

Microphysiological Systems to Advance Precision Medicine for Alzheimer's Disease (AD) and AD-Related Dementias (ADRD) Treatment and Prevention

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Acronym Definitions

AAV	adeno-associated virus
A β	amyloid beta or Abeta
ACTC	Alzheimer's Clinical Trial Consortium
AD	Alzheimer's disease
ADME-Tox	absorption, distribution, metabolism, excretion, and toxicity
ADRD	Alzheimer's disease-related dementias
ADSP	Alzheimer's Disease Sequencing Project
ALS	amyotrophic lateral sclerosis, or Lou Gehrig's disease
AMP [®] AD	Accelerating Medicines Partnership [®] Program for Alzheimer's Disease
BBB	blood-brain barrier
BMEC	brain microvascular endothelial cell
CIDP	chronic inflammatory demyelinating polyneuropathy
CNS	central nervous system
CNV	copy number variation
COPD	chronic obstructive pulmonary disease
CRO	contract research organization
DEG	differentially expressed gene
DN	Division of Neuroscience (National Institute on Aging)
EB	embryoid bodies
ECM	extracellular matrix
EMA	European Medicines Agency
EP	electrophysiological
FDA	U.S. Food and Drug Administration
FTD	frontotemporal dementia
GWAS	genome-wide association study
HEAL	Helping to End Addiction Long-term SM Initiative
iBBB	induced blood-brain barrier
IND	investigational new drug
INPP5D	inositol polyphosphate-5-phosphatase D
iPSC	induced pluripotent stem cell
ISSCR	International Society for Stem Cell Research
LOAD	late-onset Alzheimer's disease
LTP	long-term potentiation
MBP	myelin basic protein
MCI	mild cognitive impairment
MPS	microphysiological system
MRI	magnetic resonance imaging
NCATS	National Center for Advancing Translational Sciences
NDA	new drug application
NFAT	nuclear factor of activated T cells
NIA	National Institute on Aging
NIMH	National Institute of Mental Health

NMJ	neuromuscular junction
PBMC	peripheral blood mononuclear cell
PK/PD	pharmacokinetic/pharmacodynamic
PMD	Pelizaeus-Merzbacher disease
PP1	protein phosphatase 1
SNP	single nucleotide polymorphism
SORL1	sortilin-related receptor 1
SUVR	standardized uptake value ratio
TEER	transepithelial/transendothelial electrical resistance
TF	transcription factor
TREAT-AD	TaRget Enablement to Accelerate Therapy Development for Alzheimer's Disease

Executive Summary

Since the first generation of induced pluripotent stem cells (iPSCs) from human skin in 2007, microphysiological systems (MPS) have rapidly evolved as promising in vitro tools for precision medicine. The NIA Division of Neuroscience held a virtual workshop on July 19–20, 2022, to discuss the latest advances in the use of MPS in drug development for Alzheimer’s disease (AD) and Alzheimer’s disease-related dementias (ADRD).

Researchers met to review current research and development projects and exchange views on strategies to overcome challenges in identifying potential therapeutics for AD and ADRD. Three major themes emerged, related to MPS for disease modeling and therapeutic investigation:

1. Cell maturation and other ways to improve MPS’s physiological relevance to patient pathophysiology
2. Various differentiation protocols and methods for accelerating and promoting differentiation
3. MPS as a potential alternative for animal models

The first theme examined challenges in developing iPSCs and other MPS with physiological relevance to patient pathophysiology and ways to improve their pathological correlation with in vivo conditions. Inherent in iPSC development is cell immaturity, with cells that are significantly younger than AD and ADRD patients’ chronological ages. Participants discussed a variety of methods to include aging and other environmental factors in iPSC protocols to accelerate cellular age. Several presenters discussed recent advances in the development of technologies that enable more complex modeling of the CNS, such as organoids and human tissue chips.

In the second theme, participants discussed and compared various differentiation protocols and methods for accelerating and promoting differentiation. They considered the pros and cons of direct differentiation, transdifferentiation, and other strategies and techniques. They also reviewed a number of ways to evaluate and validate outcomes of the various protocols and techniques.

The third theme explored the use of MPS as a possible alternative for animal models in the future. There are translational gaps with regard to the genetic, pathological, and other differences between humans and animals, as well as ethical issues involved in animal models. Participants described some examples in which MPS could successfully replace animal models. A participant from the FDA outlined the agency’s commitment and approaches to replace, reduce, and refine animal testing (the 3Rs).

Welcome and Opening Remarks

Zane Martin, Program Director, Alzheimer's Disease and Related Dementias Translational Research, Division of Neuroscience

The National Institute on Aging hosted a virtual two-day workshop to bring together representatives from academia, biopharmaceutical companies, National Institutes of Health, and the U.S. Food and Drug Administration (FDA). They discussed advances and challenges in developing and using microphysiological systems—including organoids, organ-on-chips, and iPSC platforms—as translational tools for precision medicine research and drug development for AD and ADRD.

The workshop explored:

- Use of 2D and 3D in vitro systems for modeling disease complexity, target validation, and drug screening
- Use of MPS as tools for predictive drug development
- Overview of the National Center for Advancing Translational Sciences (NCATS) tissue chip program
- Discussion of the FDA's perspective on using MPS for therapeutic development

Introduction

Suzana Petanceska, Director, Office for Strategic Development and Partnerships, Division of Neuroscience

Since its inception in 2006, NIA's AD translational research program has worked to accelerate therapy development for AD and ADRD. A major goal is to provide a pipeline of funding opportunities through all the steps of drug development, from target identification and early validation to phase 3 clinical trials. Through these funding initiatives, the program has delivered investigational new drugs (INDs) for 13 first-in-class compounds currently in phase 1 or phase 2 clinical trials.

In addition to funding de novo drug discovery and development, the program supports drug repurposing and repositioning, particularly through PAR-20-156 Translational Bioinformatics Approaches to Advance Drug Repositioning and Combination Therapy Development for AD. Through this funding initiative, the team is integrating advanced computational methodology with experimental approaches, such as preclinical efficacy testing in animal models and early experimental trials in humans.

Given that AD and ADRD are complex, heterogeneous diseases, the team is creating an infrastructure to help accelerate the process from targets to trials. This effort involves an array of discovery programs, including the Accelerating Medicines Partnership® for Alzheimer's Disease (AMP AD) and the TaRget Enablement to Accelerate Therapy Development for AD (TREAT-AD) network. These programs are prioritizing and advancing novel targets into drug discovery.

The program also supports Model Organism Development & Evaluation for Late-Onset AD (MODEL-AD) centers, which develop novel, next-generation animal models for late-onset AD. These centers have developed more than 60 mouse models of late-onset Alzheimer's disease (LOAD) variants, prioritizing the most promising ones by aligning them with features of the human disease. The last group is the Alzheimer's Clinical Trials Consortium (ACTC), which provides infrastructure for clinical trial design and implementation.

NIA's translational research program is a work in progress, aiming to help the community overcome key barriers to successful preclinical-to-clinical translation. It supports ongoing, open, interconnected science programs, enabling a precision medicine approach to therapy development for AD. These programs share their data and tools through the AD Knowledge Portal and its associated web interfaces, Agora and Model AD Explorer.

Agora features the target nominations and associated data. The Model AD Explorer has visualizers that allow both bioinformaticians and biologists to engage with the rich data from the new mouse models.

This workshop explores how to leverage the emerging science and technologies around MPS to advance these as translational tools for precision medicine research and drug development for AD and ADRD.

Session 1: Using 2D and 3D In Vitro Systems for Modeling Disease Complexity, Target Validation, and Drug Screening

Session Chair: Tracy Young-Pearse, Harvard Stem Cell Institute

Advancements and Challenges to Modeling AD/ADRD With iPSCs: NIA iPSC Neurodegenerative Disease Initiative

Mark Cookson, Center for Alzheimer's and Related Dementias (CARD)

CARD has been developing a new approach for iPSC-based work, using genetic variants across disorders. Dr. Cookson's team began by addressing the limitations of patient-derived iPSCs. Only about 5 to 10 percent of ADRD are inherited. With single-gene disorders in single families, it is nearly impossible to access these variants because they are very rare. It is therefore difficult to formally compare intergenic relationships between mutations from different lines and different genetic backgrounds that were made by different labs using different methods.

The team took many ADRD genes involved in several conditions, including Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease, and frontotemporal dementia. They included single-gene disorders with dementia as a component and put an average of one to two variants per gene.

The team made their variant dominant, then reverted it to provide a control line to account for any changes in the genome that occur during editing. The process generates cells uniformly across all different mutations, and the team used a variety of tools to characterize the cells. The program will distribute these cell lines through the Jackson Laboratory.

An early challenge was choosing the line for engineering. The team worked with several well-known lines and assessed their ability as iPSCs to grow, be edited, and maintain genome integrity after editing. They also looked at whether these lines could make differentiated cells, because most will be used to make cells in the nervous system, including neurons, microglia, and astrocytes.

Once the team chose a line and began engineering it, they also started distributing it to many labs. They wanted to ensure that other labs could make the cells that they need using their own protocols. So far, the results have been promising: Other labs have made many neuron subtypes, microphages, astrocytes, microglia, and the like.

Throughout the engineering process, the team developed several refinements to typical Cas9 workflows to ensure an efficient process. At first, many knock-ins had very high homozygosity rates, which aren't wanted in this project. So the team worked with the Jackson team to recover more even rates of homozygous and heterozygous alleles.

They emphasized Alzheimer's alleles but also chose mutations in which dementia is part of the phenotype (Parkinson's disease, for example). Editing for about 150 lines is almost complete. The team, and some labs that have received lines, have had some early success in replicating lines and using them in studies.

A significant, ongoing challenge is that the normal human genome is not static—there are many variations. These lines have some variations, and additional variants will arise as people continue to edit the lines. The team may have to replace clones as needed.

In summary, selecting a parental line is complicated, and the perfect line probably does not exist. The team has mitigated the risk of variations by sequencing very deeply and by having multiple sex and ancestry backgrounds.

Use of iPSC Systems for Modeling Disease and Validation of Novel Targets

Valentina Fossati, The New York Stem Cell Foundation

Over the past decade, Dr. Fossati's lab has been using iPSC technology to advance disease modeling and identify and validate targets for therapeutic intervention. The team is working to address the challenges in the drug development process, which is long and costly:

- Many existing drugs don't work well in all patients and cause side effects.
- About 80 to 90 percent of drugs fail in clinical trials because they are unsafe and/or ineffective.

- Most drugs are developed through animal research, but animals and humans have critical differences.

With Alzheimer's disease treatments in particular, animal models have poor predictive power. iPSC technology provides an essential tool to complement animal research because it enables the generation of cells with the genetic makeup of many individuals.

The team is working to develop protocols for making different brain cell types, including neurons and glial cells, namely astrocytes, oligodendrocytes, and microglia. The interest in glial cells comes from clear evidence that these cells are implicated in AD pathology. Two-thirds of late-onset AD (LOAD)-related risk genes are expressed in microglia, the resident immune cells of the CNS.

Dr. Fossati's lab also has a long-term collaboration with researchers at Mount Sinai. Together, they are building a large omics data set to identify key drivers in AD pathology, many of which are in microglia and astrocytes. One ongoing project is studying TREM2 and TYROBP microglial genes, which have been strongly implicated in LOAD. A TREM2 mutation, R47H, increases the risk of LOAD, and a mouse model also shows strong evidence for a phenotype in the microglia population.

Using the edited lines and different clones from TREM2 and TYROBP, the team can generate microglia in a dish. Using these microglia, the team can determine whether there is a phenotype in the process of phagocytosis.

It is important to study microglia in a context with other cells, and the signals they send out. In current studies, researchers are integrating microglia into organoids, and the team has evidence from immunofluorescence and other analysis that microglia cells persist for more than six months inside organoids. These studies allow researchers to better understand disease modeling and how these key drivers then develop a phenotype in a dish.

Once researchers have identified potential targets for therapeutic intervention, testing them using a human platform is possible. These advanced studies using human cells in the preclinical phase (a "clinical trial in a dish") would enable researchers to validate novel targets and predict responses. The advantage of iPSC testing is that it provides a diverse population scale study, enabling better-designed clinical trials with the highest success rate.

To get to that point, researchers need to:

- Generate highly enriched cultures in a short time. Labs worldwide are developing these protocols with the transcription factors (TFs) essential for driving a specific lineage commitment.
- Automate the protocols for cell differentiation. Automated testing is important for scaling up and customizing the workflows, making them adaptable to multiple projects.

- Incorporate high-content imaging and artificial intelligence to integrate the data for the cell samples. As iPSC lines scale up, there is an ongoing need for analysis to create biobanks for community use.

Developing Stem Cell Resources for Modeling Person-Specific Molecular and Pathological AD/ADRD Phenotypes

Tracy Young-Pearse, Harvard Stem Cell Institute

Dr. Young-Pearse's lab is working to develop stem cell resources for modeling person-specific molecular and pathological AD and ADRD phenotypes, in collaboration with The New York Stem Cell Foundation (NYSCF) and Rush University. To date, the team has generated more than 50 iPSC lines from the blood of participants, all of whom have been deeply phenotyped.

Many studies of AD using human tissue or cellular model systems compare AD to “not AD.” But in actuality, AD does not have a singular phenotype, with heterogeneity occurring in several factors among individuals who develop the condition. These factors include the age of onset, pathological burden, rate of cognitive decline, and genetic risk and resilience. These factors—with similar heterogeneity—also occur in people without cognitive impairment.

For example, at death, many people who avoided significant cognitive decline have brains that are free of A β plaques. But other people with intact cognitive abilities die with high amounts of amyloid plaques and tau tangles in their brains—features that are indistinguishable from someone with AD. This comparison shows the importance of moving beyond the binary categories of AD and “not AD.”

To fully understand AD pathogenesis, researchers need to capture the heterogeneous genetic and molecular processes underlying risk and resilience. Understanding these processes requires the use of human model systems that capture all genetic risk factors, both known and yet to be identified.

Dr. Young-Pearse and her team believe that diverse combinations of genetic risk factors converge on interrelated but varied biological domains, ultimately impacting risk and resilience. This convergence could lead to differences in cognitive and pathological traits in the population.

The team's work draws on the Religious Order Study (ROS) and Memory and Aging Project (MAP) cohorts, initiated by Dr. David Bennett in the 1990s. The participants enter these two cohorts free from dementia, then undergo annual clinical evaluations that include a battery of cognitive tests. They consent to the Anatomical Gift Act and donate their brain and other tissues at death for analysis.

Data from the ROS/MAP cohorts include:

Longitudinal clinical data, including:

- Annual cognitive function test results
- Clinical and pathological diagnoses of AD and other neurological diseases

Postmortem cell and molecular phenotyping, including:

- Quantitative neuropathology for amyloid plaques, tau tangles, and other neuropathologies in the dorsolateral prefrontal cortex
- Single-cell and bulk RNA sequencing profiles
- Mass spectrometry profiles and proteomic, lipodomic, and metabolomic profiling from peripheral tissues

Genotyping, including:

- Genome sequencing
- APOE status

The first 50 iPSC lines from ROS/MAP participants show varied scores and other data—not only among those who have a clinical and pathological diagnosis of AD, but also among those who are noncognitively impaired. Already, Dr. Young-Pearse and her team are capturing heterogeneity in aging, including variations in genetic risk and resilience factors.

With iPSC-derived neurons, microglia, and astrocytes, the team is using a variety of assays to assess if and how genetic risk impacts biological domains. There are assays to quantify different forms of A β and tau, imaging-based assays to show details within cells, and functional assays to capture activity. The team also incorporates multi-omic approaches to understand pathways within cells that may be affected by various genetic variants.

The team is also using iPSC model systems in several ways to measure steps in the disease process. For example, monocultures of neurons can capture processes directly related to the genetic variants of interest. More advanced cultures, such as co-cultures and organoids, examine indirect effects on secondary biological domains and system-level dysfunction. (The study of behavioral changes tied to cognitive deficits would require animal models, of course).

Dr. Young-Pearse discussed several ways her team has used the ROS/MAP iPSC collection to identify molecular drivers of AD in a person-specific manner. The results point to potential therapeutic interventions tailored specifically to individuals who have defects in these biological domains:

- Capturing AD polygenic risk:
 - Protein phosphatase 1 (PP1): Neurons in a dish—brain tissue from each of the same people from whom the iPSC lines were derived—reflect the features of protein networks, neuropathology, and cognitive outcomes in the donors. Potential therapeutic intervention: α -A β immunotherapy.
 - Proteostasis: Dysregulated proteasome components and autophagy regulators are associated with high polygenic risk for LOAD in both ROS/MAP brain tissue and iPSC models. Potential therapeutic intervention: Proteasome activator.

- Assessing contributions of specific AD risk genes:
 - Inositol polyphosphate-5-phosphatase D (INPP5D): Chronic reduction in INPP5D levels in microglia models results in an inflammasome-mediated elevation of IL-1 β and IL-18. Potential therapeutic intervention: NLRP3 inhibitor.
 - Sortilin-related receptor 1 (SORL1): SORL1 mutations induce defective retromer trafficking in neuron models, resulting in elevated A β and phosphorylated tau (pTau). Potential therapeutic intervention: Retromer stabilizer.

The ROS/MAP iPSC cohort provides a platform for testing person-specific responsiveness to various interventions. Dr. Young-Pearse explained how to request ROS/MAP iPSC lines through the Rush Alzheimer's Disease Center (RADC) website (<https://www.radc.rush.edu/>).

Dr. Young-Pearse noted that the existing ROS/MAP cohort is limited in the diversity of existing samples and tools for studying LOAD risk. In response, her team is generating a diversity iPSC cohort of 57 lines from underrepresented ROS/MAP individuals, such as African Americans and Hispanic Americans. The lines will be ready in winter 2023, along with the multilevel omics analysis from the brain tissue of the same individuals.

