

# STEM CELL MODELS OF NEURODEGENERATION

# 2025



EDINBURGH, UK  
26-28 MARCH

 UK Dementia  
Research Institute

 CURE-ND  
Catalysing a United Response in Europe  
to Neurodegenerative Diseases

 DZNE  lucidity  EuroBrain Institute  UK Dementia Research Institute

---

# Contents

---

Pg 2	Welcome
Pgs 3 - 8	Meeting Agenda
Pgs 3 - 4	<i>26<sup>th</sup> March 2025; Day 1</i>
Pgs 5 - 6	<i>27<sup>th</sup> March 2025; Day 2</i>
Pgs 7 - 8	<i>28<sup>th</sup> March 2025; Day 3</i>
Pgs 9 - 26	Speaker Profiles
Pgs 9 - 10	<i>Plenary speakers</i>
Pgs 11 - 13	<i>2D Models</i>
Pgs 14 - 16	<i>3D Models</i>
Pgs 17 - 18	<i>Emerging Technologies &amp; Approaches</i>
Pgs 19 - 20	<i>Improving Diversity in iPSC modelling</i>
Pgs 21 - 22	<i>Selected ECR speakers</i>
Pg 23	<i>Panel 1 Discussants</i>
Pgs 24 - 25	<i>Panel 2 Discussants</i>
Pg 26	<i>Panel 3 Discussants</i>
Pgs 27 - 29	SCMND 2025 Organising Committee
Pgs 30 - 107	Poster Abstracts
Pgs 30 - 35	<i>Poster Titles</i>
Pgs 36 - 39	<i>iPSC Resources</i>
Pgs 40 - 73	<i>Group 1</i>
Pgs 74 - 107	<i>Group 2</i>
Pg 108	Sponsors

# Welcome to SCMND 2025!

Welcome to Edinburgh!

And perhaps more importantly, welcome to the inaugural **Stem Cell Models of Neurodegeneration** symposium!

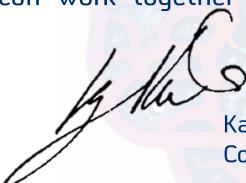
You might be wondering why we are having this meeting, and why now?

I moved to Edinburgh at the end of 2022 to start my own lab. This meeting was born out of my desire to connect with researchers and colleagues in Europe and around the world who were working with the same stem cell models, tools and resources as me – and doing it better! I *needed* and *wanted* to find that network of people to collaborate with and learn from – people who wouldn't just tell me why stem cell models are too limited or variable, and wouldn't just respond, aghast, with "*but what about the necrotic core?!*"

But I couldn't find a forum for people working with these models, specifically in the field of neurodegeneration. So, with some friends and colleagues, we set out to make one.

And, here we are! I've been blown away by the incredible support and enthusiasm the community has had for this meeting – we were able to fill a 3-day agenda with an unbelievable line-up of speakers who were kind enough to travel to Scotland for this little event they'd never heard of. We received twice as many abstracts than we expected. We sold out all registrations.

So, I would like to sincerely thank you all for your interest in and excitement for what we are trying to start with this meeting, and for being so open and sharing your work with the community. I truly hope you enjoy this meeting, find some new collaborators, and come away excited with new ideas for how we can work together to prevent, treat & cure neurodegeneration.



Kathryn Bowles  
Committee chair & Academic Lead

# Day 1 – 26<sup>th</sup> March 2025

8.00 – 8.45	Arrival & Registration
8.45 – 9.00	Welcome <i>Siddharthan Chandran - Director, UK DRI</i>
9.00 – 10.00	<b>Session 1: Plenary I</b> <i>Chair: Kathryn Bowles</i> <i>Sonia Ghandi – The Francis Crick Institute &amp; University College London, UK</i> <i>Finding your way: maps, models and mechanisms</i>
10.00 – 10.30	Break
10.30 – 12.00	<b>Session 2: 2D Models I</b> <i>Chairs: Niamh O'Brien &amp; Rachel Hodgson</i>
10.30 – 10.50	<i>Wendy Noble - University of Exeter, UK</i> Studying the dynamics of tau processing by human iPSC-astrocytes as a tool to define disease mechanisms
10.50 – 11.10	<i>Jackie Casey -University College London, UK</i> Establishing iPSC models to study the role of 4R tau and P301L mutations on axonal transport
11.10 – 11.30	<i>Andreas Hermann - University Medical Center Rostock, Germany</i> Neuron-specific IFN type 1 signalling in (FUS-) ALS induce neurodegeneration and offers new biomarker driven individualized treatment options
11.30 – 11.45	 <i>Rachel Hodgson - University of Sheffield, UK</i> Nuclear TDP-43 condensation induced by stress and C9orf72-derived DPRs leads to TDP-43 loss of function
11.45 – 12.00	<b>FLASH TALKS</b>

