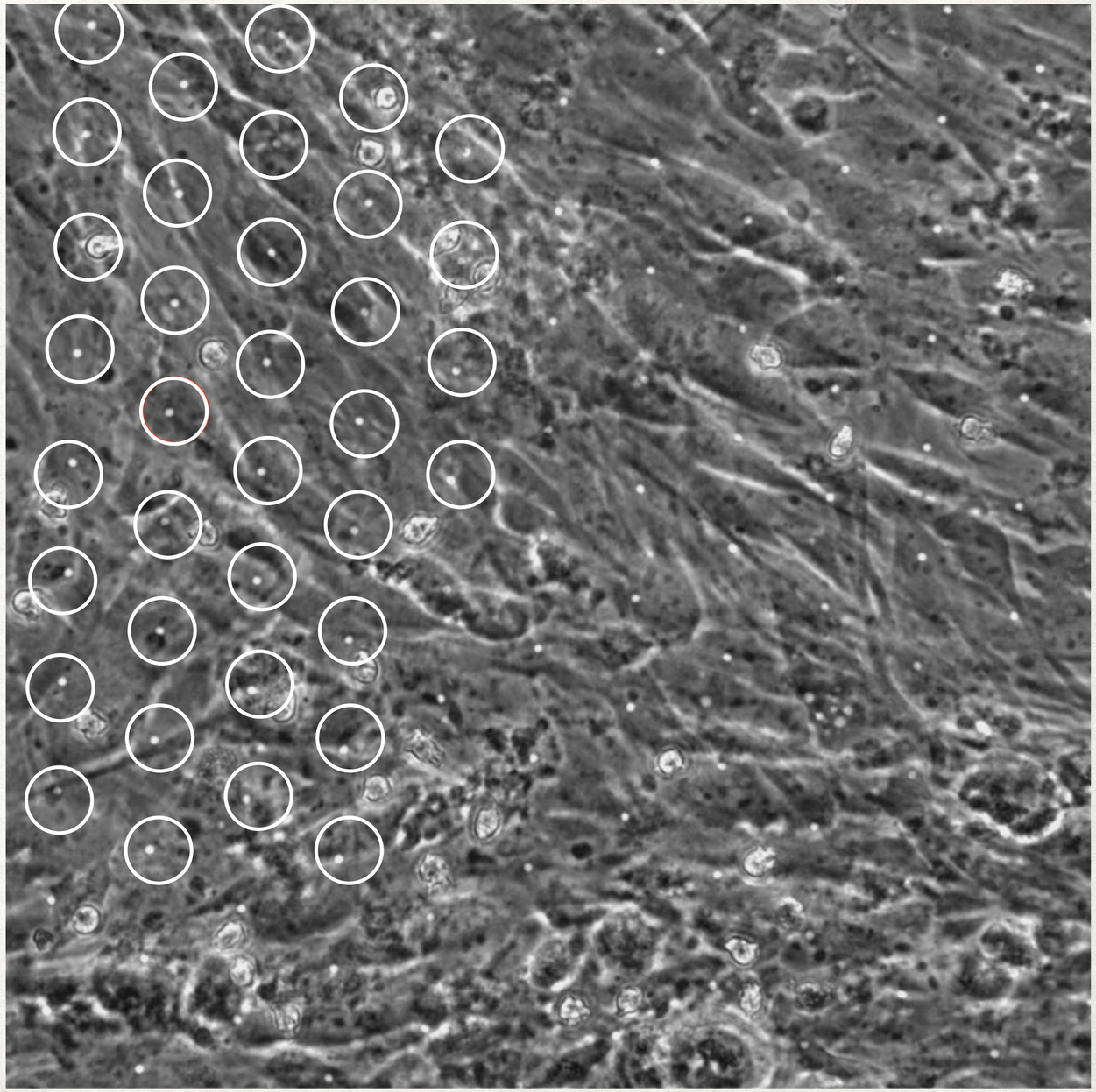
PMNs on top of BBB





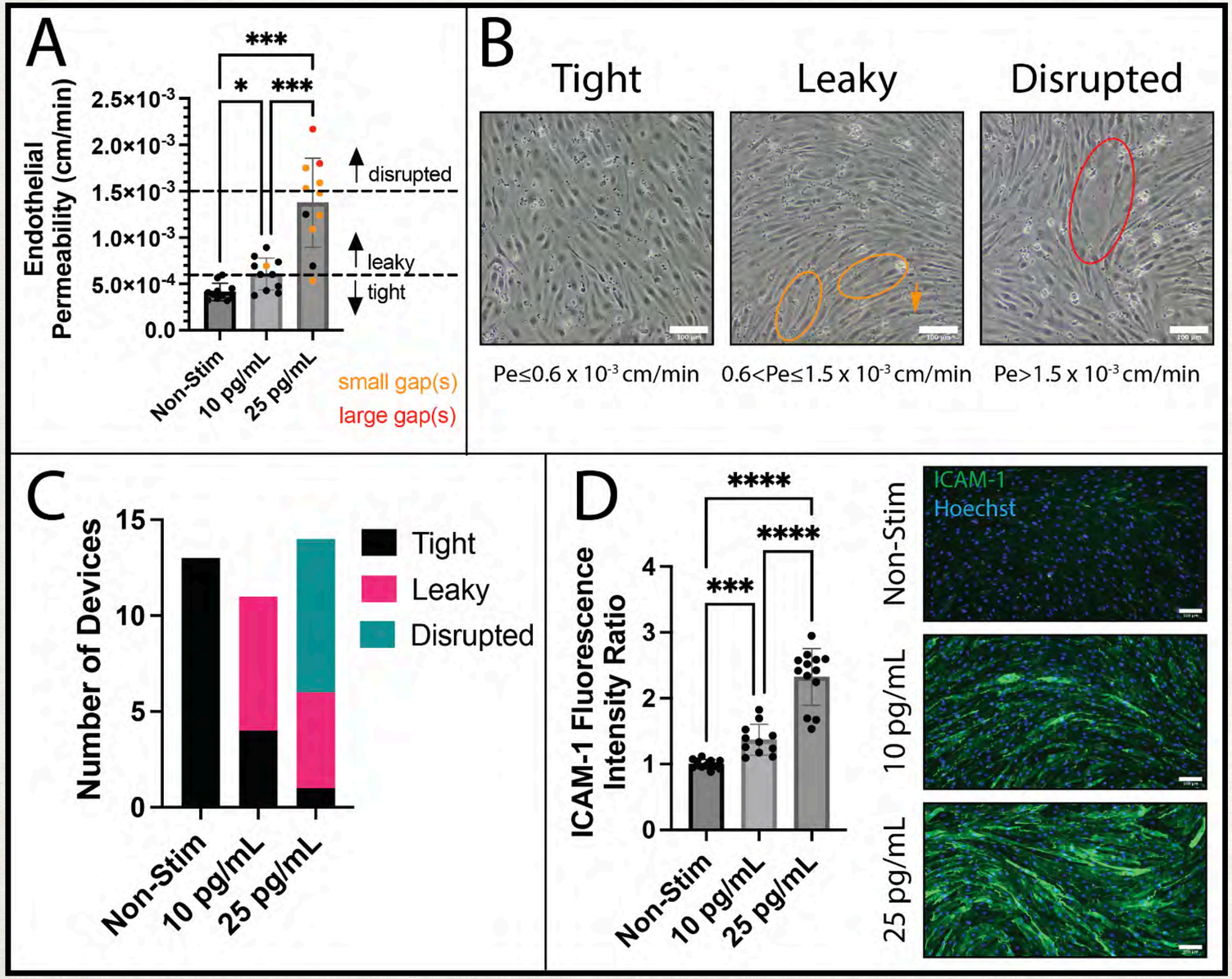
Phase contrast imaging @ 40X





BBB RESPONSE TO 'CYTOMIX' (EQUIMOLAR IL-1β; TNF-α; IFN-γ)

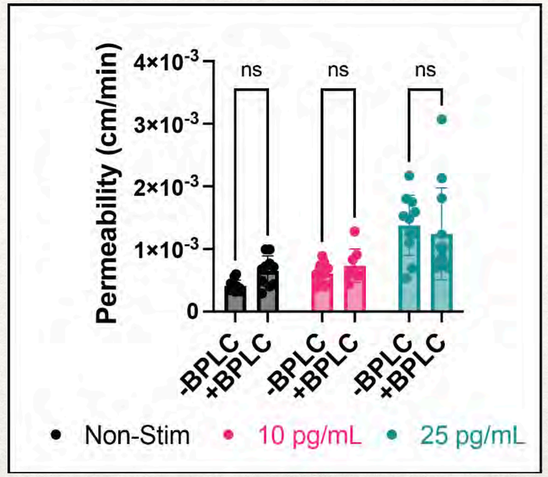
McCloskey and Ahmad et al., 2024 Biomaterials Research 28:0081

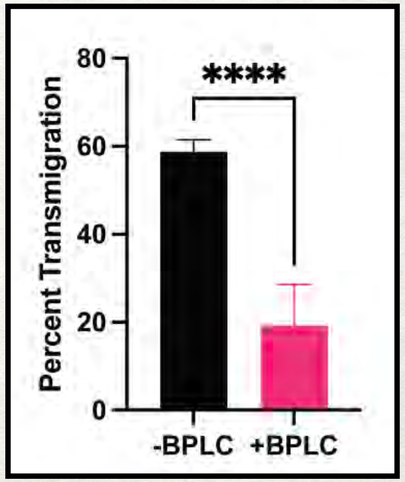


Orhun, G., et al. (2019). "Association Between Inflammatory Markers and Cognitive Outcome in Patients with Acute Brain Dysfunction Due to Sepsis." Arch Neuropsychiatry **56**(1): 63-70.

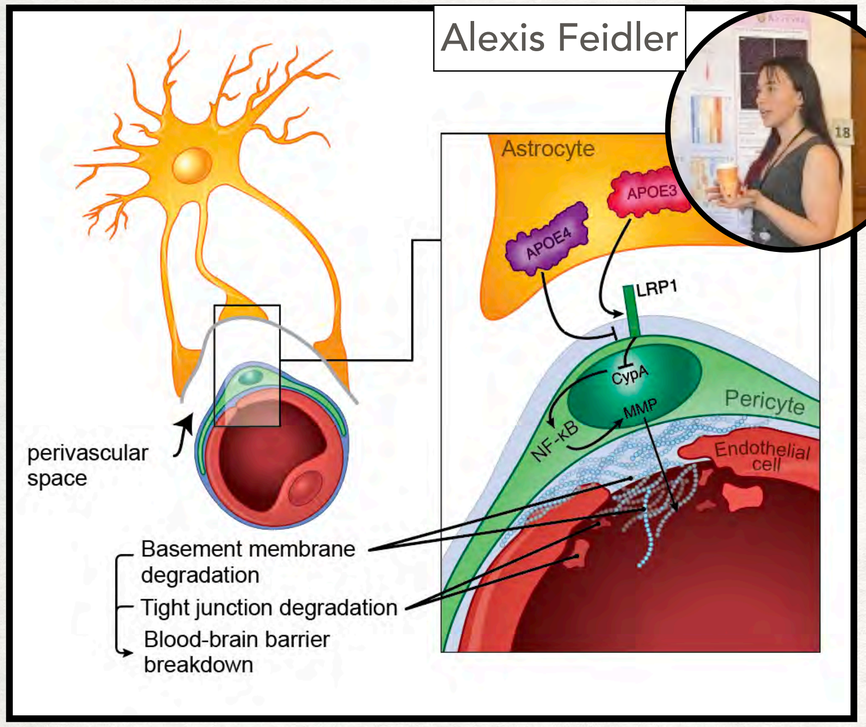
Table 2. Comparison of serum immunological and neurodegenerative parameter levels of sepsis-induced brain dysfunction patients with delirium and coma

Biomarkers	Patients with delirium (n=64)	Patients with coma (n=18)	p value
IL-1β (pg/mL)	17.3±10.9	9.7±6.8	0.277
IL-8 (pg/mL)	120.3±37.3	268.4±100.8	0.090
IL-6 (pg/mL)	43.9±6.8	90.9±21.3	**0.023**
IL-10 (pg/mL)	8.5±4.1	24.9±9.7	0.066
IFN-γ (pg/mL)	6.2±1.8	11.0±6.5	0.243
TNF-α (pg/mL)	32.1±6.0	58.6±14.1	**0.048**
IL-17 (pg/mL)	2.9±0.9	12.6±8.6	0.136
IL-12 (pg/mL)	9.1±1.1	16.6±2.6	**0.007**