To conclude, Dr. Young-Pearse summarized the limitations and bottlenecks for using iPSC experimental systems to advance precision medicine:

- Consistent differentiation across lines is key.
- Defining subtypes of AD based on functional assays in iPSCs is time consuming and expensive. A key to bringing this technology to patient care will be identifying the specific genetic variants and combinations of variants driving these subtypes.
- It is not known how many subtypes will be relevant to therapy development.
- The power to identify subtypes functionally, using the current number of lines, may be limited. Additional lines representing a greater number of people may be needed, though collaboration with large consortia projects may enable researchers to look at many lines at once.
- Validating the disease relevance of findings in a dish is challenging and may ultimately lie in engaging the target in clinical trials.

2D and 3D Systems for Modeling Neurodegenerative Disorders

Steven Finkbeiner, University of California, San Francisco

For Dr. Finkbeiner and his lab, their approach to modeling has been greatly shaped by their conception of neurodegenerative diseases. AD and ADRDs are significantly sporadic, idiopathic diseases, meaning multiple genetic mutations drive these diseases.

Nevertheless, the heritability of the diseases is quite high. The Alzheimer's Disease Sequencing Project (ADSP) estimates that heritability for AD may be up to 80 percent. Dr. Finkbeiner and his team believe that means polygenic risk is scattered throughout the genome, with multiple

variants each contributing minor effects. Those effects may be difficult to study by themselves but may converge on pathways that ultimately lead to neurodegeneration. Through cell-based modeling, researchers can get an integrated effect of the genome on these biological pathways—at the cell level.

Based on genome-wide association studies (GWAS), most genetic risk loci are in noncoding regions, which is important because those regions have evolved more rapidly for AD than other parts of the genome. In addition, conservation is relatively poor in nonhuman models. Modeling these diseases in human-derived samples, such as iPSCs, allows researchers to use patient-derived cells to make almost every type of brain cell.

For those trying to model disease, another important consideration is which phenotype to assay. The question is whether AD is one disease or many diseases—and if it is many, then how many? The answer greatly affects options for phenotypic assays. For example, the biological pathways on which the genome variants impinge may be very heterogeneous, so tailored phenotypic assays may be the best approach. However, as phenotypes funnel down into changes that culminate in neurodegeneration, it may be easier to see differences between patients and controls in general assays.

With a repository of more than 2,000 lines, Dr. Finkbeiner's lab is studying several neurodegenerative diseases, particularly ADRDs. Dr. Finkbeiner discussed the available lines, issues around variability, and strategies to manage them.

Although obtaining iPSC lines used to be difficult, today, many lines are available from patients with ADRDs from public repositories. Many maintain high levels of quality control and serve as a dependable resource. Some recommended sources for iPSC lines include the:

- Rutgers/Coriell NINDS Human Genetics DNA and Cell Line Repository
- European Bank for induced pluripotent Stem Cells (EBiSC)
- National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD)
- NYSCF Stem Cell Repository
- Cedars-Sinai Regenerative Medicine Institute

The method of reprogramming and the extent of pluripotency matter. The footprint-free methods available now can avoid many problems of older methods, such as integrated factors causing persistent reprogramming factor expression that affected differentiation.

CRISPR gene correction has also been a major step forward, particularly for Mendelian mutations, but the team strongly believes it is important to also study multiple lines from different patients. Dr. Finkbeiner's team has seen significant effects of genetic backgrounds on the phenotypes they measure, which is not helpful when studying sporadic disease.

His team's current approach is to do a primary screen in well-behaved, well-characterized iPSC lines with a robust phenotype to maximize sensitivity. They follow that with a secondary screen

in iPSC lines representing different genetic, ethnic, and clinical backgrounds and using allelic series and gene-corrected controls when possible.

Dr. Finkbeiner described techniques he and his team use to manage the inherent variability in iPSC work, including:

- Careful attention to quality control, with periodic karyotyping and cGH array analysis
- A process to ensure that cells are truly fully reprogrammed because incomplete reprogramming can cause significant variability
- Automated liquid handling, rather than hand pipetting, to ensure that multiple, small amounts of factors in differentiation protocols are added precisely

Strategies to control batch-to-batch variation in differentiation include:

- **Production of all cells at one time for large-scale screens:** The team does the differentiations to the farthest extent possible, then freezes down aliquots that can be thawed and regrown in a week for experimental use.
- **Robotic microscopy:** They use automated equipment, including a microscope, incubator, and robotic arm, to track individual cells over time. By programming the microscope to register the plate's position, the arm precisely transfers the plate between the incubator and microscope each time for imaging.
- **Biosensors as a “physical exam” of cells:** His team uses more than 300 biosensors to visualize endophenotypes and relate them to neurodegeneration. They developed a fully automated analysis computational pipeline that involves both conventional image assay tools and newer AI tools. These tools are far more accurate (nearly 100 percent) than the human eye (about 70 percent) in classifying cells that are either healthy or destined to die.

Regarding monocultures and 2D and 3D models, the development of protocols to make many disease-relevant cell types has significantly advanced. Improvements have focused on making cell types that were not previously possible and implementing a faster, more efficient process. Lorenz Studer's group recently published a paper on bioRxiv with new ways to accelerate culture maturation.

Researchers can study single cells as monocultures or combine them in co-cultures, enabling study of biology that would otherwise be difficult in single cells. Dr. Finkbeiner showed a graph in which his team had measured cytokines to examine neuroinflammation in a model of progranulin-deficient frontotemporal dementia. When grown alone, patient-derived forebrain neurons or microglia show relatively low levels of cytokines. As co-cultures, they show a dramatic increase in TNF alpha and several other cytokine levels and a dramatic change in neurodegeneration phenotypes.

His team has developed a protocol to turn a single iPSC line into both skeletal muscle and motor neurons to form a functional neuromuscular junction. It is possible to use this protocol with iPSC lines and see degeneration of that critical structure early in the process.

Dr. Finkbeiner and his team have developed models of other diseases, including Huntington's disease, amyotrophic lateral sclerosis (ALS), and Parkinson's disease, to study several disease-relevant phenotypes. With the models, they can study disease features, including:

- Calcium signaling
- Electrophysiology changes
- Gene profiling changes
- Glial phenotypes
- Neurite length
- Response to stress
- Spontaneous neurodegeneration
- Survival

In addition to cell-autonomous genetic effects, the models enable study of the interactions between environmental factors and genetic susceptibility. The team used an ALS line derived from a patient with a mutation, with an RNA binding protein to visualize stress granule formation. At baseline, without a stressor, the risk of death modestly increases in the patient line compared with the control line. Adding a stressor, such as puromycin, induces stress granule formation, creating a huge jump in the risk of death in the patient line. The experiment showed a potential gene-by-environment interaction that could be targeted for therapy.

Dr. Finkbeiner addressed a question in the Zoom chat about how many iPSC lines are needed to generate robust conclusions. Noting that most people do not do formal power analysis, Dr. Finkbeiner said that his team recently posted a paper on bioRxiv with new power analysis tools. Researchers can use the tools to calculate how many lines they need for properly powered and controlled experiments.

He briefly addressed another question in the Zoom chat about epigenetic markers. A key environmental factor for AD is aging, which has been one of the main limitations of iPSC work: The cells are immature. Dr. Finkbeiner's team has adopted a method developed by Andrew Yoo to try to better retain epigenetic markers of aging.

Instead of reprogramming cells back to pluripotency, methods have been developed to transdifferentiate adult primary cells, such as fibroblasts, into neurons or even glial cells while retaining the epigenetic markers.

The team has found that transdifferentiation can dramatically increase the magnitude of certain disease-associated phenotypes, especially those associated with protein dyshomeostasis and protein aggregation. Some colleagues have found greater signs of maturity in the transdifferentiated cells, such as more 4R or adult tau, than in iPSC cells, which produce 3R or juvenile tau.

Dr. Finkbeiner's team, in collaboration with Dr. Alex Pollen and his lab, is developing 3D organoid models of AD based on trisomy 21 cell lines. With this method, the team generated forebrain excitatory and inhibitory neurons, which both showed a significant increase in neurodegeneration. Dr. Finkbeiner noted that these organoids showed a dramatic increase in adult isoforms of tau. Both 1N and 2N4R tau were expressed strongly in these systems, which is encouraging.

A team goal in developing the organoid models was to use them as a platform for transplantation, including adding microglial cells and a way to deliver genetic perturbations using viral methods.

Dr. Finkbeiner touched on some limitations, challenges, and obstacles in developing 2D and 3D models for neurodegenerative diseases. A key question for labs: How complex does the model need to be? The answer relates to the goals that drive a research team. Will the increased complexity add relevance or just variability to a team's work?

A common disappointment with many models, including mouse models, has been the significant failure in their ability to predict what will and will not work in patients during clinical trials. Researchers must consider whether their models should be based primarily on recapitulating pathology seen in patients or on quantifying their predictive value.

In collaboration with Dr. Cookson and others in FOUNDIN-PD, Dr. Finkbeiner's team is working on functional genomics around Parkinson's disease. The team needed to know whether the cells they were making express the genes that have been linked to Parkinson's disease GWAS loci.

The team published a paper on bioRxiv about single-cell RNA sequencing on some iPSC-derived lines from Parkinson's Progression Markers Initiative (PPMI). The dopaminergic neurons or precursors they saw demonstrated that these do express the genes the team wanted to study. In this case, the model provided enough complexity to answer the question the team was trying to solve.

Dr. Finkbeiner's team studied the survival phenotypes of cells derived from PPMI, considering what they could measure in a dish that would correlate with what they measure in patients. The polygenic risk score of those patients correlated with the level of neurodegeneration the team saw in the patient-derived neurons. The highest risk was associated with some of the highest rates of neurodegeneration.

Dr. Finkbeiner finds advantages to using iPSC models: They scale well, are great for screening, and are amenable to genetic CRISPR engineering. A limitation is that epigenetic markers are lost. Transdifferentiation may be a helpful, complementary approach for retaining the effects of aging, if that is a key aspect the team is addressing.

To conclude, Dr. Finkbeiner noted that he and his team have demonstrated that patient-derived models can exhibit spontaneous phenotypes reminiscent of the disease. These can be large and robust enough to work for mechanistic studies and screening.

When deciding how complex a model system should be, researchers should carefully consider the questions and the biology they are trying to study. Certain 2D monocultures are suitable for studying cell-autonomous mechanisms—with the advantage of being less resource intensive. Monocultures can often be simpler, less heterogeneous, and more sensitive than co-cultures and organoids.

On the other hand, some biologies only emerge when two or more different cell types are together, where greater complexity is needed in testing.

Logical Network-Based Drug-Screening Platform for AD/ADRD Representing Pathological Features of Human Brain Organoids

Inhee Mook-Jung, Seoul National University

Dr. Mook-Jung discussed her team's work in developing network-based model systems using organoids to enable AD drug screening. Major pathological hallmarks of AD show extracellular deposits of beta-amyloid and neurofibrillary tangles. However, most AD cases are sporadic rather than familial, and no mass model can accurately mimic AD pathology and symptoms.

To overcome this lack of sporadic AD model systems, her team used brain organoids derived from patients who had sporadic AD to develop AD drug screening. The team has clinical and brain imaging data, including amyloid PET and MRI, for more than 1,000 patients. They also have some or whole genome sequencing data for select patients.

Using these patient data, they performed imaging genetics to choose patients for iPSC generation based on amyloid positivity, APOE genotype, age, sex, MMSE score, and other factors. They made 11 iPSCs from different patients by performing CRISPR Cas9-based editing for APOE3 to APOE4. They also maintained 1,300 brain organoids from the 11 iPSCs.

Published last year at Nature Communications, the study's results included:

1. Establishment of iPSC-derived brain organoids and their characterizations
2. AD signaling network modeling using systems biology
3. Target drug validation using brain organoids for network modeling

Dr. Mook-Jung discussed the first part: Establishing iPSCs from AD patients' blood and quality control methods. For iPSC quality control, the team used:

- Imaging-based confirmation using stem cell markers such as Oct4 and Tra1-60
- Alkaline phosphatase (ALP) staining
- Morphological quality control
- Karyotyping analysis

- MACS sorting work

Before organoid generation, Dr. Mook-Jung and her team base-edited some iPSCs using a CRISPR Cas9 system. As a result, E4 isogenic lines were made from E3 parental iPSCs. In this case, every genetic factor was identical, except for the APOE allele type. To generate iPSC-derived organoids, the team incubated 60-day continuous cultures with various cortical organoid-inducing factors, such as BDNF and NT3.

In addition, the team performed quality control of brain organoids for high-content screening (HCS). On day seven, they performed high-quality embryonic body (EB) selection again, based on organoid size, and they repeated quality control.

After manually selecting high-quality EB, the team performed automatic selection based on shape and size using HCS imaging program from molecular devices. They used custom models, including circle filter shape, and set the adaptive threshold to give minimum and maximum widths.

Also, they defined outliers with this exclusion criteria:

- Shape factor score
- Diameter length
- Average area

As a result, the team prepared high-quality, homogeneous organoids for high-content drug screening.

Dr. Mook-Jung's team compounded the brain organoid phenotypes using high-quality, homogeneous organoids. Because these organoids derive from sporadic AD patients, it is reasonable to examine the A β 40, A β 42, total tau, and pTau levels from the culture media, whether or not these organoids showed AD pathology, such as phenotype. The team selected three groups: PIB-negative vs. PIB-positive, APOE4 carrier vs. APOE4 noncarrier, and APOE3 parent line vs. APOE4 isogenic line.

The PIB positive, APOE4 carrier, and APOE4 isogenic lines showed significantly higher A β 42, A β 40, total tau, and pTau levels compared with PIB negative. The APOE4 noncarrier and APOE3 parent lines were static.

The biochemical data evaluation, compared with AD patients' brain A β deposits from the standardized uptake value ratio (SUVR), showed convincing results. The data showed significant correlation between the real human cerebral amyloid deposition and secreted hallmark protein, including:

- A β 40
- A β 42
- Ratio of A β 42 to A β 40

- Total tau
- pTau
- Ratio of pTau to total tau

These data strongly support the idea that the team's patient-derived brain organoids are a valuable model to mimic AD. And these biochemical hallmarks are reasonable to use in readouts for AD drug screening.

Dr. Mook-Jung's team also explored whether brain organoids have functional neural connections. To examine the physiological response of brain organoids, they treated fluorescence-labeled calcium dye for calcium oscillation analysis with PIB negative vs. PIB positive and APOE3 parent line vs. APOE4 isogenic groups. The PIB positive and APOE4 isogenic line groups showed higher calcium peak numbers than the PIB negative and APOE3 parent line groups, respectively. These data show that the team's brain organoids have functional neural connectivity.

The team performed transcriptomic gene ontology (GO) analysis with the two comparison brain organoid groups: the PIB negative vs. PIB positive group and the APOE3 parent line vs. APOE4 isogenic line. They incorporated the biological process and molecular function into the RNA sequencing analysis. All three categories showed down-regulated genes, which are related to synaptic function in the PIB positive and APOE4 isogenic organoid groups, compared to the PIB negative and APOE3 parent line groups.

For pathological validation of brain organoids using HCS, the team applied a 3D-clearing system using ethyl cinnamate to enable HCS imaging. After tissue clearing, brain organoids exhibited transparency and more invisibility to the naked eye.

They took the level of A β and pTau using cleared brain organoids and found significant increases in the PIB positive and APOE4 isogenic lines. From these results, Dr. Mook-Jung and her team concluded that their brain organoids could undergo effective tissue clearing and HCS imaging, and thus they could be applicable to the largest scale drug screening platform.

Dr. Mook-Jung discussed the second part: AD signaling network modeling using systems biology. The dynamics of molecular interactions in brain organoids with pathological features of AD are complicated. The team needed to construct a molecular regulatory network model using a system biology approach.

The network structure was constructed based on major signaling pathways related to AD by integrating information from public databases with information from an extensive survey of relevant experimental data on neuron cells. The network model consists of 77 nodes and 204 links. It includes five output nodes that can represent the pathological phenotypes of AD, such as A β , pTau, synaptic loss, apoptosis, and autophagy. And this network model assumes a normal aging state with no input applied.

The team performed simulations with different levels of oxidative stress mimicking the aging effect. The simulations helped validate whether the constructed network model properly represents the dynamics of AD pathological phenomena. The team performed more than 1,000 independent simulations for each oxidative stress condition from 0 to 100 percent for the network models.

They also compared the experimental database knowledge with their simulation results to validate APOE for less specific alterations relative to normal aging. For example, in an A β case, oxidative stress increased from 0 to 100 percent. A β level dramatically increases in the APOE4 allele case compared to the normal case.

Dr. Mook-Jung discussed the third part: The last step was to complete the construction and validation of a molecular regulatory network model of AD. Dr. Mook-Jung's team performed selection perturbation analysis to identify optimal candidate targets for reducing the abundance of A β and tau. Network dynamics were induced by node perturbation or analyzed by an attractor landscape. Each node perturbation eventually reached the attractor state corresponding to a specific cellular phenotype.

The team converted the activity of the output node to phenotype score, representing the degree of proximity to the desired state based on the assigned rate. For example, A β is 30 percent, pTau is 30 percent, synaptic loss 20 percent, and apoptosis and autophagy are 10 percent each. The team selected drug targets according to the priority of importance for reducing the abundant levels of A β and pTau and the degree of neurodegeneration.

The team used perturbation analysis for internal validation using public databases. The models, such as APOE4 allele, are represented by a differently wired network. The network has a distinct quality, which changes its risk factor in our disease model into the normal state model. Overall, the cellular state for a specific node perturbation, such as APOE4 allele, is defined as a phenotype score, including A β , pTau, synaptic loss, apoptosis, and autophagy.