1. Poulomi Banerjee  
*University of Edinburgh, UK*
2. James Crowe  
*University of Zurich, Switzerland*
3. James Evans –  
*Francis Crick Institute, UK*
4. Cathleen Hagemann  
*King's College London, UK*

5. Nina-Lydia Kazakou  
*University of Copenhagen, Denmark*
6. Eilish Mackinnon  
*Cardiff University, UK*
7. Arkoprovo Paul  
*University of Cambridge, UK*

8. Olivia Soper  
*University of Aberdeen, UK*
9. Eliška Waloschková  
*Sophion Biosciences, Denmark*
10. Yiyun Zhang  
*University of Exeter, UK*

# Day 1 – 26<sup>th</sup> March 2025

12.00 – 13.15	Lunch & Poster Session I
13.15 – 15.00	<b>Session 3: Emerging Models &amp; Technologies</b> <i>Chairs: Stefan Hauser &amp; Annerieke Sierksma</i>
13.15 – 13.35	<b>Linna Zhou - University of Oxford, UK</b> 3D Printing Neural Tissues for Disease and Repair
13.35 – 13.55	<b>Andrea Serio - King's College London, UK</b> Shape matters: Combining engineering, stem cell modelling and imaging to learn about neurobiology and neurodegeneration
13.55 – 14.15	<b>Renzo Mancuso – VIB, Belgium</b> Xenotransplantation models to study human microglial biology in neurodegeneration
14.15 – 14.30	<b>Annerieke Sierksma- KU Leuven, Belgium</b> Genetic diversity in human microglia modulates responses to amyloid pathology in Alzheimer's disease
14.30 – 15.00	<b>Clotilde Lagier-Tourenne – MGH, Harvard, USA</b> Genetic and pharmacological screens to identify new therapeutic targets in ALS and frontotemporal dementia
15.00 – 15.45	<b>Session 4: Panel Discussion</b> <b>Advantages &amp; Limitations of iPSC for modelling neurodegenerative disease</b> Renzo Mancuso – VIB, Belgium ( <i>Chair</i> ) Sally Temple – Neural Stem Cell Institute, USA Hilary Carswell – University of Strathclyde, UK Eunchai Kang – University of Aberdeen, UK Eva-Maria Surmann – Jackson Laboratories, USA
15.45 – 17.00	<b>Poster Session II</b>
17.00 – 18.00	<b>Drinks Reception</b>
18.00 – 20.00	<b>Optional: Scottish Dinner</b> <b>South Hall</b>



# Day 2 – 27<sup>th</sup> March 2025

8.00 – 9.00	Arrival & Registration
9.00 – 10.00	<b>Session 5: Plenary II</b> <i>Chair: Rebecca Casterton</i> Mahmoud Maina – University of Sussex, UK & Yobe State University, Nigeria From Yobe to the Globe: The African iPSC initiative to elucidate the molecular mechanisms of tauopathies across ancestries
10.00 – 10.30	Break
10.30 – 12.35	<b>Session 6: 3D Models I</b> <i>Chairs: Carles Calatayud Aristoy &amp; Julien Klimmt</i>
10.30 – 10.50	Carles Calatayud Aristoy - Leuven Brain Institute, Belgium Induced human striatal microcircuits capture dopamine modulation and functional defects induced by misfolded alpha-synuclein
10.50 – 11.10	Florent Ginhoux - Gustave Roussy, France Mapping and modelling brain macrophages
11.10 – 11.25	Julien Klimmt - LMU Munich, Germany A reproducible human brain tissue model to study physiological and disease-associated microglia phenotypes
11.25 – 11.45	Selina Wray - University College London, UK Insights into the molecular basis of clinical heterogeneity using patient specific models of familial Alzheimer's disease and Familial British Dementia
11.45 – 12.05	Anna Williams - University of Edinburgh, UK Metformin, mitochondria, metabolism and human oligodendrocytes
12.05 – 12.35	Taylor Bertucci – Neural Stem Cell Institute, USA Human iPSC vascular models to investigate APOE4 cerebrovascular contributions to Alzheimer's disease