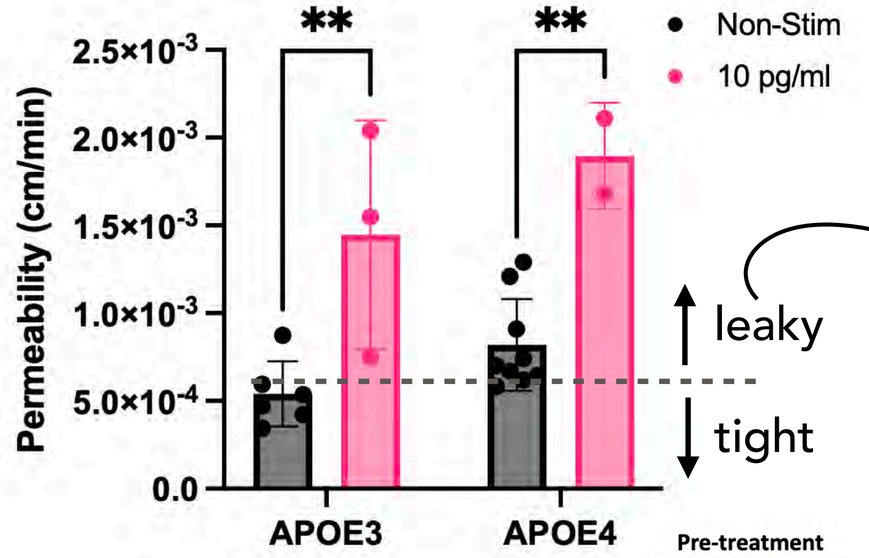
BUILDING THE VULNERABLE BRAIN … 1) BY ENGINEERING THE CELLS

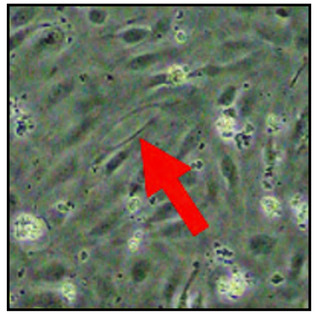


Alzheimers

BMECs only …

cytomix = 10 pg/mL
TNF-α, INF-γ, IL-1β



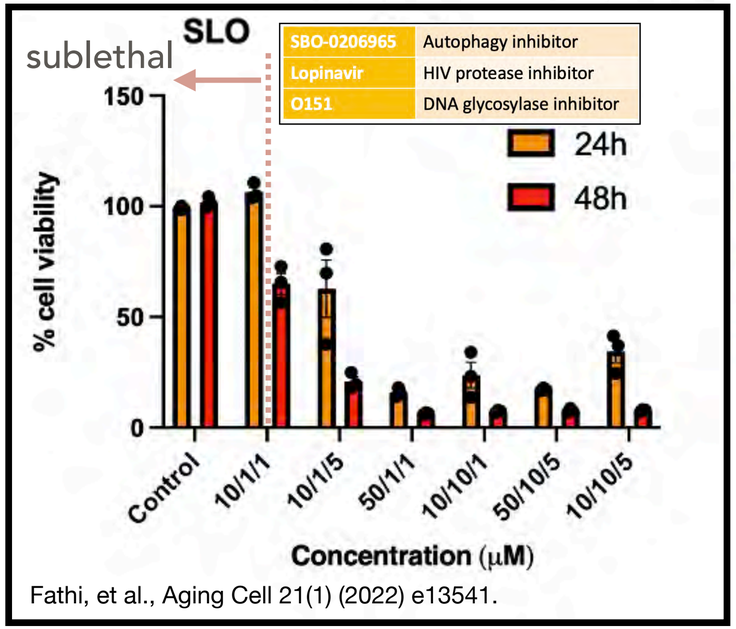


2) BY ENGINEERING THE ENVIRONMENT?

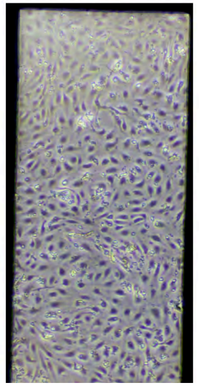
Basement membrane degradation, pericytes 'loss,' and a 'leaky' BBB are characteristics of the aging brain …

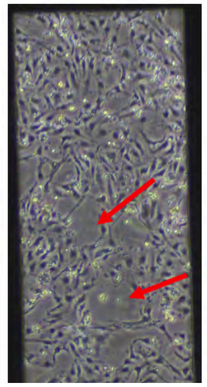
- Banks,. et al. Nat Aging 1, 243–254 (2021).
- Berthiaume, et al., Nat Commun 13(1) (2022) 5912.
- Farkas and Luiten, Prog Neurobiol 64(6) (2001) 575-611.
- Ceafalan, et al. J Cell Mol Med 23(2) (2019) 819-827.
- Nehra, et al., Fluids Barriers CNS 21(1) (2024) 29.

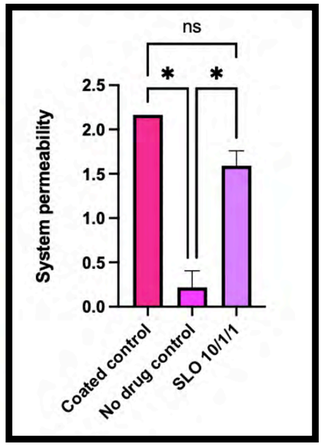
Aging

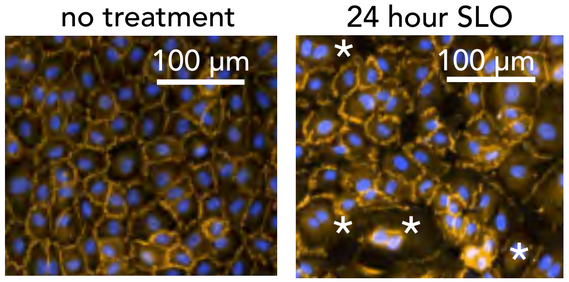


Fathi, et al., Aging Cell 21(1) (2022) e13541.