From the simulation results, Dr. Mook-Jung and her team selected targets with high phenotype scores that can readily be repositioned for approval by the FDA. The team further analyzed the alteration of signaling pathways by perturbation of a single target or double target combination. For instance, for APOE4 allele, flibanserin treatment, a PTEN inhibitor, upregulates the canonical WNT pathway. Treatment with ripasudil, a Dkk1 inhibitor, downregulates the canonical WNT pathway. Introducing these pathways subsequently decreases the production of A β and pTau.

In their candidate drug selection steps, the team validated the network-based drug screening platform using brain organoids and HCS. The team:

1. Selected the output node priority.
2. Chose the target drug based on perturbation analysis and referenced to a library of FDA-approved drugs.

3. Excluded unsuitable candidates based on their drug property such as BBB permeability, carcinogen status, and other criteria.
4. Selected six FDA-approved single or combination drugs for APOE4 isogenic lines.

Dr. Mook-Jung's team obtained large-scale drug screening results from their network-based drug screening platform using APOE4 isogenic line and PIB positive brain organoids. They found that all the candidate drugs were effective to some degree in reducing A β and tau deposition with single and/or double treatment.

In summary, the team found that these AD patient-derived organoid models introduced a reliable strategy to enable precision medicine. The models do so by engaging the convergence of mathematical modeling and pathological features of brain organoids.

Q&A for Session 1

Percentage/amount of variation within same iPSC line

Question from Florin Chirila (Zoom chat): What is a typical coefficient of variation (CV) for the genetic variability for the same iPSC line?

Answer from Dr. Cookson (Zoom chat): The best approach is to evaluate the percentage of lines that can be proven to obtain differences from the parental line. With CRISPR editing, best practices yield abnormalities in less than 5 to 10 percent of subclones, which is enough to require reagentotyping after editing. Additional issues may be missed, and his team would like to hear from end users if they find more changes.

Live discussion continued about epigenetic changes and the lack of maintenance of these epigenetic markers, based on a question from Dr. Hickman:

Dr. Cookson: Certain drivers of epigenetic changes across the genome—such as cell differentiation state, genetically defined epigenetic variation, and quantitative trait locus mapping—all appear to reestablish themselves based on genotype. However, aging and environmental exposure are epigenetic change drivers that have no markers in iPSCs, unlike in other cells, such as PBMCs.

He explained two approaches to account for this missing information in iPSCs. Given that the cells are immature, the first method is to focus experiments on things established in the immature state. The other is to determine ways to reintroduce aging and environmental exposure, for example, by rematuration of neurons or transdifferentiation.

Dr. Hickman: He clarified that his question was not necessarily about epigenetic changes that are maintained. He noted that the processes of culture and differentiation induce unintentional epigenetic changes that build up over time, through passaging and other actions they do to cells. These changes can alter the phenotype, and nobody addresses those changes.

Dr. Cookson: He concurred that these changes are underevaluated. He noted that an iPSC line is not static and because it is a biological system, it does show quantifiable changes. His team has assessed the consistency of chromosome accessibility across lines as cells mature.

Dr. Svendsen: Lorenz Studer's group discovered a set of molecules that can be added to the media and artificially matured. To obtain the molecules, they subtracted genes expressed in the young iPSC and the old, induced fibroblasts and then conducted differential analysis. Dr. Studer has called the set of molecules "gin and tonic."

Dr. Finkbeiner: To Dr. Hickman's question, he noted that it is possible to avoid accumulating unwanted epigenetic markers in iPSC lines through quality control and line management.

To Dr. Cookson's comments, he suggested that transdifferentiation could be a complementary approach because the directed differentiation system has limitations. Studies must start with a primary fibroblast or cell type prone to senescence. Also, CRISPR engineering is challenging to do on the lines because it is very difficult to scale up and more prone to batch variation. Nevertheless, several lines include both fibroblasts and iPSCs from the same patients, which can be combined to deduce the contributions of these epigenetic markers.

He pointed out that aging iPSCs to mature them does not necessarily capture the environmental influences the patient experienced. For example, aging and maturation affect a patient's risk for disease differently than smoking or other environmental exposures. He noted that it is important to figure out ways to maintain the environmental influences experienced by the patient when studying their cells.

Other methods to accelerate/promote differentiation

Question from an anonymous Zoom audience member to Dr. Finkbeiner: Are there other methods besides transdifferentiation to promote iPSC maturity and epigenetic markers?

Answer from Dr. Finkbeiner (Zoom chat): Many groups are working on different media components to accelerate or promote differentiation. BrainPhys™ medium from STEMCELL Technologies, Inc., is one such effort, and it can help cells become electrically active sooner than they would otherwise. Lorenz Studer's group recently posted a paper on bioRxiv detailing an epigenetic barrier affecting maturation that can also be targeted: <https://www.biorxiv.org/content/10.1101/2022.06.02.490114v1>. Overexpression of transcription factors is a common way to accelerate the production of more homogenous cultures that express cell-specific markers. Still, it's less clear whether they generate mature cell types faithfully.

Live discussion continued:

Dr. Tsai: Using transcription factors to accelerate maturation or differentiation effectively produces homogeneous cell populations. She recommended that when researchers have their culture of homogeneous cells, they should look at how similar or different the cultured cells are from the cell types in the brain. She noted the difficulty of comparing the transcriptomic profile of NGN2 neurons in the dish with those neurons in vivo and the difficulty of knowing how functional or mature they are. She said there are pros and cons, so it is important to consider the purpose of making a particular culture.

Cost of developing an iPSC line

Question from an anonymous Zoom audience member to Dr. Cookson: On biobanking iPSC lines, could you provide a cost estimate of producing one fully characterized line?

Answer from Dr. Cookson (Zoom chat): Starting from scratch would cost roughly USD20,000 to edit a line, genotype subclones, and revert it. He noted that the cost depends on the desired quality control. He stated that his team plans to distribute on a cost-recovery basis, so anyone using their lines will save a significant amount.

Risk genes causative for LOAD

Question from Florin Chirila (Zoom chat): Are risk genes such as TREM2 and TYROBP causative for LOAD?

Answer from Dr. Cookson (Zoom chat): The answer is no, in a strict genetic sense, because the phenotypes require aging and even then are only expressed in a subset of carriers. But for many variants, his team chose a single genetic variant as the major determinant for disease expression, so they are monogenic diseases.

Organoids and the blood-brain barrier

Question from Nikita Krishnan (Zoom chat) to Dr. Fossati: Do cortical organoids incorporate the cells of the blood-brain barrier and brain microvessels?

Live answers:

Dr. Fossati: Of the available protocols, the one her team uses does not have the blood-brain barrier or microvessels. She noted that people do want to add them because they allow a deeper study of astrocytes in their context. She said that Madeline Lancaster and other labs have been developing various protocols to address this issue, but they are not standardized yet.

Dr. Tsai: Her lab initially developed an in vitro blood-brain barrier model using iPSCs. She said that her talk later in the session would discuss how they try to integrate the blood-brain barrier with neurons and other cell types.

Pathology methods used to validate iPSC lines

Question from Florin Chirila (Zoom chat): Are the primary cell lines for iPSCs from LOAD patients validated by gold-standard pathology?

Live discussion:

Dr. Young-Pearse: That is certainly true for the ROS/MAP cohort, with extensive neuropathological workup from multiple brain regions using scoring systems such as NIA-Reagan, CERAD, and Braak staging.

Dr. Young-Pearse asked Dr. Cookson how his lab tested various lines to find the best and whether they had brain tissue or postmortem confirmation.

Dr. Cookson: He replied that they didn't, and his team deliberately chose lines without genetically encoded risk of disease to avoid biasing any potential phenotypes.

Guide RNA sequences available

Question from Dr. Young-Pearse to Dr. Cookson: Will the constructs his team uses to make their lines be available so that the lines will be independent in terms of genetic background?

Dr. Cookson: He confirmed that his team would provide the guide RNA sequences they used for the different variants.

What to do when AD pathology conclusions are not robust

Question from Dr. Mukherjee (Zoom chat): Given the heterogeneity, do you recommend a minimum number of lines needed to generate robust conclusions. He also asked the group their thoughts on AD pathology in organoids, noting that in his experience, no methods produce robust, reproducible pathology similar to that in mouse models.

Live discussion:

Dr. Young-Pearse: She built on these questions, asking the group whether it was important to see neuropathological hallmarks of a disease, such as plaques and tangles in a dish, to make insights into disease processes.

Dr. Finkbeiner: He acknowledged these problems, noting that researchers most need reliable model systems they can use to reduce risk in therapeutic strategies before testing in humans in clinical trials. With iPSC technology, they can measure the effects of various therapies in a dish, particularly in patient-derived cells, to predict things about the patient, such as disease progression and therapeutic response.

Dr. Cookson: His team now focuses on proximate biological effects of mutations rather than trying to get to a full, mature, fulminant pathology. He noted that analyzing the effects has been much more productive.

Dr. Hickman: This is one reason that MPS are an exciting development: the focus can be on function rather than cell death. His team is looking at currently approved AD drugs to see the effects in various models with MPS, which can be predictive even before animal modeling.

Dr. Lipton: In his talk later, he will discuss extensive electrophysiological (EP) characterization that recreates many features on electroencephalograms (EEGs) of AD patients, as others have shown. EP characterization provides good phenotypic data on the disease.

Dr. Svendsen: Validation is key: being able to validate any of these biomarkers in a chip, a 2D culture, or other method. He noted that a problem in AD drug development is that without great drugs to modulate degeneration, there are very few positive controls to validate anything in models.

Status of protocols using TF to derive cells from iPSCs

Question from Dr. Kostic (Zoom chat) to Dr. Fossati: Could you comment on the stage of microglia and astrocytes protocols that use transcription factors to derive microglia from iPSCs?

Dr. Fossati: Many groups are working on these protocols and publishing papers about them. However, she has not yet seen anything that could be widely, convincingly reproduced.

Ways to manage or reduce heterogeneity among iPSCs

Question from Dr. Gohel (Zoom chat) to Dr. Fossati: What are your thoughts on heterogeneity in multiple AD iPSCs that he is culturing, which are showing significant growth and phenotypic differences at all stages, despite using the same protocol for all.

Dr. Fossati: Some heterogeneity occurs in lines derived from different patients. Her lab has found that variations among iPSC clones from the same patient look much more alike than lines from different patients. Noting that inherent patient-specific variability can be managed only by studying many patients, she mentioned a paper that her team put on bioRxiv to help with power analysis for this purpose. She suggested that some variations could be reduced with careful attention to the methods and extent of original reprogramming and routine, periodic quality control to detect new genetic variations not present in the patient. She noted that automated liquid handling is also helpful and that CRISPR gene correction is a great option for Mendelian causes.

Organoids as a confirmed method or protocol in development

Question from Dr. Baca (Zoom chat): Should organoids be considered a method, like animal models that labs order from a supplier or protocols that still need a lot of time and effort to be developed?

Answer from Dr. Finkbeiner (Zoom chat): It depends on the specific application. The methods for making organoids are standardized enough that larger labs can reliably produce them. Small groups or groups that will use them only as an assay platform may want to start with organoids made by companies to determine their utility for their specific applications.

Role of proteasome in ADRD and proteostasis

Question from Dr. Yang (Zoom chat) to Dr. Young-Pearse: Could you elaborate on the role of proteasome in ADRD and proteostasis?

Live answer from Dr. Young-Pearse: Her team had examined the proteomic profiling of the neurons in a dish, the astrocytes in a dish, and the brain tissue of the same individuals. They saw this elevation with AD pathology and with increasing AD pathology. They also saw changes in dysregulation and proteasome components and could show differences in the capacity of proteasome activity in the different genetic backgrounds of neurons.

Antibody or marker used for amyloid characterization

Question from an anonymous Zoom audience member to Dr. Mook-Jung: Which antibody or marker are you relying on for amyloid characterization?

Answer from Dr. Mook-Jung (Zoom chat): Her team uses A β antibody D54D2 to capture all isoforms of A β for imaging. For measuring A β levels from conditioned media, they use SIMOA kit for A β 42 and 40.

Cost of iPSC lines from NIA Laboratory of Neurogenetics

Question from Dr. Cai (Zoom chat) to Dr. Cookson: Will your lab distribute lines with a cost and, if so, what would the cost be?

Answer from Dr. Cookson (Zoom chat): The cost would be about USD300 to cover the banking, maintenance, and expansion of lines.

Same or different assays to model risk factors vs. pathogenic mutations

Question from Dr. Benitez (Zoom chat): When using iPSC-derived cells for modeling risk factors vs. pathogenic mutations, should we use the same assays even though we expect a milder effect?

Answer (Zoom chat): A risk factor is less potent than a pathogenic mutation, so, at least in the patient, the pathogenic mutation can produce disease in any genetic background, whereas a risk factor cannot. So the effect may be smaller and weaker. But results depend on the assay used, when the factor acts, and which pathway it acts on. Researchers may be able to devise a system that can detect an effect of a risk factor more sensitively than a pathogenic mutation.

Oligodendrocyte organoid model

Question from Dr. Cai (Zoom chat) to Dr. Fossati: In the oligodendrocyte organoid model with additional microglia, does this model recapitulate nodal structures in addition to myelination? Have you converted this model into an AD model to look for features like amyloid deposits?

Live discussion:

Dr. Fossati: Myelination is difficult because it takes more than six months to achieve and is highly variable from line to line. Her team is now beginning to further investigate nodes and delve into myelin biology. But the model is not quite there yet.

Dr. Svendsen: The protocols depend on the cell type because making microglia is a shorter, simpler protocol than the more challenging protocol for making oligodendrocytes. He added that astrocyte protocols are much more complicated; in particular, the directed differentiation protocols have been challenging to reproducibly take a fibroblast through.

Dr. Young-Pearse asked Dr. Svendsen to clarify his point about the difficulty in getting these transdifferentiation protocols to work.

Dr. Svendsen: His team tried many fibroblast-based protocols, down into, for example, differentiated dopamine neurons or motor neurons, and that it was difficult to get them. So the team went back to iPSC as the base to do directed differentiation, which they have found to work more efficiently.

Dr. Lipton: Quality control for every cell type is critical for rigor and reproducibility between labs to compare data. He noted that four, five, or even 10 markers are not enough and that even single-cell transcriptomics with a thousand markers is better. He said it is important to see which transcripts are expressed with mass spec, so it is still a work in progress.

How to distinguish signs of maturity vs. aging

Question from Dr. Cortes-Perez (Zoom chat): Maturation and aging are different: How do we know we are addressing one and not the other?

Answer from Dr. Finkbeiner (Zoom chat): It is often difficult to untangle, but new tools to assess signs of maturity and aging may make it easier to distinguish the two.

Different findings in iPSC-derived cells vs. patient brain tissue: Artifacts?

Question from Dr. Benitez (Zoom chat): What would be your interpretation of different findings, such as transcripts or proteins, in iPSC-derived cells that are not in the brains of patients with the same genetic changes? Would they be considered artifacts?

Answer from Dr. Finkbeiner (Zoom chat): There are many possible reasons for these differences: Some are artifactual and could relate to incomplete reprogramming, new genetic mutations in the lines that are not in the patients, immaturity, lack of epigenetic markers of aging, or lack of important environmental cell nonautonomous contributions.

However, there are also many nonartifactual technical explanations. Perhaps the changes in the iPSCs were in the patient in early life at a state of maturity more similar to the iPSC model, but they disappeared in adulthood or late-stage disease. Most patient brains are from patients with late-stage disease and will have many additional changes that reflect cell loss and maladaptive changes that may not be present in the culture.

Reference for “gin and tonic” molecules

Question from an anonymous Zoom audience member to Dr. Svendsen: Could you share the reference for the “gin and tonic” molecule work?

Dr. Svendsen: He posted a link to the paper:

<https://www.biorxiv.org/content/10.1101/2022.06.02.490114v1>.

Ways to accelerate iPSC neuron maturation

Question from Dr. Liao (Zoom chat): Many iPSC neurons are immature and lack the appropriate clustering of postsynaptic receptors. How can we accelerate maturation of iPSC neurons so they will have functional responses similar to animal neurons?

Answer from Dr. Finkbeiner (Zoom chat): The main approaches have been using media like BrainPhys that accelerate electrical maturation, opting for more complex cultures like organoids or co-cultures with glia, and maintaining the cultures/organoids for very long lengths of time.

How to interpret data from relatively immature cultures

Question from Dr. Mukherjee (Zoom chat): Could you discuss the maturity of the cells, specifically about what cautions the group recommends when interpreting the data? When

studying these neurodegenerative disorders, the data are from relatively immature cultures that are less like the adult brain and more like a developing brain.

Answer from Dr. Finkbeiner (Zoom chat): Because many of these diseases are highly heritable, some signal from the genome must lead to cell dyshomeostasis that culminates in neurodegeneration. The closer the phenotypic assay is to the genomic roots causing the disease, the easier it should be to detect, even in immature cells. And in some cases, such as AD caused by trisomy 21, it is possible to find patients who develop symptoms very early, meaning that aging is not required. Dr. Finkbeiner pointed out that although transdifferentiation is not as easy as iPSC work, it is reliable enough to use to deduce contributions of aging. Transdifferentiation can dramatically affect disease phenotypes, especially those related to protein dyshomeostasis.

Live discussion continued:

Dr. Young-Pearse: Among the precautions her team takes are looking in the actual adult brain, when possible, to see if there are concordant findings. She noted that the group will likely return to this question in later sessions.

Dr. Cookson: His team is interested in transcriptional fidelity to brain cell types and said that his team gets close, when making microglia, with a Pearson's r of about 0.9, more than is seen from person to person. He noted that other cell types his team makes, such as astrocytes, are less transcriptionally similar to brain astrocytes. He said that they cannot map to an adult brain and then to a fetal brain, a finding that has been very consistent across their studies. The idea is to use the cells for purposes that they fit.