# Day 2 – 27<sup>th</sup> March 2025

12.35 – 13.45	Lunch & Poster Session III
13.45 – 15.30	<b>Session 7: 2D Models II</b> <i>Chairs: Natalie Connor-Robson &amp; Eliona Tsefou</i>
13.45 – 14.05	<b>Sally Cowley - University of Oxford, UK</b> Tau uptake, processing and secretion by human iPSC-microglia
14.05 – 14.35	<b>Tracy Young-Pearse - Harvard Medical School, USA</b> Human iPSC-based experimental systems for capturing genetic risk and resilience for Alzheimer's disease
14.35 – 14.55	<b>Rik van der Kant - Vrije University Amsterdam, Netherlands</b> iPSC-models to study and target lipid metabolism in Alzheimer's disease
14.55 – 15.10	<b>Eliona Tsefou - University College London, UK</b> A novel iPSC-derived model recapitulates 4R tau expression and produces endogenous seed-competent tau
15.10 – 15.30	<b>Hazel Hall-Roberts - Cardiff University, UK</b> Exploring Alzheimer's disease polygenic risk through iPSC-derived microglia models
15.30 – 16.15	<b>Session 8: Panel Discussion</b> <b>How can we translate iPSC models to drug discovery, development &amp; therapeutic approval?</b> Marc-David Ruepp – King's College London, UK (Chair) Katerina Gospodinova – ARUK drug discovery institute, UK Elise Malavasi – Concept Life Sciences, UK Emma Jones – Medicines Discovery Catapult, UK Ana Carreras Mascaro – Neurospector, Netherlands Zhi Yao – LifeArc, UK
16.15 – 17.00	Poster Session IV
17.00 – 18.00	Drinks Reception



# Day 3 – 28<sup>th</sup> March 2025

8.00 – 9.00	Arrival & Registration
9.00 – 10.00	<b>Session 9: Plenary III</b> <i>Chair: Stefan Hauser</i>  <i>Sergiu Pașca – Stanford University, USA</i> Instructions included: Assembling human neural circuits to study disease
10.00 – 10.30	Break
10.30 – 12.20	<b>Session 10: Improving diversity in iPSC modelling</b> <i>Chair: Rebecca Casterton</i>  10.30 – 11.00 <i>Mubeen Goolam – University of Cape Town, South Africa</i> Defining the roles of biochemical and physical cues in driving neural differentiation in African stem cells 11.00 – 11.30 <i>Bradford Casey – Michael J Fox Foundation, USA</i> Building global networks, addressing a global disease 11.30 – 11.50 <i>Aleksander Rakovic - LMU Munich, Germany</i> Refining patient-derived iPSC models of X-linked dystonia-parkinsonism and genetic Parkinson's disease using genome editing 11.50 – 12.20 <i>Maneesha Inamdar – inStem, Bangalore, India</i> Diversity in a Dish: Opportunities and challenges in utilizing an Indian cohort of pluripotent stem cell lines.
12.20 – 13.00	<b>Session 11: Panel Discussion</b> <b>How can we promote diversity in iPSC modelling?</b> <i>Mahmoud Maina – University of Sussex, UK &amp; Yobe State University, Nigeria (Chair)</i> <i>Mubeen Goolam – University of Cape Town, South Africa</i> <i>Maneesha Inamdar – inStem, India</i> <i>Chris Morris – University of Newcastle, UK</i> <i>Daniel Paull – New York Stem Cell Foundation, USA</i>

---

# Day 3 – 28<sup>th</sup> March 2025

---

13.00 – 14.00

Lunch & Poster Session V

---

14.00 – 15.45

**Session 12: 3D Models II**

*Chairs: Kathryn Bowles & Alba Ortega Gascó*

14.00 – 14.20 **Dries Braeken – IMEC, Belgium**

Nanotechnology enabling advanced *in vitro* human models

14.20 – 14.40 **Bhuvaneish Selvaraj – University of Edinburgh, UK**

Human stem cell organoid models to study cell-cell communications in neurodegenerative diseases