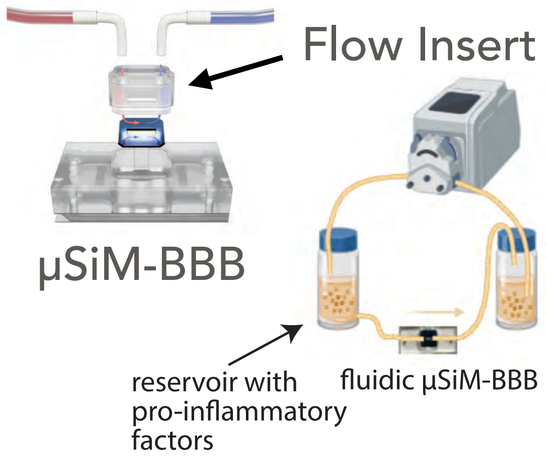


w/ Alexis Felder and Chris Proschel

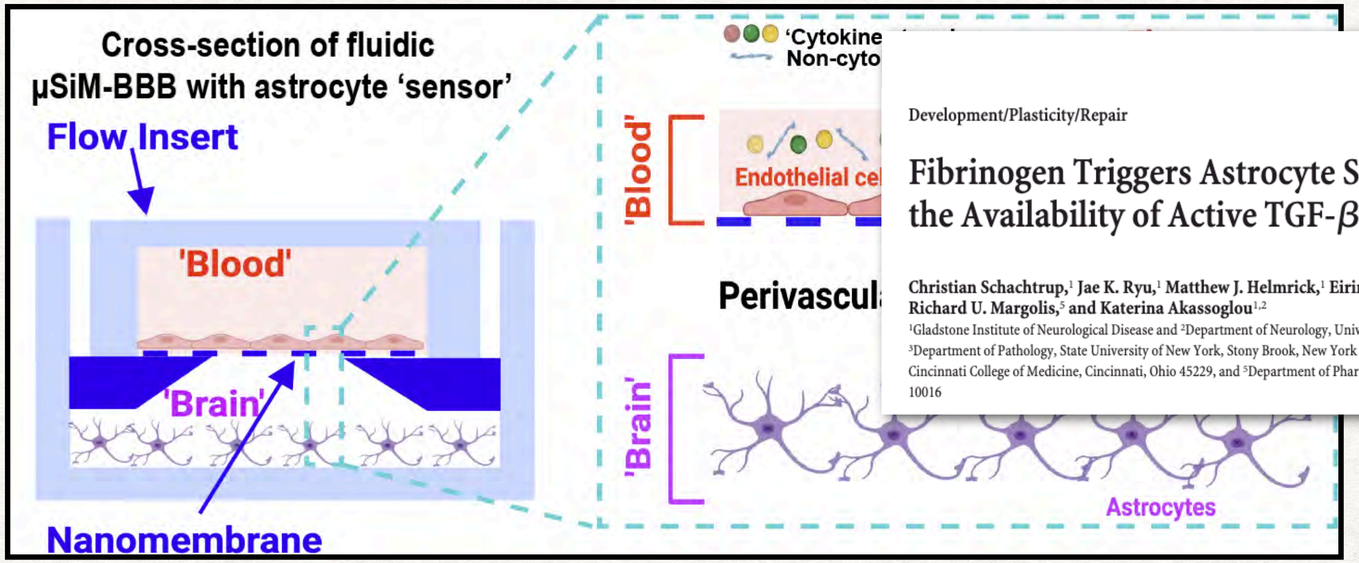
SHEAR CONDITIONING PROMOTES MICROVASCULAR ENDOTHELIAL BARRIER RESILIENCE IN A HUMAN BBB-ON-A-CHIP MODEL OF SYSTEMIC INFLAMMATION LEADING TO ASTROGLIOSIS

First Successful Transduction of Systemic Inflammation into Neuroinflammation in the μSiM-BBB

Kaihua (Chloe) Chen



- Shear conditioning of BMECs for 48 hours improves barrier function and dampens several inflammatory responses
- ± Introduction of pro-inflammatory factors after conditioning
- Measurement of barrier function and astroglyosis marker (GFAP)



The Journal of Neuroscience, April 28, 2010 • 30(17):5843–5854 • 5843

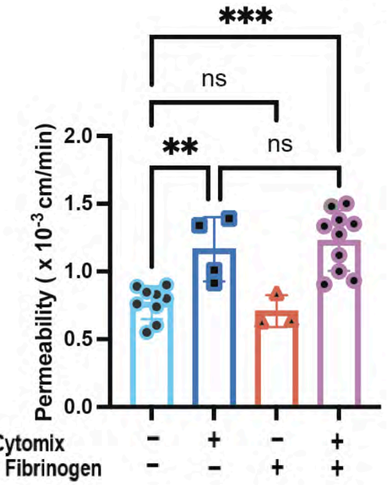
Development/Plasticity/Repair

Fibrinogen Triggers Astrocyte Scar Formation by Promoting the Availability of Active TGF-β after Vascular Damage

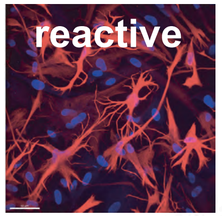
Christian Schachtrup,[1] Jae K. Ryu,[1] Matthew J. Helmrick,[1] Eirini Vagena,[1] Dennis K. Galanakis,[3] Jay L. Degen,[4] Richard U. Margolis,[5] and Katerina Akassoglou[1,2]

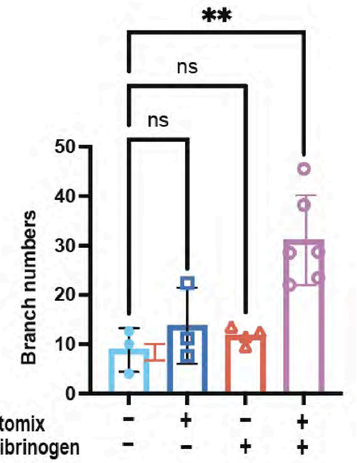
[1]Gladstone Institute of Neurological Disease and [2]Department of Neurology, University of California, San Francisco, San Francisco, California 94158, [3]Department of Pathology, State University of New York, Stony Brook, New York 11794, [4]Children's Hospital Research Foundation and the University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, and [5]Department of Pharmacology, New York University Medical Center, New York, New York 10016