How long until the clinical use of iPSCs for treating diseases

Question from Dr. Omole (Zoom chat): How far we are from the clinical use of iPSCs for treating diseases?

Answer from Dr. Finkbeiner (Zoom chat): There have already been trials for transplantation.

Dr. Young-Pearse restated the question: Approximately how many years it will take for a drug that they find in tests, in iPSCs in a dish, to be tested in humans in clinical trials, specifically for AD and ADRD?

Dr. Hickman: His group has already done it: They did an IND filing with Sanofi for a therapy for a rare neurodegenerative disease. The application was to repurpose a current drug for a new indication, and it went directly into a phase 2 clinical trial.

Dr. Young-Pearse asked Dr. Hickman: Do you think the application may have received special consideration because it was for a rare disease with no treatments?

Dr. Hickman: He agreed that was probably true. He also pointed out that they could reproduce the in vivo human function deficits in patient sera samples and show that their molecule reversed them.

Dr. Svendsen: He noted the example of Dr. Hideyuki Kano, who has gone through the same process with the Japanese FDA for ropinirole. The drug, currently approved to treat Parkinson's disease, is now in a clinical trial for treating amyotrophic lateral sclerosis (ALS).

Dr. Finkbeiner: iPSC and other MPS could solve a key problem: whether researchers must use mouse models even if they do not accurately predict human response.

Dr. Svendsen: He added an example of liver chip models that accurately predicted human toxicity—that animal studies did not detect—for drugs later halted in clinical trials. The company is now pushing the FDA to accept chips as toxic models because many substances that are toxic to humans are not toxic to mice, rats, and other animals used in studies.

Dr. Hickman: MPS have an advantage in rare disease research: With 7,000 rare diseases, only 400 research programs are active because there are no animal models. Without an animal model for comparison, the FDA and other regulatory agencies may be more likely to accept phenotypic data from iPSC, chip, and other microphysiological models. He noted that it will be important to combine phenotypic systems with target-based marker biology to advance the process with the FDA and other agencies.

Dr. Fossati: AI will probably accelerate the process as well because researchers can now use it to uncover new phenotypes.

Question from Dr. Omole (Zoom chat): He clarified that he had meant how long it would take for drugs to go from trials to official use in clinics.

Answer from Dr. Finkbeiner (Zoom chat): It has already been done, although it has mostly been repurposing previously approved drugs and limited to relatively rare and aggressive diseases.

Session 2: Using Microphysiological Systems as Tools for Predictive Drug Development

Session Chair: James Hickman, Hesperos Inc.

Using 3D Cerebral Organoids and AI to Identify Candidate Therapeutics for AD

Stuart Lipton, Scripps Research Institute

Dr. Lipton discussed one of the many approaches to drug discovery for AD and ADRDs that he and his team are working on. It is an artificial intelligence (AI) approach based on transcriptomic differences, mainly in inherited AD but also applicable to sporadic cases.

It is widely believed that A β does not correlate very well with the degree of eventual severity in dementia, although it is important in triggering and spreading the disease. Altered excitability and progressive neuronal synaptic loss are the best correlative to neurocognitive decline, as researchers have shown, beginning in the early 1990s. Yet no effective drugs have been developed to protect synapses.

Most AD patients have a hyperexcitability phenotype and even subclinical seizures in many cases. Dr. Lipton's team has worked to make reproducible organoids as a tractable human model, and they can mimic many brain regions. The organoids can express neurons, astrocytes, and microglia, although they are immature. Dr. Lipton believes researchers can take advantage of that in many cases.

He and his team have reported and published misfolded proteins and synaptic loss in their AD organoids, so they do resemble the disease pathology. They then use these organoids for high-throughput screening.

Dr. Lipton's team is working with iPSC lines from Dr. Marc Tessier-Lavigne's lab at Stanford University and Dr. Lawrence Goldstein's lab at the University of California San Diego. They are testing familial forms of AD vs. isogenic wild-type controls, always using different genetic backgrounds. They also test sporadic forms once they find a drug that works.

But for primary screens, he believes the power of the isogenic control vs. the familial form is worth taking advantage of, even though the neurons are immature and lack epigenetic markers. He believes epigenetic markers may be less important in genetic disease.

His team based their differentiation of cortical organoids mainly on the Pasca protocol with some changes. A major change from the Pasca protocol is putting the organoids on a shaker to prevent fusion. The team recently published their nine-day microglia protocol and also recently published that they can see into the organoids. Their organoids have many features that are important in immature brains, such as little ventricles forming and many markers showing a plethora of cell types.

Maybe more important is their single-cell RNA seq analysis, which the team worked on with Dr. Nicholas Schork and his group at TGen. The neurons are mostly immature, with some maturing neurons, but none are fully mature. However, they can see a variety of phenotypes and analyze the differentially expressed genes between their AD lines and the isogenic controls for each cell type on various genetic backgrounds.

Analysis on different genetic backgrounds is powerful because it can show the differences between AD and the wild-type, isogenic, gene-corrected controls. Although age is a major risk factor for AD, an interesting feature of immature organoids is that they allow study of AD's early features. Because a goal for the field is early treatment of AD, immature organoids may be a suitable model to screen for drug development. However, the organoids must have adequate phenotypes that resemble the disease.

Dr. Lipton's approach, called gene expression complementarity studies, compares differentially expressed genes between the AD organoids and the isogenic controls. The team typically sees some transcripts that are decreased and some that are increased. They then superimpose these transcriptomic profiles on library screening that has been performed on various cell types.

A weakness of the screening is that most cells in the library are cancer and other cell types, not neural cell types. However, several drugs have been screened on various lines, so the team can evaluate how that changes the transcriptomic profile. They then try to match the changes to normalize the transcriptomic profile that's abnormal in the AD cell, called the connectivity map.

The team uses two drug libraries:

- **ReFRAME drug library:** Unique to Scripps, ReFRAME contains more than 13,000 drugs, which have been synthesized by Scripps' chemists. ReFRAME contains every drug approved by the FDA, EMA, and other such agencies in the world, and every drug that has completed at least a phase 1 safety trial.
- **CMAP/LINCS L1000 database:** This library contains FDA drugs and other compounds.

The aim is to find drugs by gene expression complementarity approach, with either the ReFRAME or CMAP/LINCS L1000 libraries, that will normalize a transcriptomic profile in the AD iPSC cells in organoids.

The team superimposes a secondary screen to examine the hyperelectrical activity profile previously mentioned. The activity appears in the AD cerebral organoids but not in the isogenic controls and has been shown to contribute to synaptic damage. This hyperelectrical phenotype is present on EEGs of patients with AD. Blocking the activity prevents synaptic damage to a large extent.

The strategy is to use the differentially expressed gene sets that are either up or down. The team signature matches by connectivity, analyzes the drugs, and validates the drug matches. They examine differences in particular cell types in AD vs. the isogenic control GABAergic interneurons and glutamatergic excitatory neurons. They also do the screening for astrocytes,

microglia—all the various cell types—to select drugs that correct the transcriptome for a particular cell type.

To test the hyperelectrical activity, they use a multi-electrode array (MEA). They put a 96-well dish in each one with a cerebral organoid and each one with a microarray. This technique enables the team to simultaneously look at organoid activity, like examining an EEG in an AD patient.

The resulting data shows low activity at four months in the wild-type array. The organoids do mature, and their activity increases, which can be seen in quantifying action potentials on one point in the array. Organoids with a PSEN mutation show much more activity by nine months, and those with an APP mutation show even more activity despite early maturation. The AD cerebral organoids show much more activity than the wild-type isogenic controls, as the team has seen in tests using different genetic backgrounds.

To curate the differentially expressed genes, the team evaluates each cell type for statistical significance of a difference in AD mutations in both sets of lines. For example, if cells in one line show a PSEN1 or APP mutation, cells in the other line must also show that mutation in order to choose drugs. The team examines each cell type one at a time, such as excitatory neurons, inhibitory neurons, astrocytes, and microglia.

The cells must also have the same direction of change in a glutamatergic neuron. The team has upregulated and downregulated differentially expressed genes (DEGs), which they then superimpose on, for example, the ReFRAME drug library. The team looks for drugs that will normalize the DEGs and develop a connectivity map, a series of drugs. They often prioritize the drugs by looking for drugs that make sense on a GO analysis or other systems approach to see if they might work in treating the disease. The team then tests the cells with their MEA recordings to see whether the drugs not only normalize the transcriptome but also correct the phenotypic abnormality.

Dr. Lipton showed an example of a promising match from the ReFRAME database. The drug lowered activity in the treated cells compared with the untreated cells. The activity returned after a couple of weeks, so the team treated and monitored the organoids regularly, maintaining them for a long time. The findings indicated that this drug candidate might normalize the hyperelectrical activity by lowering it toward normal.

Another way the team examines activity is with 2D cultures or 3D cultures, such as calcium imaging. Dr. Lipton showed an example of neurons compared with an AD organoid with a heterozygous mutation in PSEN1. The neurons showed relatively low activity at baseline, whereas the organoid showed much more activity due to high extracellular glutamate triggered by A β and other substances.

The team developed a drug called NitroSynapsin, which they turned on in the experiment. The before and after videos showed how NitroSynapsin absolutely normalized the activity. The drug

recently received funding to go into a clinical trial, probably at the end of 2022 or first quarter of 2023. The clinical trial will test the drug for several diseases with this hyperelectrical activity.

The team can also test combinations of drugs, such as drugs that correct the abnormal DEG profiles in excitatory neurons, inhibitory neurons, astrocytes, or microglia. They can also verify the drug effects with at least one phenotypical approach on MEA recordings to normalize the hyperelectrical activity.

Dr. Lipton's team has other approaches to examine autophagy, mass spec analysis for the proteome, histological synapses, and various other profiles. They also have several kinds of phenotypes, including lipidomics and proteomics. This electrophysiological phenotype may be closer to the disease, actually a functional readout.

In conclusion, Dr. Lipton summarized his team's AI approach:

- Find DEG profiles in each cell type of AD vs. isogenic control cerebral organoids.
- Find potential therapeutic drugs by DEG complementarity approach using the ReFRAME and CMAP/LINCS L1000 drug collections that normalize the transcriptomic expression profile in an AD cell type.
- Verify the drug effect phenotypically on MEA recordings by normalizing hyperelectrical activity profile that contributes to synaptic damage in AD cerebral organoids.

Construction of an Integrated Immune, Vascular, and Brain Chip as a Platform for Drug Discovery and Development for AD

Li-Huei Tsai, Massachusetts Institute of Technology

Dr. Tsai discussed her team's unpublished work to construct an MPS for drug discovery and development for AD and ADRD. She noted that the mechanisms for AD and other neurodegenerative diseases are highly complex, with multiple genetic and environmental risk factors involved in their etiology. To understand these mechanisms, the field conventionally analyzed these factors in control systems, such as mass models, in vitro cell cultures, and cell lines.

It is possible to model the obvious disease pathologies, such as A β plaques and tau tangles, when the model overexpresses APP or tau. But this approach relies on preexisting knowledge of disease mechanisms with many genetic risk factors whose functions have yet to be discovered. Patient-derived iPSC technology represents the only model available that can fully recapitulate the total complexity of the genetics of human diseases.

Dr. Tsai noted that researchers want models to have mature cells, various brain cell types, preserved tissue architecture, and be able to model sporadic diseases. Previous speakers discussed the strengths and limitations of 2D iPSC-derived cell lines and organoid models. Dr. Tsai and her lab are working to develop a model that could enable modeling of sporadic diseases and serve as a platform for developing precision medicine.

Current protocols for building MPS use cells harvested through biopsy, which researchers reprogram into iPSCs. They further induce iPSCs to differentiate into different cell types, which they then process and embed in a culture system. The cultures usually incorporate a soft matrix to support the microfluidic devices.

The cells grow and mature into cellular structures, but the terminal and functional differentiation of cells in the cultures continues to be challenging. Work also remains to maximize the homogeneity and reproducibility of these cultures and enhance their ability to mimic cell microenvironments.

Brain tissue develops in a stereotyped architecture with neurons and glia arranged outside the blood vessels. Extracellular matrices provide the scaffolding to maintain this structure and enable cell function. The most used matrices in MPS, however, do not contain the disaccharides, proteins, or proteoglycans that are in the brain.

Most researchers use Matrigel®, a slurry of about 1,800 proteins derived from tumors induced in mice. So there is much variability from lot to lot. Matrigel is also prone to fast degradation and suffers from poor tunability. Another popular matrix, fibrin, is usually used to form vascular networks in MPS. However, fibrin is neurotoxic and has deleterious effects on glial cells. It has also been linked to inflammation of the central nervous system and suffers from fast degradation and poor tunability.

Dr. Tsai's team's first attempt to develop an MPS was to create a 3D neurovascular model called induced blood-brain barrier, or iBBB. The team differentiated iPSCs into brain endothelial cells, mural cells such as pericytes, and astrocytes and co-cultured them together.

Over time, the cells assembled into a 3D cerebral vasculature, with endothelial cells forming networks and mural cells becoming vessels. Astrocytes extended endfeet onto the vessels, increased their expression of aquaporin-4, and strengthened the barrier properties of the iBBB.

The team used transepithelial/transendothelial electrical resistance (TEER) assays, permeability assays, and other assays. They demonstrated that the iBBB exhibits increased barrier function with a tighter regulation than endothelial cells alone. Using isogenic APOE3 and APOE4 iPSCs to create the iBBB, the APOE4 iBBB appears to be much clearer than the APOE3 iBBB in increasing the permeability of molecules of different molecular weights.

The team could further harness this iBBB to elucidate drug mechanisms, applying the iBBB to study cerebral amyloid angiopathy (CAA). They created the iBBB from isogenic iPS APOE3 and APOE4 lines and then analyzed which cell type specifically contributes to CAA. They did so by mixing the three cell types that make up the iBBB and by mixing up their genetic backgrounds.

From this permutation experiment, the team identified mural cells from the APOE4 line that were key to forming cerebral amyloid angiopathy. They then performed RNA sequencing of the

APOE4 mural cells and compared them with APOE3. The team identified the calcineurin M5 signaling pathway, which was abnormally elevated in these APOE4 mural cells.

With this information, the team turned to repurposed drugs, such as the calcineurin NFAT inhibitors FK506 and cyclosporine A, to test their effect in reducing CAA formation in the APOE4 iBBB. They showed that treatment with these calcineurin inhibitors efficiently reduced CAA formation in this culture.

Dr. Tsai's team built on this iBBB model to create a brain extracellular matrix because, as previously mentioned, most existing matrices are not ideal. They wanted to create a brain extracellular matrix that can mimic the brain matrix and support the integration of neurons into the iBBB culture.

Dr. Alice Stanton, a postdoctoral fellow in Dr. Robert Langer's lab at MIT, engineered a soft matrix that mimics extracellular matrix proteins in the brain. She brought cross-polymer formations to identify a composition that can support most cell types in the brain and created a formulation she referred to as Neuromatrix.

Dr. Tsai showed examples of culturing the neurons and iBBB in Matrigel vs. Neuromatrix. The cells showed more robust growth in Neuromatrix than Matrigel. Neuromatrix has longer-term structural integrity, remaining intact for a longer period compared with Matrigel, which rapidly degraded.

The team also found that Neuromatrix enhances the functional maturation of neurons. Using MEA to record electrical activity, they compared neurons cultured in Matrigel with those cultured in Neuromatrix. The neurons in Neuromatrix showed greater spiking activity than those in Matrigel.

Using Neuromatrix, Dr. Tsai's team could upscale their iBBB into an integrated brain model called multicellular integrated brain (miBRAIN) model. The goal is for the miBRAIN model to contain all the resident cell types of the human brain, including the vasculature, neurons, microglia, and oligodendrocytes.

Over several years, her team has developed protocols by optimizing those of other pioneers in the field. They further optimized the protocols to include all the cell types in the miBRAIN system, integrating neurons with the iBBB to form a neurovascular unit.

The team differentiated each cell type from the iPSCs, performed quality control, and often purified each cell type before co-culturing them together in Neuromatrix. Dr. Tsai showed an example of the integration of the induced microglia-like cells. The cells readily expressed high levels of P2RY12, a marker for homeostatic microglia. The video also showed the microglia's motility in the culture. The team sought to understand how much of the microglia were in the miBRAIN system compared with microglia in vivo in the brain.

Currently, many transcriptomic profiles of microglia are being published, both from in vitro from the iPSC systems and from in vivo. Dr. Tsai's team uses data sets published by Dr. Christopher Glass and Dr. Matthew Burton-Jones. Dr. Tsai showed a slide with microglia from their labs: in vitro cells vs. the cell profile from in vivo.

The team then examined their induced microglia cultured alone in 2D vs. their co-culture with the miBRAIN. Microglia from the miBRAIN showed transcriptomic profiles that are closer to in vivo microglia than in vitro microglia.

About myelination, Dr. Tsai showed a slide of the miBRAIN system with myelin basic protein (MBP) labeled along the neurofilament positive axons. Through single-cell RNA sequencing, the team could identify the cluster of cells more likely to be oligodendrocytes that are positive for MBP, MOBP, and PP1.

The team also used the miBRAIN system to try to produce Alzheimer's pathology, such as amyloid plaques. They produced miBRAIN systems from APOE3 vs. APOE4 isogenic iPSC lines. After long-term culturing, they examined fibrillar amyloid and aggregated amyloid. The APOE4 culture appeared to be much more prone to developing amyloid pathology than the APOE3 miBRAIN.

They also used a protocol from Dr. Virginia Lee's lab to look at tau pathology, applying tau fibrils to the miBRAIN culture. They found that APOE4 miBRAIN showed phosphorylated tau.