Barrier function affected by cytokines, not fibrinogen



Astrocytes are activated by fibrinogen *after* BBB breakdown





Fibrinogen in Brain Injury and Neurodegeneration (Akassoglou Lab)

- Dean T, Mendiola AS, Yan Z, Meza-Acevedo R, Akassoglou K. Fibrin promotes oxidative stress and neuronal loss in traumatic brain injury via innate immune activation. *Acta Neuropathol*. 2024.
- Petersen MA, Ryu JK, Akassoglou K. Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. *Nat Rev Neurosci*. 2018;19(5):283-301.
- Schachtrup C, Ryu JK, Meyermann R, et al. Fibrinogen inhibits neurite outgrowth via β3 integrin-mediated transactivation of the EGF receptor. *Proc Natl Acad Sci USA*. 2007;104(26):11814-11819.
- Wood H. Fibrinogen links vascular pathology to cognitive decline. *Nat Rev Neurol*. 2019;15:187.
- Davalos D, Ryu JK, Merlini M, et al. Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration. *Nat Immunol*. 2018;19(12):1315-1323

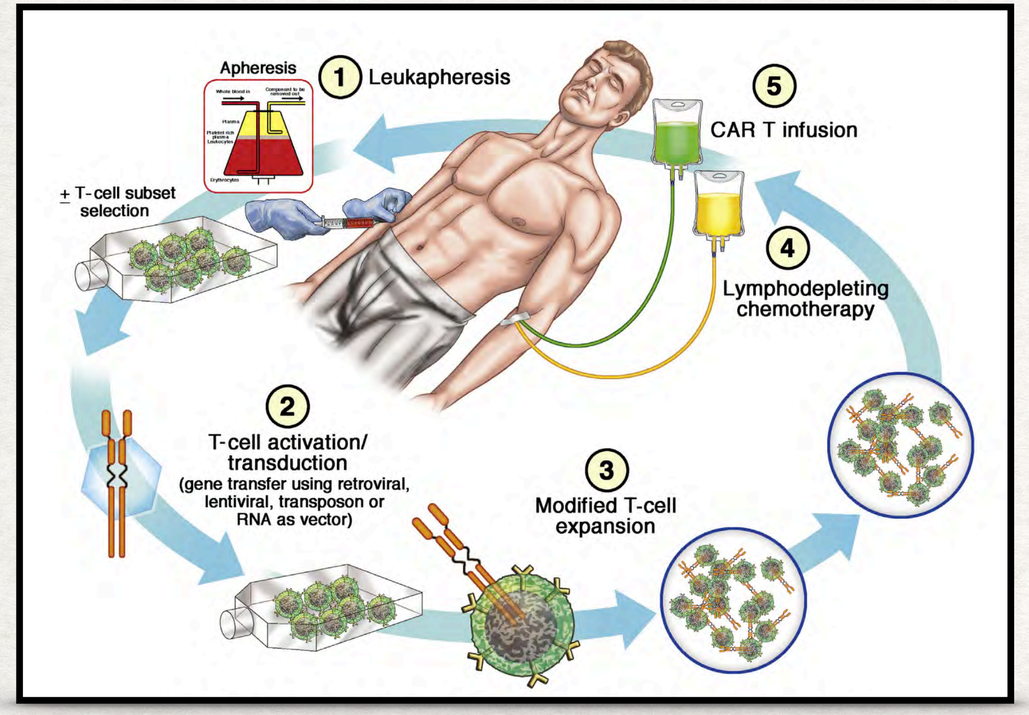
CAR T IMMUNOTHERAPY HAS MAJOR SIDE EFFECTS THAT WE CAN MODEL

Current perspectives

Cytokine release syndrome and neurotoxicity following CAR T-cell therapy for hematologic malignancies

Check for updates

Craig W. Freyer, PharmD, BCOP,[a] and David L. Porter, MD[b,c] *Philadelphia, Pa*

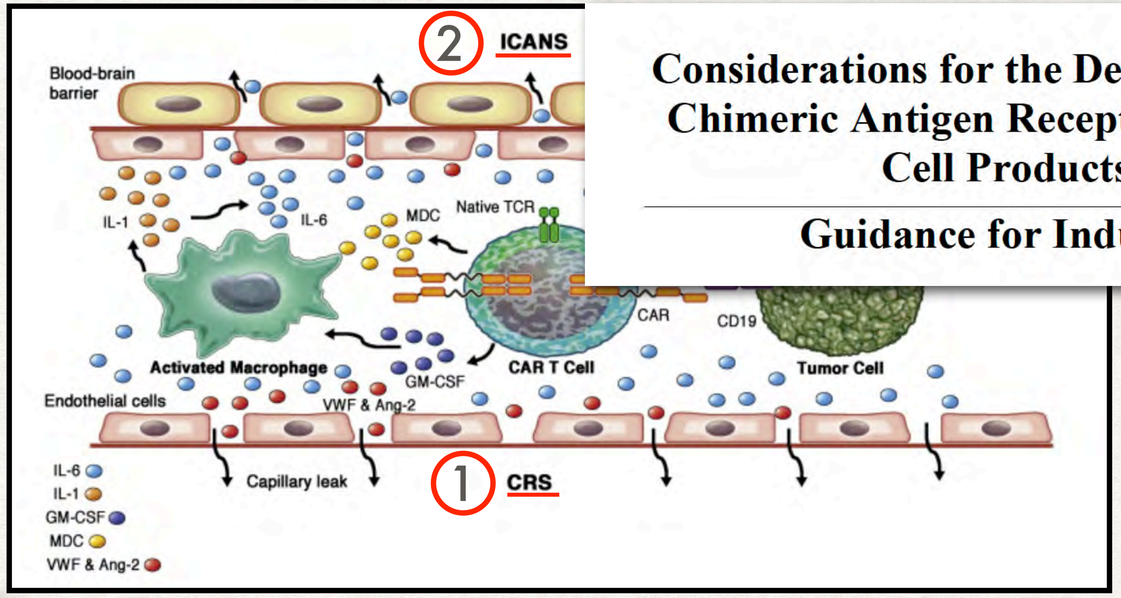


R/R = relapsed / refractory B-cell lymphomas

WARNING: CYTOKINE RELEASE SYNDROME, NEUROLOGIC TOXICITIES, AND SECONDARY HEMATOLOGICAL MALIGNANCIES

See full prescribing information for complete boxed warning.

- **Cytokine Release Syndrome (CRS), including fatal or life-threatening reactions, occurred in patients receiving BREYANZI. Do not administer BREYANZI to patients with active infection or inflammatory disorders. Treat severe or life-threatening CRS with tocilizumab with or without corticosteroids (2.2, 2.3, 5.1).**
- **Neurologic toxicities, including fatal or life-threatening reactions, occurred in patients receiving BREYANZI, including concurrently with CRS, after CRS resolution, or in the absence of CRS. Monitor for neurologic events after treatment with BREYANZI. Provide supportive care and/or corticosteroids as needed (2.2, 2.3, 5.2).**
- **T cell malignancies have occurred following treatment of hematologic malignancies with BCMA- and CD19-directed genetically modified autologous T cell immunotherapies, including BREYANZI (5.8).**
- **BREYANZI is available only through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS) called the BREYANZI REMS (5.3).**



Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products

Guidance for Industry

FDA

CRS = Cytokine Release Syndrome. More frequent but well-managed.
ICANS = Immune Effector Cell-Associated Neurotoxicity Syndrome. Downstream of CRS. More dangerous risk.

FDA U.S. FOOD & DRUG ADMINISTRATION

← Home / News & Events / FDA Newsroom / Press Announcements / FDA Announces Plan to Phase Out Animal Te

FDA NEWS RELEASE

FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

More Press Announcements **For Immediate Release:** April 10, 2025

Content current as

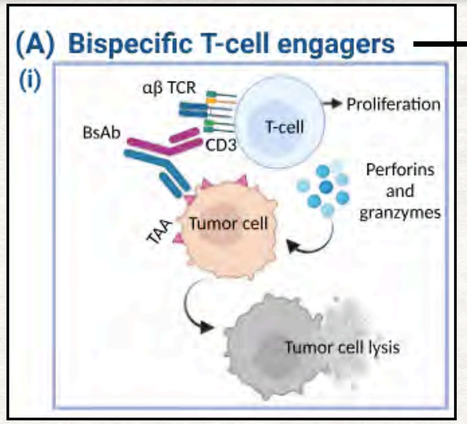
Nonclinical Evaluation of the Immunotoxic Potential of Pharmaceuticals
Guidance for Industry

Bispecific Antibody Development Programs
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration

...le, tandem ...bodies where each ...g domains are ...match the ...et al. 2015).

...uch as quality, ...rized and the ...noclonal antibody ...ld be studied, including antigen specificity; affinity and on- and off-rates; avidity (for bispecific antibodies that target two molecules on the same cell); potency; product-related impurities such as aggregates, fragments, homodimers, and other mispaired species; stability; and half-life. For example, in vitro and in vivo pharmacology studies may provide information on the relative binding activity and on- and off-rates for each target. Design of the potency assay(s) will depend on the target product attributes. Early in vitro studies may inform selection of an expression construct with optimal affinity and stability properties. The relative amounts of homodimers should be assessed. This evaluation is particularly important for effector cell engaging constructs where homodimers of the anti-CD3 or anti-Fc engaging arm may lead to cytokine release. Also, novel structures could potentially lead to increased immunogenicity.



→ BiTEs

Herrera, M. *et al.* Bispecific antibodies: advancing precision oncology. *Trends Cancer* **10**, 893-919 (2024). https://doi.org/10.1016/j.trecan.2024.07.002

Whole blood assay produces CRS →

Would facilitate CRS generation … Patient specific testing in clinical trials), multiplicity of donors for pre-clinical safety

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

Dissecting the Mechanisms Underlying the Cytokine Release Syndrome (CRS) Mediated by T-Cell Bispecific Antibodies

Gabrielle Leclercq-Cohen[1], Nathalie Steinhoff[1], Llucia Albertí Servera[2], Sina Nassiri[2], Sabrina Danilin[2], Emily Piccione[3], Emilio Yángüez[1], Tamara Hüsser[1], Sylvia Herter[1], Stephan Schmeing[1], Petra Gerber[1], Petra Schwalie[2], Johannes Sam[1], Stefanie Briner[1], Sylvia Jenni[1], Roberta Bianchi[1], Marlene Biehl[1], Floriana Cremasco[1], Katerina Apostolopoulou[1], Hélène Haegel[2], Christian Klein[1], Pablo Umaña[1], and Marina Bacac[1]

ABSTRACT

Purpose: Target-dependent TCB activity can result in the strong and systemic release of cytokines that may develop into cytokine release syndrome (CRS), highlighting the need to understand and prevent this complex clinical syndrome.

Experimental Design: We explored the cellular and molecular players involved in TCB-mediated cytokine release by single-cell RNA-sequencing of whole blood treated with CD20-TCB together with bulk RNA-sequencing of endothelial cells exposed to TCB-induced cytokine release. We used the *in vitro* whole blood assay and an *in vivo* DLBCL model in immunocompetent humanized mice to assess the effects of dexamethasone, anti-TNFα, anti-IL6R, anti-IL1R, and inflammasome inhibition, on TCB-mediated cytokine release and antitumor activity.

Results: Activated T cells release TNFα, IFNγ, IL2, IL8, and MIP-1β, which rapidly activate monocytes, neutrophils, DCs, and NKs along with surrounding T cells to amplify the cascade further, leading to TNFα, IL8, IL6, IL1β, MCP-1, MIP-1α, MIP-1β, and IP-10 release. Endothelial cells contribute to IL6 and IL1β release and at the same time release several chemokines (MCP-1, IP-10, MIP-1α, and MIP-1β). Dexamethasone and TNFα blockade efficiently reduced CD20-TCB–mediated cytokine release whereas IL6R blockade, inflammasome inhibition, and IL1R blockade induced a less pronounced effect. Dexamethasone, IL6R blockade, IL1R blockade, and the inflammasome inhibitor did not interfere with CD20-TCB activity, in contrast to TNFα blockade, which partially inhibited anti-tumor activity.

Conclusions: Our work sheds new light on the cellular and molecular players involved in cytokine release driven by TCBs and provides a rationale for the prevention of CRS in patients treated with TCBs.