Dr. Tsai and her team believe we can leverage this culture to study and consider precision medicine. They can use it to test drugs longitudinally or through staged drug administration. They can also test combinations of drugs and different doses in the system. In people with different genetic backgrounds, their miBRAIN could respond very differently to these drugs.

We now have the chip format of the miBRAIN. We can perfuse different factors and drugs into our miBRAIN and collect the efflux from the miBRAIN. Eventually, we can test pharmacokinetic/pharmacodynamic (PK/PD) properties and biomarker monitoring. We can perfuse nanoparticles into the vasculature in the miBRAIN.

Dr. Tsai concluded with a summary of her talk:

- The multicellular integrated brain (miBRAIN) model incorporates multiple cell types found in the brain in an engineered matrix that mimics aspects of the extracellular matrix (ECM) of brain tissue and enhances neuronal properties and maturation of other cell types.
- miBRAIN contains several features absent in alternative models, including:
 - A fully integrated BBB within the neuronal network
 - Enhanced microglia cell phenotypes and myelination
 - Biomimetic tissue architecture
 - Transcriptional signatures, indicating that miBRAINS capture enhanced diversity of cell types

- We are harnessing this model to address neurodegenerative disease, aiming to identify new drug targets and conduct in vitro clinical trials.

Construction of Integrated Human-On-Chip Systems for AD Drug Development for the Central Nervous System and Peripheral Nervous System

James Hickman, Hesperos Inc.

Dr. Hickman discussed his company's work to build human-on-a-chip systems for AD drug development in both the central nervous system (CNS) and peripheral nervous system (PNS).

In the context of cellular research, clinically relevant functional readouts are measurements of cellular functions such as muscle contraction and electrical activity. For both neurons and cardiac cells, Dr. Hickman's team evaluated neuromuscular junction (NMJ) systems. They also assessed barrier integrity for TEER for the kidneys, BBB, and gastrointestinal (GI) tract.

These readouts permit noninvasive functional analysis, as the team can put cells in an incubator and study them for at least 28 days without disturbing them. Clinically relevant functional readouts also allow the team to:

- Perform mechanistic termination of toxicity and target identification
- Pattern cardiomyocytes on top of MEAs
- Put cantilevers on MEAs and place skeletal-muscle and cardiac cells on them to track
- Look at liver biomarkers, kidney TEER, and other systems

For about 20 years, Dr. Hickman and his team have studied long-term potentiation to understand cognitive deficits and published a paper in Stem Cell Reports explaining how they can differentiate iPSCs into mature cortical neurons.

Dr. Hickman's original training was in surface chemistry, which provides an advantage in understanding how the cells in question interact with their surfaces and the ECM. His team can subsequently control the spatial organization of the cells on surfaces. They put down a monolayer and place a stain lithography, before shine a light on the mix and adding a second monolayer.

To initiate the long-term potentiation (LTP) process, his team plates neurons on a surface, which then search for broken lines. Communicating throughout this pathfinding, the neurons find a cell adhesion site, with translocation of the neuron cell bodies taking place. The cells put out neurites that become axons and dendrites. In effect, Dr. Hickman's team uses surface chemistry to control not only cell differentiation, but also cell placement.

During experiments, the team also showed that their neuronal cultures formed paired patterns on MEAs and demonstrated activity. In the cultures, the team saw all the appropriate markers signaling that these are cortical neurons. They added lidocaine to confirm that they were getting biological electrical signals off the MEAs.

The team then treated the systems with NBQX, which blocks AMPA receptors, to confirm synaptic connections between the two electrodes. (These connections go down with time.) Similarly with an NMDA receptor (D-AP5), they could show biological synaptic signals in the cultures.

In these systems, the team can show maturation on the MEAs using immature or fetal-like cells. Normally, LTP cannot be induced in 21 days. However, the team's immunochemistry demonstrates that the immature culture has a mixture of inhibitory and excitatory neurons. By blocking inhibitory neurons with picrotoxin, it is possible to induce LTP. At about days 35 through 40, the team can again induce LTP in the system as inhibitory neurons convert into excitatory neurons or die off. The team can thus monitor the cells as they change from a fetal phenotype to a mature cortical phenotype in the system.

It is well known that isogenous A β oligomers will block synaptic connectivity and those effects. The team did not get an effect using patch-clamp on scrambled A β . But within the one-to-one correlation on the MEAs, the scramble produced strong collections of action potentials on the MEAs. The A β oligomers abolished most of the currents so there was no spontaneous activity. In this case, the team treated with A β plus an approved AD drug (donepezil) to recover some activity in the system. The team can reproduce this effect on the MEAs and get much higher throughput, looking at the actual potential, than with patch-clamp. In other words, they validated their results against the gold standard in electrophysiology.

In collaboration with Dr. David Morgan's group at Michigan State University, Dr. Hickman's team could show that, using A β oligomers and the A β scramble, they could maintain LTP. However, they blocked the ability to do LTP with A β in these systems. They achieved the same results with tau oligomers, where with the control, they could maintain LTP, but LTP is blocked with tau oligomers. The team published this work in *Alzheimer's & Dementia* a couple of years ago.

Dr. Hickman pointed out that the team is not seeing cell death in these systems. The systems could, therefore, be good assays for mimicking some deficits seen in mild cognitive impairment (MCI) or even pre-MCI. In the later stages of MCI, significant cell death occurs—about 20 to 30 percent. In full-blown AD, cell death is at least 30 to 40 percent, if not more.

The team began these experiments using donepezil to block LTP, then repeated the assays with the other three approved AD drugs: saracatinib, memantine, and rolipram. All block the ability of the A β oligomers to shut off the LTP by attacking the synapses between the neurons.

In experiments using tau oligomers, however, only memantine and donepezil worked to block them—saracatinib and rolipram did not. It is well known that tau comes up later than A β when looking at AD longitudinally. Dr. Hickman's team believes that they will be able to achieve similar effects when looking at the lifetime of AD, from very early stages to very late stages. With ratios and dosing controlled, the team plans to test the drugs on combinations of A β and

tau oligomers. They expect to see all four drugs working for A β oligomers, but only two working for tau oligomers. The results should provide some ideas on how the drugs will work in vivo.

The team has also examined PSEN1, PSEN2, and APOE4 in iPSCs and has seen good survivability in the PSEN1 and wild-type cultures. They used patch-clamp to examine spontaneous firing in the systems. The results showed little effect—while repetitive firing was seen in the PSEN1 cultures, that action can be monitored. In the wild-type cultures, repetitive firing was delayed.

PSEN1 is a hyperexcitable phenotype, but that trait dies down with activity. PSEN2, a severe phenotype, shows activity only in its sporadic forms. With APOE4, also a severe phenotype, the team initially saw some activity, but it went away with time.

When memantine was introduced, it provided some rescue in the system, showing that the drug addresses A β and potentially tau. Memantine was effective in PSEN1 and APOE4 but less so in PSEN2.

With another project, Dr. Hickman and his team are studying how A β affects the peripheral nervous system, with an examination of the reflex arc to potentially find functional biomarkers for AD. The team has created a functional NMJ system for personalized ALS models and drug testing that they will use with AD. They can put the disease models into a chamber where the motoneurons are electrically and chemically isolated from each other for muscle sculpting. The team could then:

- Grow axons through the tunnels to innervate
- Perform motion capture when motoneurons are stimulating the muscle
- Compare effects in wild type and ALS mutations and show reversal with a holistic treatment, the Deanna Protocol

For a clinically relevant functional readout in this project, the team worked with researchers running an ALS clinical trial at Massachusetts General Hospital. The team's goal was adapting clinical physical function assessments, such as hand grip tasks, to reproduce in their model across several phenotypes.

The project began with a clinical assessment in which patients were asked to do tasks faster with their hands, leaving their muscles spastic and jittery and their grip strength weakened. In the models, Dr. Hickman's team could increasingly stimulate motoneurons, driving them into tetanus. The results showed deficits in function similar to clinical measurements in the patients.

The system had a control: With the deficits occurring at the NMJ, the team could place electrodes with the muscle. Across all mutations, they saw that the muscle stimulation was fine, as demonstrated by increased firing frequency. Dr. Hickman noted that no cell death occurred in the models.

The team created another clinically relevant readout, a fatigue index, which went up with the mutations as expected. They introduced a holistic treatment that has shown some benefits in

ALS, the Deanna Protocol, and achieved some reversal in the systems. The team published this work in *Advanced Therapeutics* in 2020, with Xiufang Guo as lead author. The team will apply this system to AD, treating A β and tau, to understand the functional deficits and begin creating biomarkers for AD.

This team created the first human-to-human myelination model, a conduction velocity model to examine multifocal motor neuropathy and chronic inflammatory demyelinating polyneuropathy (CIPD). In CIPD, the body creates antibodies for node of Ranvier, which activates the complement system. This switch causes pores in the node of Ranvier that lead to a gradual loss of conduction velocity.

This NMJ model uses patient sera samples, not just mutations or iPSCs, for testing a Sanofi drug molecule. The team put electrodes in the tunnels through motoneuron axons, then monitored the action potentials in the tunnels to calculate conduction velocity. They compared results among untested and untreated serum, serum plus isotope control for the molecule, and serum with the drug molecule.

The results showed that the isotype control with the serum shuts off conduction velocity, but the drug molecule with the serum protects conduction velocity. As Dr. Hickman discussed during the Q&A for Session 1, Sanofi put the drug into an IND and applied for and received authorization to go to a clinical trial, which started in April 2021. This was the only phenotypic data that was added to the application, because the drug already had safety data from another trial. It was a notable example of repurposing drugs to target rare diseases.

Dr. Hickman described his team's human-on-a-chip models for other organs, including a system they created for the HEAL Initiative through NCATS. This five-organ system includes iPSC neurons for the liver, heart, skeletal muscle, pre-BötC, and kidney to look at overdoses. The team has also done overdose response curves for four opioids to show recovery with naloxone. They are now looking at chronic naloxone treatment and off-target toxicity with their systems.

The chips incorporate a pumpless and tubeless system that uses gravity to circulate the media. This gravity-based approach allows the team to create low-volume systems to look at both the parent and the metabolite in the same system (in the liver, for example). The team has created PK/PD models to predict in vivo outcomes from these in vitro systems.

Other models that Dr. Hickman's team has developed include:

- Immune system on a chip that they expect to show both M1 and M2 phenotypes
- Barrier tissue organ system, which has a skin mimic plus four other organs
- Three-organ system with blood-brain barrier, GI tract, and CNS to study oral, IV, and intrathecal dosing

To conclude, Dr. Hickman summarized his key points:

- Development of a long-term potentiation (LTP) model for a clinically relevant functional model of AD utilizing A β and tau oligomers to create an AD phenotype

- Evaluation of AD therapeutics utilizing the LTP phenotypic model
- Characterization of PSEN-1, PSEN-2, and ApoE4 AD models derived from induced pluripotent stem cells (iPSCs)
- Development of a neuromuscular junction (NMJ) model to be utilized for AD PNS evaluation
- Development of barrier tissue and PK/PD models to translate in vitro to in vivo outcomes

Construction of a Multicellular Organ-On-Chip to Inform FTD/ALS Clinical Trials

Clive Svendsen, The Regenerative Medicine Institute, Cedars-Sinai

Dr. Svendsen disclosed that Cedars-Sinai, his employer, owns a minority stock interest in Emulate, a company that produces the chips he's going to discuss.

He discussed his team's work over the past five years in disease modeling on multicellular organ-on-chip systems. His team used tissue chips, which are scalable and provide substantial physiological output, to begin more detailed research into iPSC-derived cells.

An early project combined BMECs with neurons in co-cultures to enhance maturation. Using the same iPSC line, the team created spinal cord neurons and BMECs. They then made an organ chip system in which the top channel has the neurons, and the bottom channel has the BMECs.

Dr. Svendsen found that they could create these co-cultures efficiently by using chips from a company called Emulate. They decided to use commercially available chips rather than creating their own to reduce potential variations in the chip design.

The chips provide a way to model the BBB with separate channels, or layers, for different types of cells. The bottom layer with vasculature creates an intact vessel all the way around, and it is possible to flow blood through that layer. With neurons on the top layer, researchers can simulate damage to the BBB by adding TNF alpha to the bottom layer. They can also simulate test drug perfusion by adding drugs to the bottom layer.

With this technology, Dr. Svendsen's team can simulate the drugs that should cross over to the brain and those that should not. Another study simulated an MCT8 deficiency and found that the BBB, not the neurons, was the main problem. That work led to a gene therapy trial for the disease.

The Emulate system to create the chips is vacuum based, without tubing, and mostly automated with relatively high throughput. The machine contains 12 pods on top of the chips to deliver the fluids. Emulate has developed chip models for sporadic ALS and Parkinson's disease, and the project Dr. Svendsen discussed is MPS models for ALS and FTD.

ALS and FTD occur because of a mutation in the gene C9orf72 that causes a hexanucleotide repeat. At one end of the spectrum is ALS and the death of motor neurons in the spinal cord. The other end is FTD with neuronal loss in the frontal and/or temporal lobes, cognitive language deficits, and neuropsychiatric issues.

His team used iPSC-derived cells from control subjects, C9orf72 ALS subjects, and C9orf72 FTD subjects to make isogenically matched iPSCs to compare patients and their isogenic lines. Repeat expansion of the C9orf72 hexanucleotide of 1,000 indicates full-on disease. Dr. Svendsen showed mechanisms that underlie the visible part of the pathology, which is the loss of motor neurons or damage to the frontal cortex.

The repeat also caused a gain in function, with RAN peptides and dipeptides that could be toxic to the neurons and create RNA foci in the nuclei. These powerful FTD biomarkers will be a focus of the team's study in the dish.

Their iPSC lines for ALS came from the Answer ALS cohort, and the iPSC lines for FTD came from Synapticure. The iPSC control lines came from the Lothian cohort developed at the University of Edinburgh, a group of patients who reached age 80 with no brain diseases or major comorbidities and with normal IQs.

The team has the neuropathology and RNA foci data from the brains of the patients from whom they derived the iPSCs. They used a single-cell nuc RNA seq to pull individual neural phenotypes from the chips. The team hopes to see pathology in the C9orf72 organ chip system and see if pathology in a patient's iPSC chip correlates with the patient's pathology.

As previous speakers described, Dr. Svendsen's team can make the various types of brain cells from iPSCs. Their differentiation protocol has produced the various phenotypes they want to put on the chip. The team also created a protocol to clear opacity in cortical chips, which develop a thick layer of cells, using a SCALE technique.

To create phenotypes, the team is collaborating with another lab to analyze dipeptide repeats. To target their drug assays, the lines must have robust polypeptide repeat production, similar to that in symptomatic carriers.

The team has conducted a thorough comparison of all types of neurons using bioinformatics, big data, and AI to understand more about how the cells look. They used single-cell RNA seq to analyze the various cell types relevant to FTD and ALS. Imaging is important for analysis, including imaging the cell activity, especially during drug administration. The team uses live calcium imaging to observe fluid flowing through chips and high-content imaging and effluent for several other markers.

The chips allow modeling of several biomarkers, including lactic dehydrogenase or ELISA for neurofilaments, dextran permeability for BBB function, and metabolite studies with mass spec studies. The team can also do transcriptome studies.

Companies interested in working with Dr. Svendsen's lab to develop models include Denali Therapeutics for RIPK1 inhibitors, AcuraStem for PIKFYVE inhibitors, Mitochondria in Motion for MFN2 agonists, and Ionis Pharmaceuticals for C9orf72 and ATXN2 ASOs.

Dr. Svendsen noted that the future for tissue chip systems could be interactive models in the chips, with blood flowing through one side. Dynamic models with flow could be used to study a wide range of targets. He showed a video with an example of blood cells flowing through seven-micron pores in the chip to defeat infection on the top layer.

He discussed current challenges in developing organ-on-chip models:

- The Emulate chips are stable but expensive.
- Survival for more than 21 days in the chip has been hard: Cells start to peel off.
- Consistent cell production and generation of frozen stocks is hard work.
- Generation of BMECs are identical to in vivo. Ours have some epithelial markers.
- Drug absorption to PDMS (although this problem reduces if fully coated with cells).
- Standardizing assays of effluent needs to be highly sensitive and reliable.
- ***Validation of any in vitro model in clinical trial.***

He also noted opportunities:

- Next-generation chip technology provides the possibility of "patient on chip."
- It may be possible to flow blood through the blood channel and test the effect on neurons in the neural channel to see the effect of drugs in real time and in a powerful model.
- If scale can be established, this could be a precision health strategy—each ALS/FTD patient could get their own chip to test drugs that work on their brain cells.

Q&A for Session 2

Panel discussion

Action potentials and long-term potentiation firing frequency

Live question from Dr. Lipton to Dr. Hickman: Action potentials are not long-term potentiation (LTP) firing frequency because you would need to look at the slope of the excitatory postsynaptic potential (EPSP). Your model is certainly looking at electrical activity, but it may not be an LTP model. We can discuss further offline.

Live answer from Dr. Hickman: In terms of LTP from patch-clamp analysis, Dr. Lipton is correct that his team is not doing that. In terms of calibrating the slope, it has been accepted in many publications that maintaining the increased firing frequency for at least an hour provides a correlative for LTP. His team has done that.

Dr. Lipton: There are other reasons for excessive firing rate besides LTP, so it is something to keep in mind.

Live question from Dr. Hickman to Dr. Lipton: In your systems, are you looking only at activity? And just increased or lower activity?

Live answer from Dr. Lipton: His talk today showed the action potential firing rate. His team also does 1/f activity with bioengineers at Dr. Brad Voytek's lab at University of California San Diego to see a variety of parameters. They can see theta and gamma activity, and gammas are important for memory consolidation.