See related commentary by Luri-Rey et al., p. 4320

BLINCYTO (blinatumomab) for injection 35 mcg single-dose vial

----------INDICATIONS AND USAGE----------

...pecific CD19-directed CD3 T-cell engager indicated for the treatment of adult and pediatric patients one month and older with:

- CD19-positive B-cell precursor acute lymphoblastic leukemia (ALL) in first or second complete remission with minimal residual disease (MRD) greater than or equal to 0.1%. (1.1)
- Relapsed or refractory CD19-positive B-cell precursor acute lymphoblastic leukemia (ALL). (1.2)

WARNING: CYTOKINE RELEASE SYNDROME and NEUROLOGICAL TOXICITIES including IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXICITY SYNDROME
See full prescribing information for complete boxed warning.

- **Cytokine Release Syndrome (CRS), which may be life-threatening or fatal, occurred in patients receiving BLINCYTO. Interrupt or discontinue BLINCYTO and treat with corticosteroids as recommended. (2.4, 5.1)**
- **Neurological toxicities, including immune effector cell-associated neurotoxicity syndrome (ICANS), which may be severe, life-threatening, or fatal, occurred in patients receiving BLINCYTO. Interrupt or discontinue BLINCYTO as recommended. (2.4, 5.2)**

epkinly epcoritamab-bysp SUBCUTANEOUS INJECTION 4mg 48mg

---------- INDICATIONS AND USAGE----------

...Y is a bispecific CD20-directed CD3 T-cell engager indicated for the ...t of adult patients with relapsed or refractory diffuse large B-cell ...lymphoma (DLBCL), not otherwise specified, including DLBCL arising from indolent lymphoma, and high-grade B-cell lymphoma after two or more lines of syst...

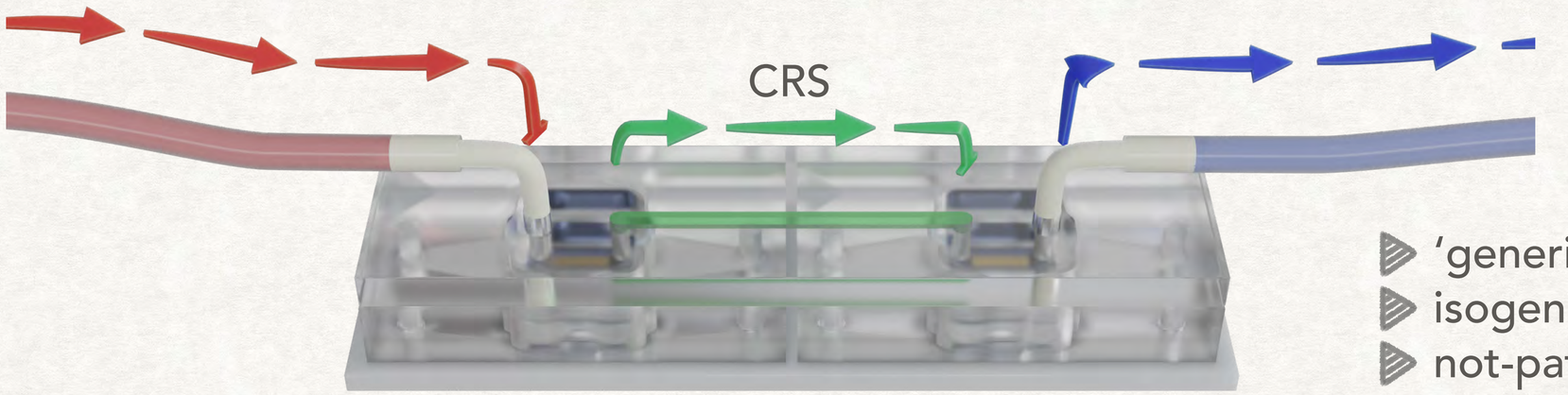
This in... and du... contin... confir...

WARNING: CYTOKINE RELEASE SYNDROME and IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXICITY SYNDROME
See full prescribing information for complete boxed warning.

Cytokine release syndrome (CRS), including serious or life-threatening reactions, can occur in patients receiving EPKINLY. Initiate treatment with the EPKINLY step-up dosing schedule to reduce the incidence and severity of CRS. Withhold EPKINLY until CRS resolves or permanently discontinue based on severity. (2.1, 2.2, 2.6, 5.1)

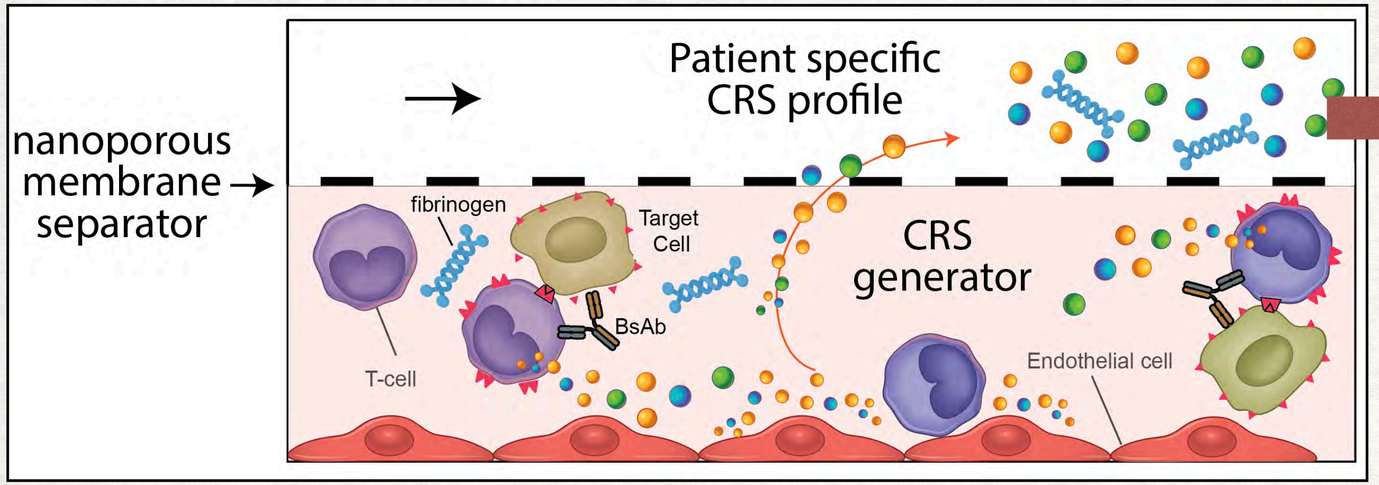
Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), including life-threatening and fatal reactions, can occur with EPKINLY. Monitor patients for neurological signs or symptoms of ICANS during treatment. Withhold EPKINLY until ICANS resolves or permanently discontinue based on severity. (2.1, 2.2, 2.6, 5.2)