Possibility of modeling resilience with AI

Live question from Dr. Tsai to Dr. Lipton: Based on your model with AI methodology, are you looking at resilience? Do you think that can be modeled?

Live answer from Dr. Lipton: He thinks it can, but it may not be possible to look at resilience in terms of a centenarian in an organoid model because the organoids are immature. His team may see some other early signs, but the gene expression is different.

Organoid models may be good for very early disease, and early treatment is certainly a goal for AD. He noted Dr. Finkbeiner's earlier discussion of ways to environmentally age organoids and mentioned that his team is working on models of environmental factors such as pollution.

Recent advances integrating interneurons

Live question from Dr. Tsai to the group: Today's talks covered neurons, microglia, and other cell types, but not interneurons. Interneurons should be in the picture when discussing microcircuits or oscillatory activities. Are there recent advances integrating interneurons?

Live discussion:

Dr. Lipton: A group at University of California Los Angeles has fused dual organoids (a germinal matrix, iP inhibitory interneuron organoid, and a cortical organoid). His team is doing similar work to try to enrich for GABAergic interneurons, and they get a fair number from RNA seq data and electrophysiologic recordings.

Dr. Svendsen: Dr. Sergiu Pasca at Stanford and Dr. Lorenz Studer at Memorial Sloan Kettering Cancer Center have assembloids now. A key question regarding organoids is how to standardize their development so they are all high quality. Another question is how to efficiently vascularize an organoid: It is possible to get things into them but not to flow blood through them. Dr. Tsai and her team are close to solving these questions.

Dr. Lipton: He said they all need to work on perineuronal nets, extracellular matrices around iPSCs. They need these structures to affect gamma oscillations properly. There are features of perineuronal nets, but he has not seen a real one yet.

Dr. Finkbeiner: In his talk, he showed some data with interneurons in his trisomy 21 model. The interneurons do exhibit neurodegeneration phenotypes that are even slightly stronger than the cortical cells.

Dr. Tsai: She replied to Dr. Finkbeiner that after neurons adopt the interneuron phase, they can show certain features. She noted that it has been challenging to express the classical interneuron subtype markers in vitro.

Why AD drugs lose effectiveness over time

Live question from Dr. Hickman to Dr. Lipton: Why, in your opinion, do memantine and the other three approved AD drugs stop working after some time?

Live answer from Dr. Lipton: The disease progresses, and these drugs are stopgap measures. Newer AD drugs work much better.

A related question is the type of target needed for a drug to be disease modifying. Protecting synapses could be considered disease modifying because synapse loss is the strongest correlative for neurocognitive decline in AD. Protecting synapses may be the gold standard for AD drug development.

Questions from Zoom audience members

Potential for perfusion in the BBB components in miBRAIN

Question from Tracy Chung (Zoom chat) to Dr. Tsai: Do the BBB components in miBRAIN have the potential for perfusion? Could this bring the model even closer to ex vivo cell signatures?

Live answer from Dr. Tsai: Yes, her team is working toward this goal. They have now combined this chip with their miBRAIN system, and initial results show that it is possible to perfuse the iBBB embedded in the miBRAIN system. A next step is to take influx and efflux into the miBRAIN system to see whether certain biomarkers start to appear in the disease model.

Dr. Hickman showed how his team had developed a tight neuromuscular junction with expression of certain biomarkers. It is important to look at the maturity of the iBBB, in addition to TEER and other permeability assays. With the iBBB embedded in the miBRAIN, the question now is whether they can start characterizing the PK/PD properties of molecules.

Dr. Tsai mentioned her team's collaboration with Dr. Ed Boyden and his lab to screen a large library of recombinant viruses and for adeno-associated viruses (AAV) that have a better BBB penetration.

How to measure TEER in vasculature

Question from Xiufang Guo (Zoom chat) to Dr. Tsai: With the random distributing vasculature structure in the culture, how is TEER measured?

Live answer from Dr. Tsai: The TEER value she showed in her talk was from the iBBB system before it was embedded into the miBRAIN. Her team has not yet measured TEER in the miBRAIN after the iBBB was embedded.

iPSC-derived neurons to model the peripheral nervous system

Question from Subhash Kulkarni (Zoom chat) to Dr. Hickman: Could you explain the neurons you use for modeling for the peripheral nervous system? Are these iPSC-derived neurons?

Live answer from Dr. Hickman: Yes, the motor neurons his team is modeling for the ALS system are derived from iPSCs for the mutations and the wild type. They published the first protocol about 10 years ago and are using it now.

Screening for permeability across iPSC lines

Question from an anonymous Zoom audience member to Dr. Svendsen: Have you screened across permeability across lines?

Answer from Dr. Svendsen (Zoom chat): Yes, in the paper, we used three lines. It works if the differentiation of the BMEC is done right.

Best way to position chips seeded with iPSCs

Question from Anne Taylor (Zoom chat) to Dr. Svendsen: What is the benefit of having the iPSC neurons and blood vessels stacked in the z-direction vs. side-by-side, which is easier to stain and visualize?

Live answer from Dr. Svendsen: When seeding the chips, it is important to get the cells to stick, but it does not matter which way the chips sit. He finds the two layers (top and bottom) easy to work with.

Increased excitability due to loss of inhibition

Question from Anne Taylor (Zoom chat) to Dr. Lipton: Are you thinking of adding a percentage of inhibitory neurons to your MEA assay of activity? Increased excitability in diseases such as AD may be due to loss of inhibition.

Live answer from Dr. Lipton: His team published a paper (Ghatak et al) on eLife about a year or two ago, and they found that both types of neurons are the cause. Both the excitatory and inhibitor neurons have intrinsic membrane properties that are different.

Whole-blood perfusion in organ-on-chip systems

Question from Tracy Chung (Zoom chat) to Dr. Svendsen: Regarding perfusion of whole blood in these systems: You mentioned a focus on incorporating drugs into these perfusates, but has the disease-modeling potential of the perfusate been studied in these models? Potentially using samples from disease patients in healthy cell systems to identify systemic triggering components? This would be interesting, especially in the healthy but aged Edinburgh cohort.

Live answer from Dr. Svendsen: The isogenic approach for blood from the same patient must be done in the hospital because the patient is local. So his team may be unable to do that with the Lothian cohort. In addition, the lab must have the right safety protocols for using human blood, and anticoagulants must be added to the blood to stop clotting.

He noted that his team's process is detailed in the paper. A key point to keep in mind while modeling is that drugs behave differently in blood than in lab media. People can begin modeling with clear fluid, then use blood to get more refined. But if they get the same validated readout without blood, they do not need to overcomplicate the model.

Commercially available BBB-on-a-chip models

Question from an anonymous Zoom audience member: For brain-targeted nanoparticles for AD therapy research, can someone on the panel recommend a commercially available BBB or brain on a chip?

Live discussion:

Dr. Hickman: The Emulate system could probably be adapted for BBB because they have published on it. He asked Dr. Svendsen if Emulate marketed their system for BBB or just GI tract and lung at this point.

Dr. Svendsen: Emulate recently published a paper (in Science Translational Medicine, he thought) about their brain chip. This generic brain chip had dopamine neurons, and they did some synuclein work on it. They are working on a BBB model, but it is not yet available. People could order the chips from Emulate, then use his team's paper as a guide for creating their own BBB chips.

Sourcing the right brain microvascular endothelial cells (BMECs) is challenging because they are difficult to make and validate. They may have functional effects, but if people do RNA seq, the cells start expressing epithelial markers they should not have. But if the cells absorb the right drugs into the brain, the model is still effectively reproducing things that happen in vivo, even without a perfect RNA seq.

Dr. Hickman: His team typically shows functional data, but people often ask whether they did the RNA seq. He believes that measuring the function directly shows actual function, whereas RNA seq shows RNA expressions for potential function that may or may not happen. He asked Dr. Lipton for his thoughts.

Dr. Lipton: He believes both are important, such as a functional assay and the multi-omics approach. The combined approach provides multiple pathways to a function. People can show whether these pathways actually exist in the disease and then see whether each pathway has a correlating functional readout. He agreed with Dr. Hickman that the multi-omics approach alone is not good enough: A functional assay is needed.

Dr. Tsai: She agreed that the multi-omics analysis can help provide further insight into pathways and mechanisms and could uncover potential biomarkers.

Compatibility between iBBB and myelination culture

Question from Yifei Cai (Zoom chat) to Dr. Tsai: Are iBBB and the myelination culture compatible? How well do different cell types incorporate with each other?

Live answer from Dr. Tsai: They are compatible. One of her slides showed labeling of myelin basic protein along the axons and single-cell RNA sequencing from the miBRAIN culture. That shows the maturation of oligodendroglia and myelin basic proteins decorating the axons, and the BBB is definitely there. She attributed this possibility to the development of Neuromatrix.

Using Neuromatrix with the right supporting soft matrix somehow enables all the cells to assemble into a kind of in vitro unit. Then they further support each other physically or by secreting paracrine growth factors to promote further functional maturation of each cell type.

Translatability between AD/ALS mouse and stem cell models

Question from Satheesh Ravula (Zoom chat): Do any experimental drugs show translatability between the AD/ALS mouse model and AD/ALS stem cell models?

Live discussion:

Dr. Svendsen: There are no drugs that work for ALS and FTD. Right now, ALS has only two FDA-approved drugs on the market, riluzole and edaravone, which both work through glutamate toxicity mechanisms. Unfortunately, they extend lifespan only by about three months.

Positive controls—powerful drugs to slow neurodegeneration—would provide a platform that researchers could base other therapies on. It would be good to have a proteasome inhibitor or something that could successfully attack a mechanism of neurodegeneration. There are disease-modifying drugs such as memantine and L dopa, and several multiple sclerosis drugs that modulate the immune system. But after all these years, there is no positive control that gets at the core of neurodegeneration.

Dr. Lipton: For the peripheral nervous system, his colleague Dr. Jeffrey Kelly developed tafamidis for transthyretin (TTR) amyloidosis. So in principle, the data exists even though it is in the peripheral nervous system and heart, not in the CNS yet.

Session 3: National Center for Advancing Translational Sciences (NCATS) Tissue Chip Program

Session Chair: Zane Martin, NIA

Introduction

Suzana Petanceska, Director, Office for Strategic Development and Partnerships, Division of Neuroscience

Based on the comments and discussion from yesterday's meeting and focusing on the limitations with preclinical animal models, today we highlight the NIH's approach to increasing

the rigor and visibility of studies on animal models and their predictive efficacy in AD-related applications. Existing animal models are designed based on familial AD-risk variants and, therefore, fail to accurately replicate the features of sporadic LOAD.

To address this, the MODEL-AD program was developed to leverage genetic discoveries related to LOAD and generate specific phenotype/genotype mouse models (to date: >60 mouse models) using CRISPR knock-in technology. Translation-relevant models are subsequently developed by not only using humanized AD-related genes but also introduction of prioritized AD-risk variants. Additionally, genetic diversity is introduced by exposure to different environmental variables, thereby expanding the potential research focus of each model for various AD phenotypes. All findings related to the models are distributed to the research community via Jackson Laboratories and the AD Knowledge Portal.

Following model establishment, preliminary screening determines their molecular homology with human AD, followed by deep phenotyping of the most promising strains. This process is conducted in mice through 24 months of age and in both genders, with most of the associated analyses performed at multiple sites to evaluate/confirm reproducibility. In addition to knock-in models, there exists a variety of “strong-risk” strains that include coding and noncoding variants, as well as knockout strains.

These processes support the NIA/NIH Translational Research Pipeline for AD/ADRDs:

- 1) Establish and implement guidelines for rigorous phenotyping and preclinical testing in LOAD models using standards/rigor comparable to clinical trials;
- 2) Align complex datasets from models and humans to better inform model development, characterization, and utility for preclinical drug studies;
- 3) Provide a resource for standardized therapeutic efficacy testing of preclinical drug candidates that prioritizes translational biochemical and physiological endpoints (e.g. PET/MR) over behavioral measures using best practices.

Notably, transcriptomics and metabolomics alignments of human AD features with those of the established models have revealed age-based correlations between identified genotype/phenotype features. Additionally, we observed concordance of molecular features of the animal models with LOAD based on environmental variables. Specifically, models fed a high-fat diet revealed an increased likeness to the transcriptomic signatures of LOAD. Furthermore, these evaluations are performed along with metabolomics and proteomics analyses of both tissue and blood specimens, with the goal of producing translatable biomarkers critical for driving preclinical drug development.

Importantly, the preclinical testing pipeline includes a method for evaluating promising compounds by matching them with the most appropriate model based on the mechanism of action. This infrastructure is available to the community through the STOP-AD Compound Submission Portal, which allows submission of a *de novo* compound or repurposable drug

candidate. The pre-submission inquiry will be evaluated by a committee of experts and, if approved, moved forward for further assessment at no cost to the contributing investigator. In addition to the AD Knowledge Portal, we have launched the MODEL-AD Explorer, which houses all of the data from the various models and allows their visualization in a way that supports biologists and bioinformaticians in selecting the model best suited for their research question or preclinical therapeutic development program. Notably, MODEL-AD teams are not only generating new mouse models suitable for modeling LOAD but have also deeply characterized existing models (e.g., 5XFAD mice) across many data modalities in a longitudinal fashion. Furthermore, a course is available annually that teaches principles of rigorous experimental design for preclinical efficacy testing studies. The course is very hands-on, and we particularly recommend it for postdoctoral fellows, early career faculty, and even undergraduate and graduate students.

To address some specific comments yesterday on the historical utility of the current animal models, we did a deep search of the AlzPED Knowledge Base (now ~7-years old) in order to review the study designs presented in >20 years of the previous literature. AlzPED has curated summaries of published studies and “graded” the experimental design. There are 24 elements of study design that are fairly typical for preclinical efficacy testing studies, with nine of these considered core elements of rigorous scientific design. Our review found that the reporting frequency across some of the most important elements of study design is poor. This reinforces the fact that although no model is perfect, they are simply meant to be useful; however, the data suggest that what largely contributes to the poor predictive value of animal models is poor rigor in study design. The 5-year trend in use of the majority of these nine elements remains poor, and we believe that if these things are not reported, it is highly likely that they have not been conducted. As noted yesterday, we hope that iPSC models do not suffer the same fate as animal models in AD. To avoid this, we need to reinforce the use of rigorous study designs and ensure transparent reporting at the outset. As a brief aside, I would like to note that researchers can also submit their unpublished studies and particularly those that may have arrived at a negative finding, given that it is very challenging to publish such results in journals due to the bias for publishing only positive findings.

Overview of the NCATS Tissue Chip Program

Passley Hargrove-Grimes, NCATS

The development of new drugs is extremely slow and costly, with a single drug potentially requiring >10 years and \$2.6 billion for market realization. Although these data are from 2016, the numbers continue to worsen, reinforcing the need for alternative tools to improve this process. Despite significant developments in computational and *in vitro* biology and toxicology, only ~7% of human diseases actually have available therapies. This suggests that current preclinical models used to test drug safety, efficacy, and toxicity are unable to accurately replicate human organ and tissue responses. This highlights the potential utility of tissue/organ chips to transform these areas of research.

Tissue/Organ-on-a-chip (or electrophysiological systems) describes a tiny bioengineered device capable of recapitulating key functional aspects of organs and tissues. They range from about the size of a USB thumb drive to that of multi-organ systems (comprising a 96-well plate) and present three major features that separate them from other conventional models: a 3D tissue configuration similar to that of a normal human organ; multiple cell-type composition; and the inclusion of biomechanical forces, such as those on the lungs during breathing. Moreover, tissue chip technology is more physiologically relevant than conventional models owing to their ability to reconstitute organ microarchitecture and dynamic tissue–tissue interfaces, as well as control the fluidic environment and tissue oxygenation and nutrition. Furthermore, dynamic mechanical cues can be evaluated along with spatiotemporal chemical gradients. Notably, we have started adding circulating immune cells, features of the microbiome, and biochemical signaling molecules and metabolites.

The mission of NCATS is to remove or bypass scientific and operational hurdles that prevent the rapid development of therapeutic options for patients. The NIH Tissue Chip Program, as a banner program for NCATS, has grown rapidly in the previous 10 years and addressed multiple areas, including safety and toxicity, disease modeling, and efficacy testing, and gradually moved into the personalized medicine space.

The Tissue Chips for Drug Screening (TC 1.0) program was established in 2012 and subsequently demonstrated its efficacy for predictive safety and toxicity testing. We recently moved into disease modeling and efficacy testing, and our Tissue Chips in Space program has accelerated our research using tissue chips under a microgravity environment. Additionally, the Tissue Chips for Disease Modeling and Efficacy Testing initiative (established in 2017) has moved forward through partnerships with the NIDDK (evaluating their utility to model diabetes) and the NIBIB (creating functional immune systems on chips). Moreover, we are working with the HEAL Initiative to help end long-term addiction by using tissue chips to model nociception, addiction, and overdose, and in the previous 2 years, have moved into the personalized medicine space via clinical trials.

All of our initiatives are biphasic. In the first phase, teams develop and validate *in vitro* disease models of both rare and common diseases. In the second phase, the teams demonstrate the utility of these models to identify novel treatment mechanisms. The list of diseases is extremely broad in terms of the tissues and organs being studied; however, I will focus today on a project from Don Ingber's group at the Wyss Institute at Harvard.