μSIM-ICANS DRUG DEVELOPMENT TOOL

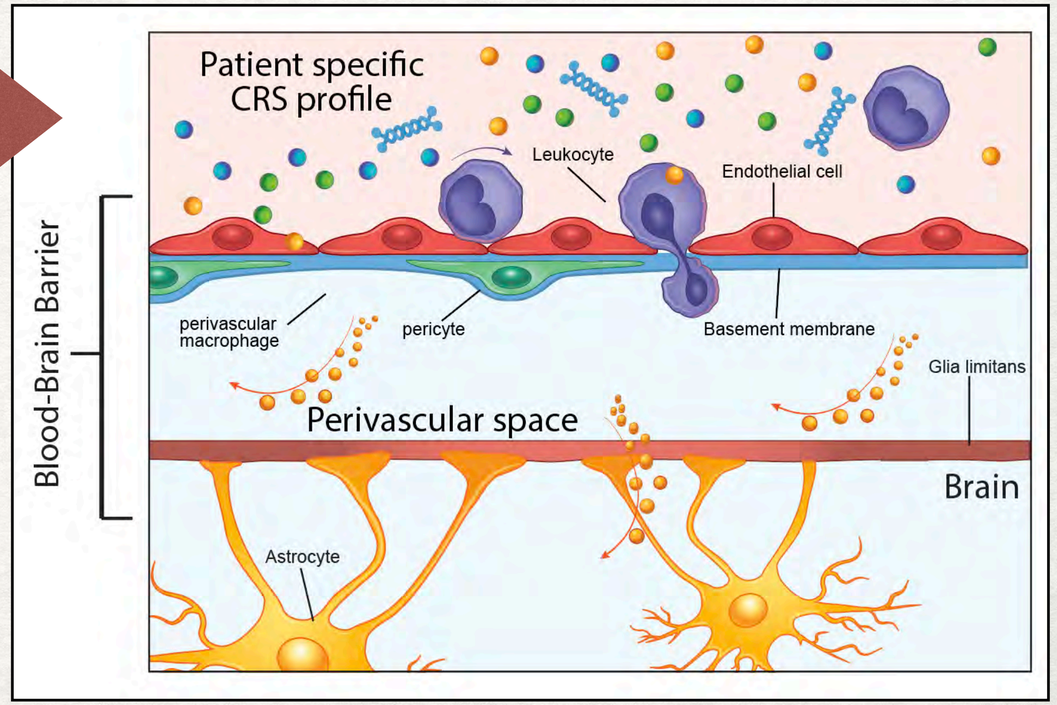


- 'generic' BBB
- isogenic cells from iPSCs
- not-patient specific

CRS Chip



ICANS Chip



Metrics

1. *Patient specific CRS plasma**
2. *BBB permeability***
3. *Fibrinogen leakage**
4. *Leukocyte infiltration ***
5. *Astrocyte activation (Morphology, GFAP, S100B)***

Methods: *integrated sensors; ** imaging

CONTEXT OF USE

PRECLINICAL SAFETY

The μSiM-CRS-ICANS will be used to evaluate the safety of monoclonal antibody (mAb) immunotherapies, including bispecific antibodies (bsAbs), targeting hematologic malignancies to enable risk assessment of CRA and ICANS, and to inform dose justification for Investigational New Drug (IND) applications prior to first-in-human trials.

TAKE HOMES

1. Why Non-Animal Models?

- **TGN1412 failure** is a stark case study: rodent and monkey immune systems lacked key human CD28 biology, leading to catastrophic first-in-human trial results. Even "humanized" animals cannot fully replicate human physiology.
- Animal data gaps contribute to high late-stage drug failure rates (over 90%), **costing $100B+ annually.**

2. Regulatory and Societal Drivers

- **FDA Modernization Act 2.0 (2022)**, and the upcoming 3.0 version, enable use of validated non-animal alternatives in drug development.
- The **FDA Roadmap** proposes an aggressive shift to non-animal models (**animals testing is rare by 2029**).
- Funding streams are shifting, with both regulatory bodies and animal-welfare organizations directing support toward non-animal model development**.**

3. The Landscape of Non-Animal Models (NAMS)

- **MPS = Organ-on-Chip + Tissue Chips; Organoids; Computational Models**
- Collectively, these form the "**Wheel of NAMS**"—a toolbox of complementary approaches**.**

4. A Strategic Imperative to Medical Research Institutions

- Non-animal models are not only ethically attractive but also **scientifically necessary** to overcome the translational gap in many cases.
- They align with **regulatory momentum** and are gaining traction even amid funding headwinds in biomedical research.
- Institutions like **TraCe-bMPS** position themselves at the intersection of engineering, biology, and regulatory science to push these tools toward qualification and adoption**.**

Thank you!

For all the work in Year 1

μSiM-CA

Michael Klaczko
Dan Ahmad
Lindsay Rathbun
Ahmet Gurcan
Ann Gill
Yojong Choi
Jeanne Holden-Wiltse
Anthony Corbet
Andrew Watts

Executive Committee

Ben Miller
Joan Adamo
George Truskey
Hani Awad

hVasc-SKM

Mingzhi Xu

Admin

Bonnie Lipari
Molly Van Wyk

Partners

SiMPore
ALine
Etaluma

UR Ventures

McKenna Jo Geiger

Graham Marsh
Nick King

For joining the team in Year 2

Jenny Chen (Duke)
Vinay Abhyankar (RIT)
Aityyah Hosseinifakhr (RIT)
Louis Widom (RIT)
Sami Farajollahi (RIT)
Dinindu De Silva (RIT)
Tom Gaborski (RIT)
Jon Flax, MD
Richard Waugh, PhD
Carla Casulo, MD
Lauren Covert, MD

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