Don's group studies lung infection and COPD and is focused on developing a human lung alveolus chip that contains cells from the human lung air sac and blood vessels. They have successfully applied cyclic stretching to the chip in order to mimic physiologically rhythmic breathing and are working with Cantex Pharmaceuticals, a clinical-stage company, to assess the efficacy of a repurposed drug to treat inflammatory lung diseases, including those related to COVID-19 infection. Mechanistically, their findings demonstrated that a small-molecule inhibitor of the RAGE receptor significantly blocks the production of proinflammatory cytokines following viral infection, thereby reducing lung inflammation. Cantex Pharmaceuticals will

conduct a phase two clinical trial to test this drug in hospitalized COVID-19 patients with severe disease, as well as patients with COPD and steroid-refractory asthma. Notably, this small-molecule RAGE inhibitor was previously tested on AD and diabetic nephropathy patients and showed high levels of safety in phase three clinical trials with >2,000 patients. This is exciting for the NCATS, because it is the first submission of tissue chip data as an IND proposal to the FDA and represents a significant leap forward in terms of potentially qualifying tissue chips as a drug-development tool.

The clinical trials-on-a-chip initiative addresses the slow speed and high cost of drug development driven largely by high attrition rates in clinical trials and a likely a product of poor trial design. This situation is likely exacerbated in trials related to rare diseases, neural diseases, and diseases that affect vulnerable populations, such as pediatric populations, where trial enrollment and recruitment are extremely difficult. Importantly, for many patients, enrollment in one clinical trial often precludes their future eligibility for other trials, even if they received no therapeutic benefit from the current clinical trial. Therefore, the goals of this initiative are to 1) inform clinical trial design and execution while establishing patient-recruitment criteria that help stratify patients based on their response (or lack thereof) to candidate therapeutics and 2) develop clinically relevant biomarkers. Our goal is to change the way clinical trials are conducted and perhaps shorten the time required for their completion by using tissue chips as patient surrogates.

In the first phase of this initiative, teams were tasked with developing and validating rare and common disease models using patient-derived cells (mostly iPSCs). We understand the limitations that exist with using iPSCs in place of primary tissue; however, the goal was to use a renewable cell source that could be seeded onto the chips. Almost all of the teams have since transitioned successfully to the second phase, where they are now testing potential drugs for efficacy and safety and the establishment of clinical trials. Notably, all of these groups compare their tissue chip data with clinical data in order to broaden the understanding of the utility of tissue chips for clinical trial-design planning and execution.

Of particular relevance to the NIA, the project overseen by Yu Shrike Zhang has established a premature vascular aging-on-a-chip model at the Brigham and Women's Hospital. This project is focused on Hutchinson–Gilford progeria syndrome, which is an extremely rare (affecting ~1 in 18 million people) and fatal syndrome that manifests as progressive premature aging with no known cure or treatment. This is an autosomal dominant disease, in which death usually occurs between the ages of 14 and 15 from accelerated atherosclerosis. The goal of this project is to develop a tissue chip model containing fibroblast smooth muscle cells and endothelial cells derived from patient iPSCs for drug screening.

The team initially worked in the first phase to engineer a thermoplastic chip and apply a range of fluid shear stresses and mechanical strains. They are now moving into the second phase, where they will develop 15 patient-specific chips with inline sensors and test a variety of different retrospective and prospective drugs. The study cohort of 15 cases represents from 6%

to 7.5% of the global population for this disease, making this assessment unique, especially for a rare disease.

The ultimate goal with this program is to increase confidence in this technology. Since 2012, we have demonstrated an ability to capture organ structure and function on tissue chips, as well as their ability to respond accordingly to reference compounds with known toxicity in humans but not in animals. We subsequently moved into analytical-stage validation in 2017, where we funded these independent tissue chip-testing centers, as well as establishment of the the MPS database, to evaluate the chips for robustness, reproducibility, reliability, and relevance using specific compounds, biomarkers, and assays.

We have since moved into industrial validation to promote the use of this technology by both industry and regulatory agencies with proprietary sets of compounds. Our tissue chip-testing centers have now evolved into their own privately funded entities. Javelin is now a contract research organization (CRO) business model, whereas Texas A&M represents a pay-for-play model that incorporates leaders in the field from academia, government, and industry. The NIH has also supported a number of spin-off and startup companies centered around tissue- and organ-on-chip technology. We believe that democratization of this technology will allow pharmaceutical companies and other end users to choose among ~30 different companies for CRO-like services and purchase various platforms. Moreover, increased confidence in this field has increased the use of tissue chips by the FDA, as well as our pharmaceutical partners. The FDA has established an alternative methods working group engaged in advancing useful tools, such as tissue chips, to support alternative methods for traditional toxicity and efficacy testing or consider them as a novel drug-development tool.

Additionally, we've worked extensively with the IQ MPS Affiliate, a consortium of pharmaceutical companies, on their co-authorship of a series of organ-specific manuscripts outlining the guidelines required for context of use in applying tissue chips for their own decision making. These organotypic manuscripts have focused on liver, kidney, lung, gastrointestinal, skin, and cardiovascular applications, with additional articles in the planning stage to address tissue chip uses for ocular toxicity, disease modeling, the blood–brain barrier, and the immune and reproductive systems.

We have three major goals that we would like to accomplish in the future: 1) sustain and increase the utility and adoption of this technology, 2) work toward global harmonization of regulatory use and standardization of these platforms, and 3) ensure the appropriate training of the next generation of MPS and tissue chip scientists and practitioners. One way that we are trying to accomplish some of these goals is through formation of the MPS World Summit, which we are funding for the next 3 years. The first summit met ~1.5-months ago in New Orleans, and the next will be held in Berlin in June 2023. We hope that this will support the establishment of an international MPS professional society to promote the training of future scientists for tissue chip development.

The success of NCATS has been a direct result of collaborations, as well as partnerships with other NIH institutes and centers. Their guidance and expertise have enabled our progress. We are currently making inroads with leaders in the field in other countries, including Europe, Asia, and Australia.

Microphysiological Systems Database Center

Mark Schurdak, University of Pittsburgh

My name is Mark Schurdak, Director of Operations at the University of Pittsburgh Drug Discovery Institute and co-founder of BioSystics, a company formed to continue development of the MPS database. We have since transitioned the name of the database to the BioSystics Analytics Platform based on its expanded scope as a complete data and modeling pipeline from discovery to human preclinical trials.

The concept of patient digital twins describes computational models of individual patients created from both their clinical data and data from MPS models using their iPSC-derived cells. Patient digital twins enable an understanding of disease mechanisms at the individual patient level, with this directly supported by the use of patient-derived iPSCs for model creation. This concept also enables the study of mechanisms of disease progression, drug activity, and drug toxicity according to different biological and genetic backgrounds, which facilitates the design of optimal precision therapeutics and selection of cohorts for clinical trials.

The BioSystics platform captures, manages, and analyzes *in vitro* and *in vivo* experimental data, as well as aggregates information from publicly available sources, including databases housing clinical data and drug compounds. Additionally, the platform includes tools capable of data analysis and computational modeling and provides information on the experimental models, compound activity, disease mechanisms, and compound-specific mechanisms of action. The system enables translation of this information into actionable knowledge in terms of designing optimized experimental models; improving predictions of absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox); predicting therapeutic drug candidates; designing clinical trials; and ultimately creating patient digital twins. The platform addresses the challenges inherent in the increased complexity of live-cell and tissue experimental models used for ADME-Tox disease modeling, specifically in how large datasets are managed and meaningful insights extracted.

During the development of the tissue chip testing center, the database evolved from a simple data repository to a streamlined workflow that guides the establishment and implementation of study protocols, followed by statistical analysis of the outcomes to determine reproducibility and the generation of computational models. Application of this system in a centrally located database supports easy sharing of data in a secure manner.

There are two versions of the database. The one currently available is the cloud version (i.e., our academic version) that was developed alongside the tissue chip testing centers. This version is accessible online, with data access controlled by the data provider. Data can either be kept

private or shared with collaborators/stakeholders; however, we would eventually like to have the data made publicly available to benefit the research community. The second version currently under development has the same functionality as the academic version but is designed for installation behind company firewalls, where data access is controlled by corporate policies. To date, the system has >400 registered users with access to data supplied by 27 centers. Furthermore, it currently includes 163 publicly accessible studies covering 12 different organ systems.

The interface is a simple, icon-driven graphical design that provides an interface with study components. Three of the icons comprise aspects required to establish a study protocol (e.g., the models being used, the compounds employed, and the samples that will be used to establish the model). The other three icons are used to access studies currently housed in the database and perform data analysis and computational modeling.

The database currently contains 150 experimental models covering 23 organs. The associated data include a link to the developer of the model and possibly an outline of the model with more detailed information. For example, this could include locations on the model for the introduction of cells, application of reagents, sample collection, and/or image acquisition. Additionally, we include information on different versions of the model, as two different models may be identical, except for the cell-type used for model establishment. In regard to compounds, the database includes links to external repositories that extract information on compound properties (e.g., bioactivity and preclinical/clinical findings) and clinical trials in which that compound was used.

This system also has access to the FDA Adverse Events Reporting System (allowing insight into adverse events associated with various compounds) and the Centers for Disease Control (to obtain data on compound usage). Importantly, normalizing compound-specific adverse events against their usage offers a more accurate picture of the relative activity of a compound with reported adverse events. This can aid in model or study design when selecting a range of toxic activities to calibrate or validate a given model.

The system workflow guides users in entering the appropriate data, including setting up the groups being tested and labeling the chips. For plate-based assays, the workflow will aid in the design of plate maps for the assay and determination of what analytes or targets will be measured, as well as how those measurements will be obtained. Furthermore, when analyzing study outcomes, the system includes graphing tools that facilitate data visualization (either raw data or those normalized against a given control). Furthermore, because the data are housed in a database, comparison of outcomes between studies or models is relatively straightforward, allowing a more in-depth evaluation of possible pros and cons for a given study approach. One requirement was an ability to assess reproducibility across studies and/or laboratories. The system enables this analysis and assigns grades of “excellent”, “acceptable”, or “poor” reproducibility based on the statistics. Notably, “poor” does not necessarily mean that a study is bad but rather that a closer assessment of the data is likely in order to determine possible

shortfalls. The system also offers an ability to perform this analysis by drilling down into the details.

We've implemented power analysis to both enable evaluation of the statistical power of results and support the design of subsequent experiments based on those studies. For example, if a power of 0.8 is required, a time-series assay involving a different number of days will change the number of chips required to obtain that statistical power.

In cases involving the use of -omics and/or microarray data, we included flexible tools that establish gene-expression thresholds in order to limit the search space to only the most interesting or statistically relevant genes. Additionally, gene clustering is an available analytical option using either raw or normalized expression data. Users can subsequently extend the analysis to search various databases and ascertain pathway or ontology enrichment in a given dataset in order to obtain biological insights.

As noted, almost any data type can be analyzed, including mass spectrometry, plate-reader, and -omics data; videos; and images and their associated metadata. Therefore, when analyzing quantitative data, the images used to obtain the quantified results can be evaluated. Additionally, we have integrated cytokine profiling and computational modeling of liver clearance, which enables calculation of the intrinsic clearance of compounds using a liver model and potential prediction of blood levels under a given dosing regimen.

We've also developed a COVID-19 disease portal that provides information on different models and resources to allow the establishment of studies and/or determine reproducibility standards. As part of this portal, we included an iPSC portal, which specifically lists the iPSCs or iPSC-derived cells that have been used successfully used in COVID-19 studies.

The platform can accommodate *in vivo* studies specifically by allowing direct comparisons of *in vitro* versus *in vivo* outcomes. We provide a web API for programmatic access and an extensive help page for people accessing the database. We're currently working on integrating clinical concordance analysis for liver toxicity and designing a preclinical trial portal that would represent a repository for data from clinical trials on chips. The BioSystics platform is an NIH-supported scientific data repository specifically designed to manage and share data from tissue chips and other complex models. Therefore, it is ideally suited for the changes being implemented to the scientific management and sharing policy in January 2023. If help is needed in preparing any data-management and -sharing plans for compliance with this policy change, we are available to provide this help upon request. Furthermore, we have a number of webinars available through the help menu on the platform and are happy to schedule individual interactive online training or demonstrations.

Q&A for Session 3

Live Discussion

Sustainability and funding model

Question from Suzana Petanceska: What is your sustainability and funding model? Work is

involved, I'm assuming on your end to curate the data or collaborate with the teams on bringing the data in, do the contributing teams support, fund that, or what is the model, of?

Live answer from Mark Schurdak: Currently we've set up BioSystics, Inc. as the analytics company for further development and support. And we're currently working on a couple of different models at the moment. I'm not at liberty to say publicly, but we are working to actually develop the, behind the firewall version, as it were, the on-premises version, which would support, financially the development of the platform. But it would also allow for the academic users to still use it at no cost.

Alignment of database

Question from Zane Martin: The database, you mentioned that you align with human data as well. Can you talk on that, in how that is aligned, such as Omics, biomarkers, stuff like that?

Live answer from Mark Schurdak: For the preclinical trial portal, or just in general? Because the database, as I mentioned, it accesses a number of public sites to bring in the human data, that you could, so you don't have to go through many Google searches to try to find the information that you need to set up a study. So we try to bring that all into one place that you can do that here. So with the portal, we're trying to design that, so that we can pull in the clinical data, for example, and then match that up to the MPS data. That we haven't implemented yet. That's something down the road.

Aging component of diseases

Question from Zoom Chat: Besides immune system playing a key role in homeostasis, the biggest problem is that many diseases of interest, including AD, have a significant aging component. Given that humans living for 80 years on average, how do you add that component?

Live answer from Passley Hargrove-Grimes: Two things. The space program has made some really great strides in terms of that. For example, kidney stones take years to develop on earth, but we've been able to create kidney stones up in space, through accelerated aging and micro gravity, very rapidly, within a matter of weeks. And so we feel like the space program has contributed a lot to our understanding of accelerated aging.

Also, NASA, along with NIH and a few other institutes, have created a long life tissue chip program, whereby they're using tissue chips to try to create functional tissue chips for six months or longer, to help try to address this question. We most likely will never be able to keep tissue chips alive for 80 years, but we hope to make some inroads by taking tissue chips out beyond just the typical kind of four week stance that we normally do.

The immune system component is something that we're still lacking. And it is something that we recognize is going to be a lot harder to add into these systems. BARDA has an immune chip

plus program, but they're really interested in taking chips that are really ready to be commercialized, that have these immune chip components, so that we can truly understand more facets of the immune system and immunosenescence and immune ageing.

Longevity tissue chip

Question from Zoom Chat: Is the longevity tissue chip specific for one tissue type or do you have multiple tissues?

Live answer from Passley Hargrove-Grimes: This is going to actually be multiple tissues. We're looking at a lot of different tissue chips. So eight research projects to extend longevity of tissue chips to six months. Some of the projects will include:

- a multicellular integrated model of the human brain.
- a neurovascular MPS to model chronic inflammation related neuro generation.
- stressors of atherosclerosis and using mRNA therapeutics to combat that.
- a multiorgan tissue platform to model the response of human tissues to various stressors
- a multiorgan repair post hypoxia
- radiation exposure and engineered heart and vascular tissues
- an extended culture of a kidney MPS to model acute and chronic exposures to drugs and environmental toxins.

Most common biological biomarkers to test

Question from Zoom Chat: What are the most common necessary biological biomarkers we must test to evaluate the potential platform for safety on animals before the trial?

Live answer from Passley Hargrove-Grimes: Biomarkers are going to be really distinct to each organ and tissue system. And so we're not going to probably be able to find biomarkers that are kind of homogenous, I should say, between perhaps the heart and the liver, or the liver and maybe the brain. And so a lot of these teams are coming up with really elegant ways to find biomarkers and then to test them within these tissue chips. And so, we're not going to probably have a one size fits all in terms of biomarker discovery. It's going to probably be very unique and tailored to each specific organ system.

Live answer from Mark Schurdak: To answer that question really is to get the data, the clinical data and the chip data together and say, well, what are the correlates between the disease and the clinic and what I can be measuring? Because obviously you can measure a lot more in the chip than you can in the clinic, at least down at the molecular level. And so yes, I agree, that's going to be a case by case in terms of disease specific assessment. But putting the clinical data together with the chip data is going to be critical to being able to identify what those biomarkers might be.

How does postmortem drive cell lines

Question from Zoom Chat: Patient derived iPSCs have obvious benefits for studying disease

pathology. How does the use of postmortem drive cell lines such as astrocytes and microglia compliment this? Would this address the aged in vitro model question or does it compound additional considerations?

Live answer from Passley Hargrove-Grimes: I think postmortem derived cell lines will be really beneficial. I know that you all had Clive Senson speak yesterday and I don't know if he, I don't think he's here, but he is actually taking a lot of, iPSC lines from a patient cohort called the Lothian Birth Cohort. This is a group of Scottish individuals who lived well into their 80s and 90s, who developed absolutely no CNS disease pathologies. And so they were of sound mind, you could say, when they kind of were elicited to give their, iPSCs. Clive's group is doing a lot of work as well, with postmortem tissue and trying to kind of understand how they could relate that to some of the responses they're seeing within their tissue chips.

I think this will help us understand the aged component, but it will make things more difficult as well. We can't tease apart all the variables that are going into this. Another major problem is that iPSCs are lovely, but in a lot of iPSC derived kind of CNS tissues, these cells aren't really functionally or phenotypically mature, and we can take them out for a year or longer and we still don't see the same level of maturity that we would see with primary tissues. I was just on a call yesterday where they were showing us some, iPSC derived sympathetic neurons, and they've done RNA sequencing analysis. And the RNA profile is completely different than what we would see in primary sympathetic neurons.

And so the major question remains, do you need a similar RNA profile or do you need something to just be functionally equivalent? Right. And that's going to be very hard to tease out, especially when we now know that the RNA profile doesn't always really be... It's not always correlative to the protein itself. And so that's, it creates a lot of confounding factors.

Live answer from Mark Schurdak: The iPSCs obviously, are less mature and that's been an issue for, iPSC all along. I do think, however, that protocols such as trans differentiation that were discussed yesterday, may offer a better model because it does retain a lot of the age related state of the cell. And I think that's something that people should be really looking into, to get these aged cells, especially for neurons, because you can't take a postmortem neuron and keep it forever, for example. You do need to have a readily supply of cells for experimenting.

NCATS' mechanism to qualify a newly developed tissue

Question from Zoom Chat: Does NCATS have a mechanism to qualify a newly developed tissue chip for use by other groups?

Live answer from Passley Hargrove-Grimes: The problem with tissue chip development at this stage is that we cannot, as a group, as a field, agree upon the word qualification or standardization. We do not have harmonization in terms of what regulatory officials want, what end users want, what developers want. We really are leaning on the FDA, which you'll hear

from in your next session, to create these types of guidelines for qualification. The FDA has created the IStand program to try to see if some of these tools such as tissue chips might be able to be qualified as drug development tools.

But every single tissue chip that's going to be qualified and validated is going to only be validated for one or two specific contexts of use. We're never going to have a tissue chip that can do everything. No model is perfect. Every model just answers basically one or two specific questions. At this point NCATS does not have a mechanism, nor do we feel like it's our job to qualify these for use by other groups. We hope that these groups can go to the MPS database, they can go to BioSystics online, they can look at the data themselves, they can say, "Oh, okay, well, this seems to be reproducible. Maybe we'll try to reproduce it in our lab and see if it works in our hands."

We're really hoping that that qualification and validation will take place in the future, but we're going to have to agree as a community on what it means for something to be successfully validated. That's something that, because we're such a young technology, we have not been able to do.

Live answer from Mark Schurdak: I think what will help there is this type of concordance analysis, where a model, a relatively large number and range of compounds which have clinical activity. Are tested in the models. And right now I don't see that happening a lot, because a lot of these are very expensive experiments to do, and you can't run 100 or 200 compounds in a lot of these models to generate that type of data. But I think that's the type of data that's needed to be able to make this type of assessment.

Session 4: Regulatory Perspective on Microphysiological Systems for Therapeutic Development

Session Chair: Nadezda Radoja, NIA

FDA's Predictive Toxicology Roadmap for New Predictive Toxicology Methods in Regulatory Reviews

David Strauss, Center for Drug Evaluation and Research (CDER)

There are multiple opportunities for MPS to impact the regulatory evaluation of drugs in terms of predicting safety in patients and drug-drug interactions. Simultaneously, there exists potential to reduce the number of clinical drug-drug-interaction studies and clinical trials, as well as predict drug-specific efficacy in patients. I'm going to provide examples where *in vitro* data rather than MPS data were used to not only predict efficacy but approve drugs and expand the labels for certain rare diseases.

In terms of safety applications, one context of use that can be applied is advancing development-level drugs that show potentially false-positive safety signals. The Innovation and

Quality Consortia conducted an industry-wide survey for the attrition of small-molecule drugs due to unacceptable toxicity in animal studies. During the late discovery phase, these occurrences were most prevalent in organ systems.

I'm now going to provide an example in which complex *in vitro* model data were submitted to the FDA as part of a new drug application (NDA) and where these data contributed to the overall decision-making process. This was for a class of drug in which other drugs in that class were discontinued from clinical development due to liver toxicity, as well as reports of elevated liver enzyme levels in rat studies. The sponsor conducted experiments using complex *in vitro* models with 3D spheroids combined with *in silico* modeling. The methods were able to reproduce the observed liver toxicity observed in the other drugs and suggested that the new drug showed a significantly reduced risk of liver toxicity. These data contributed to the liver toxicity assessment as described in the supervisory pharmacology toxicology review for the NDA and were linked to the approval package and the public review.

In terms of applications in clinical pharmacology, the problem with reducing clinical drug–interaction studies is that it is impractical to evaluate every drug combination in clinical trials. FDA guidance documents how *in vitro* studies in combination with physiological pharmacokinetic modeling, PK/PD modeling, and *in silico* modeling inform the need to conduct clinical drug–drug–interaction studies. However, there remain limitations that also present opportunities. Conventional *in vitro* models have limitations, as they underpredict clinical effects. Additionally, there are limitations with evaluating transporter-mediated drug–drug interactions. These represent potential opportunities for MPS to fill in some of the gaps and potentially reduce the need for clinical drug–drug–interaction studies beyond the current paradigm or at least alter the timing of such studies to allow their delay until later in development.

In terms of efficacy, *in vitro* models for expanding drug approvals for rare diseases have been used previously. Rare diseases introduce challenges in drug development. The small number of patients coupled with the thousands of genetic variants precludes opportunities to enroll a sufficient number of patients required to test the efficacy of a drug against all variants. In such situations, an innovative approach would be to test drug efficacy in cell models harboring each genetic variant. This was successfully employed in a study on cystic fibrosis, where a specific drug had been approved for 10 genetic variants but was subsequently expanded to approval for 24 additional variants based on cellular models in the absence of clinical trials. Additionally, there was a clinical trial for a new drug targeting Fabry's disease that included 63 patients with 40 genetic variants; however, after leveraging *in vitro* data and evaluating different genetic variants in an *in vitro* model, the drug was ultimately approved for use against 348 genetic variants.

The labels for these drugs included indications for use that referred to drug responsiveness based on the presence of a specific genetic mutation according to clinical and/or *in vitro* assay data. In these cases, extensive assays performed in FDA laboratories were critical for assessing the quality and reproducibility of the results. In many cases, we did not actually perform the experiments but rather received the raw data and were able to reproduce the findings.

Moreover, we applied a level of review normally employed for patient-level data from clinical trials. There was a summary publication in *Clinical Pharmacology and Therapeutics* that described this process for each of these two drugs.

In terms of the liver MPS, the FDA published a review on applications for predicting and evaluating drug effects using liver MPS and then another publication on characterizing the reproducibility of liver MPS. We specifically performed comparisons between using MPS data versus 2D cultures and 3D organoids. The question was whether criteria for ensuring the reproducibility of results could be developed to allow MPS to be used for regulatory applications in drug development.

In terms of hepatocyte function, we assessed the SIP 3A4 activity between the liver MPS, 3D spheroids, and sandwich cultures (the traditional 2D method). The hepatocytes in the MPS were more functionally stable than those in other culture platforms. The SIP 3A4 activity and albumen secretion remained prominent for >18 days, and functional decline was delayed with the liver MPS relative to that observed in the other systems. In terms of quality control based on assaying the culture media, we collected culture medium supernatant samples and looked at outcomes before the experiment and after excluding statistical outliers, followed by assessment of microscopy results after disassembling the system. The findings suggested the importance of establishing quality control criteria that enable exclusion of specific wells in advance of running a drug experiment.

The liver MPS reproduced the hepatotoxicity of a drug withdrawn from the market due to causing idiosyncratic acute liver failure and death. Co-dosing with lipopolysaccharide to induce inflammatory signaling and administration of trilofloxacin resulted in cytotoxicity, whereas levofloxacin administration did not produce this effect. This suggested that the liver MPS detected inflammation-induced drug toxicity. Subsequent assessment of reproducibility between two sites using different batches of Kupffer cells identified unique quality control criteria for those specific cells. This again provided a proof of principle for establishing quality control criteria, which is highly important when answering regulatory questions.

We developed general considerations and recommendations for establishing quality control parameters to ensure proper assembly and preparation of functional systems and appropriately testing cellular properties to enable the intended system use. Subsequent papers outlined examples of the liver MPS-development guidelines for safety risk assessment and provided guidance on best approaches to benchmark liver MPS based on three stages of characterization. Qualifying an MPS or other drug-development tool for regulatory use depends on the context of use.

We also performed applied research assessing differences in drug response between 2D and 3D approaches to culturing iPSC-derived cardiomyocytes, evaluating contractility endpoints, and assessing calcium-cycling endpoints. Additionally, we performed work on a heart–liver combination system that was developed at UC-Berkeley to characterize the functional reproducibility of additional liver and heart MPS that utilize iPSC-differentiated cells for testing interconnected heart–liver systems. This specifically looked at the effects of liver metabolism,

drug interactions, and cardiotoxicity or dual liver–heart toxicity. We have since initiated work on different lung MPS. To date, MPS have proven capable of yielding reproducible results when the system-preparation and drug-administration and measurement schedules are carefully planned.

Discussion on material presented at the FDA science board meeting on advancing alternative methods for regulatory use.

Transforming toxicology is a key FDA goal that was highlighted in 2011. There was an FDA predictive toxicology roadmap released in 2017, and there was a publication describing the goal of advancing new alternative methodologies at the FDA in 2021. The FDA has multiple working groups overseen by the Office of the Chief Scientist. The toxicology working group, alternative methods working group, and modeling and simulation working group are relevant to advancing new alternative methods. There are multiple national and international collaborations, including those with the NIH, different centers, and NCATS in the MPS space.

Regarding international collaborations, working with other global regulators and the International Council for Harmonization (ICH) is critical for introducing alternative methods into drug development. Among these methods, advances in systems biology, stem cell biology, tissue engineering, applications that improve the ability to predict risk and efficacy, and MPS promote further steps forward. These advances can aid faster drug development and commercialization while preventing products with increased toxicological risk from reaching the market.

However, multiple steps are required to translate these new technologies into regulatory use and maintain the same standards of safety, efficacy, and quality in FDA-regulated products. Although we are unable to replace all animal testing, there are opportunities for alternative methods to replace, reduce, and refine animal testing for specific contexts of use. In the President's FY2023 budget for the FDA, there is a proposal for new funding to implement a cross-agency alternative methods program in order to promote the adoption of alternative methods to improve the productivity of non-clinical testing and streamline the development of FDA-regulated products.

It is necessary to qualify these alternative methods. Using medical development as an example, developers of medical products can submit data derived from alternative methods as part of an IND, device, or marketing application; however, the suitability of the alternative method would need to be evaluated in parallel. Unfortunately, there is typically not enough time to do this, and this process introduces significant uncertainty for the developer. Therefore, qualification describes a process that allows for an alternative method to be endorsed by the FDA in advance for a specific context of use. A qualified context of use defines the boundaries within which the available data adequately justify use of the tool. This is a similar concept to drug or medical device indications for use, which describe what types of patients for which a given therapy is indicated. After completing the qualification, medical product developers can then use the alternative method for the qualified context of use with confidence that it is an acceptable method.

We have current FDA qualification programs in the Center for Drugs and the Center for Biologics (Combined Drug Development Tools Qualification Program). These include biomarker qualification and the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program. Notably, MPS or other *in vitro* methods can go through the existing biomarker-qualification program in cases of biomarker-based outputs.

In the Center for Devices, there exists the medical device development tools qualification program, which includes a non-clinical assessment model category. For the drug development tools qualification publication programs, all information about submissions to the program are public according to the transparency requirements of the 21st Century Cures Act. As part of this transparency, all FDA responses to letters of intent are made public on the FDA website regardless of acceptance or denial. The FDA is currently assessing whether qualification programs are appropriate in other FDA products areas, including foods, veterinary medicines, and tobacco products.

As noted, the IStand pilot program is designed to expand drug-development tool types to those outside the scope of other programs, including MPS for assessing safety or efficacy questions. As *in vitro* methods, MPS can undergo biomarker qualification. For medical devices, the Center for Devices and Radiological Health qualification program is a nonclinical assessment model that measures or predicts device function or *in vivo* device performance.

The proposed FY2023 budget program includes guidance to stakeholders for developing alternative methods. This guidance involves the qualification process, topical guidance on specific safety or development areas, guidance on assessing the credibility of specific types of alternative methods, and what to include in regulatory submissions. For the Center for Devices, there is draft guidance on assessing the credibility of computational modeling and simulation in medical device submissions. In the Center for Drugs, we have guidance on physiological pharmacokinetic analyses. These elements of guidance describe the expectations of how data are to be presented in IND or NDA submissions.

Since 2005, regulatory guidelines have relied on a nonspecific test for predicting drug-induced abnormal heart rhythms. A new approach was introduced involving a comprehensive *in vitro* pro-arrhythmia assay initiative using laboratory cell-based models and their integration into a computer model for predicting pro-arrhythmic risk in patients. Together with the Health and Environmental Sciences Institute, we collaborated on a study of devices for assessing iPSC-derived cardiomyocytes, with this study published in *Cell Reports* in 2018.

The proposed New Alternative Methods Program has a goal of spurring the adoption of new alternative methods for regulatory use that can address the three Rs and improve the predictive efficacy of nonclinical testing. As noted, we cannot develop and implement alternatives alone but rather need to work with our partners. Through this initiative, we want to expand our qualification processes, develop policy and guidance to streamline qualification and implementation, and fill information gaps for the applied research.

At the science board meeting last month, we discussed how we plan to seek input from the science board and how the agency can enhance its existing approaches to support the development, qualification, and implementation of alternative methods for regulatory use. We plan to charge a science board subcommittee to work on this topic.

Q&A for Session 4

Live discussion

Question from Nadezda Radoja: When you are thinking from regulatory perspective, what would you put as regulatory priorities when you are thinking about developing MPS for certain use? When you say that context of use, and letter of intent, and qualification package, and then full qualification package, if you can break that down into more... What are the things that we all should think about?

Live answer from David Strauss: Thinking about one, is trying to understand what are the bottlenecks in drug development, regulatory decision making. What are the limitations of certain technologies? And can we get it down to a narrower question? Maybe it's a certain class or classes of drugs that have a... If we're thinking in the toxicity space. Or it's figuring out an understanding if you have this signal, how do you de-risk this specific potential safety signal that emerged. Thinking narrower. And then, how would you develop a robust set of studies to support qualification for that context of use? That's much more likely to be successful. You can always expand the context of use further down the road.

Question from Nadezda Radoja: Some organ or tissue models require one type of cells, primary cells, which is always good to go. But sometimes you have to, because of some limitations, to go with iPSC assays. Can say something about flexibility of the agency to review a lot of information, data, maybe systematic literature reviews, as a package of these qualification applications?

Live answer from David Strauss: We have received submissions into the IStand program, and it is a pilot program right now, and that it's not really funded specifically through any mean, but we are receiving submissions. The example I talked about in the in vitro method, using a iPSC assay, not a full MPS system, but for developmental reproductive toxicity. So that was accepted into the biomarker qualification program because there are biomarker outputs from this in vitro cellular system.

Regarding the flexibility of cell types, we're not limited to one cell type or another, but it has to be defined and reproducible, whatever the method is, and you have to generate that evidence to support the context of use for what you're seeking qualification. But there's definitely flexibility beyond that.

Appendix A: Agenda

All times listed are Eastern Daylight Time.

Day 1: Tuesday, July 19, 2022

10:00 a.m. Introduction: National Institute on Aging AD Translational Research Program, *Lorenzo Refolo and Suzana Petanceska, NIA*

Session 1: Using 2D and 3D In Vitro Systems for Modeling Disease Complexity, Target Validation, and Drug Screening

Session Chair: Tracy Young-Pearse, Harvard Stem Cell Institute

10:15 a.m. Advancements and Challenges to Modeling AD/ADRD With iPSCs: NIA iPSC Neurodegenerative Disease Initiative

Mark Cookson, Center for Alzheimer's Disease and Related Dementias

10:35 a.m. Use of iPSC Systems for Modeling Disease and Validation of Novel Targets

Valentina Fossati, The New York Stem Cell Foundation

10:55 a.m. Developing Stem Cell Resources for Modeling Person-Specific Molecular and Pathological AD/ADRD Phenotypes

Tracy Young-Pearse, Harvard Stem Cell Institute

11:15 a.m. 2D and 3D Systems for Modeling Neurodegenerative Disorders

Steven Finkbeiner, University of California, San Francisco

11:35 a.m. Logical Network-Based Drug-Screening Platform for AD/ADRD Representing Pathological Features of Human Brain Organoids

Inhee Mook-Jung, Seoul National University

11:55 a.m. Q&A

12:25 p.m. Break

Session 2: Using Microphysiological Systems as Tools for Predictive Drug Development

Session Chair: James Hickman, Hesperos Inc.

12:40 p.m. Using 3D Cerebral Organoids and AI to Identify Candidate Therapeutics for AD
Stuart Lipton, Scripps Research Institute

1:00 p.m. Construction of an Integrated Immune, Vascular, and Brain Chip as a Platform for Drug Discovery and Development for AD

Li-Huei Tsai, Massachusetts Institute of Technology

1:20 p.m. Construction of Integrated Human-On-Chip Systems for AD Drug Development for Central Nervous System and Peripheral Nervous System

James Hickman, Hesperos Inc.

1:40 p.m. Construction of a Multicellular Organ-On-Chip to Inform FTD/ALS Clinical Trials

Clive Svendsen, The Regenerative Medicine Institute, Cedars-Sinai

2:00 p.m. Q&A

2:30 p.m. End of Day 1

Day 2: Wednesday, July 20, 2022

Session 3: National Center for Advancing Translational Sciences (NCATS) Tissue Chip Program

Session Chair: Zane Martin, NIA

- 10:00 a.m. Introduction: MODEL-AD: Model Organism Development and Evaluation for Late-Onset Alzheimer's Disease
Suzana Petanceska, NIA
- 10:15 a.m. Overview of the NCATS Tissue Chip Program
Passley Hargrove-Grimes, NCATS
- 10:45 a.m. Microphysiological Systems Database Center
Mark Schurdak, University of Pittsburgh
- 11:05 a.m. Q&A

Session 4: Regulatory Perspective on Microphysiological Systems for Therapeutic Development

Session Chair: Nadezda Radoja, NIA

- 11:35 a.m. FDA's Predictive Toxicology Roadmap for New Predictive Toxicology Methods in Regulatory Reviews
David Strauss, Center for Drug Evaluation and Research (CDER)
- 12:20 p.m. Q&A
- 12:50 p.m. Wrap Up and Adjourn

Appendix B: List of Participants

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