14.40 – 15.00 **Andras Lakatos – University of Cambridge, UK**

Early cell network vulnerability revealed by a human 3D organoid ALS model

 ECR talk

15.00 – 15.15

**Alba Ortega Gascó - University of Barcelona, Spain**

Cortico-hippocampal assembloids: a novel tool for studying Alzheimer's disease

15.15 – 15.45

**Sally Temple – Neural Stem Cell Institute, USA**

Cortical Organoids: A Window into the Development of FTD-tau

---

15.45 – 16.15

Poster prizes & Closing remarks

---

# Meet the Speakers

## Plenaries



### Sonia Gandhi

Sonia Gandhi is an MRC Senior Clinician Scientist and Professor of Neurology, a Senior Group Leader of the Neurodegeneration Biology Laboratory, and Assistant Research Director at The Francis Crick Institute. She established and co-leads the UCL Queen Square Movement Disorders Centre, which seeks to accelerate translation from scientific discovery to patient benefit, through the discovery of biomarkers, therapeutic targets and clinical trials. Her research program focusses on understanding the molecular mechanisms that cause neurodegenerative diseases. Her laboratory has adopted an interdisciplinary approach combining single molecule biophysics, single cell sequencing approaches and human stem cell systems to understand how proteins misfold in the brain, and the cellular consequences of protein aggregation.

### Sergiu Pasca

Sergiu P. Pasca is the Kenneth T. Norris, Jr. Professor and the Uytengsu Director of Stanford Brain Organogenesis. He seeks to understand the rules governing human brain assembly and the mechanisms of disease. His laboratory pioneered assembloids, introduced the use of instructive signals to create regionalized neural organoids, and developed integrated human circuits following transplantation. These models have been adopted by hundreds of laboratories worldwide, and he systematically applied them to gain insights into physiology and disease and, more recently, to develop therapeutic approaches. He was named a New York Times' Visionary in Medicine and Science. He is a Knight of the Order of Merit, holds a Doctor Honoris Causa, and was a TED-2022 Speaker. His work was recognized with the Vilcek Prize, the American Philosophical Society's Daland Prize, the 12th IBRO-Kemali Neuroscience Award, the ISSCR Momentum Award and the Schaller Prize in Translational Neuroscience.



# Meet the Speakers

## Plenaries



### Mahmoud Maina

Dr Maina holds dual roles as a Director and Group Leader at the Biomedical Science Research and Training Centre (BioRTC) at Yobe State University, Nigeria, and as a Senior Research Fellow leading a small team at the University of Sussex Neuroscience Department in the UK. His team's research focuses on generating induced pluripotent stem cells (iPSCs) from indigenous Africans for open access biobanking and investigating the molecular mechanisms of tauopathies, with a special emphasis on the contribution of African genetic backgrounds to disease mechanisms. In particular, Dr Maina's previous work identified a critical function of tau in nucleolar stress response and rDNA transcription. An important direction in his lab now focuses on deciphering the mechanisms of nucleolar dysfunction in tauopathies and understanding the implications of ancestry-driven rDNA variations in this process. Dr Maina is a Wellcome Trust Career Development Investigator and a Tau Consortium Investigator. With over a decade of experience in initiatives to strengthen African science, he sits on multiple local and international committees and serves as a Science Adviser to the Yobe State Government, providing strategic guidance to various institutions and funders within and beyond Africa. His contributions to neuroscience and African science development have earned him several prestigious recognitions, including the ALBA-FKNE Diversity Prize for the promotion of basic neuroscience. In recognition of his dedication to education, research, and knowledge advancement, Dr Maina was recently conferred the traditional title of "Shettima Ilmube" of Damaturu (akin "Guardian of Knowledge") by His Royal Highness, the Emir of Damaturu in Nigeria.

# Meet the Speakers

## 2D Models



### Wendy Noble

Wendy Noble is Professor of Molecular Neurobiology at the University of Exeter. Her work is focussed on understanding the molecular mechanisms underlying neurodegenerative diseases, with a particular interest in tau.

Wendy graduated with a BSc Honours degree in Anatomy from the University of Edinburgh in 1996, followed by a PhD at University College London. Wendy began to work in the field of neurodegeneration research, with a focus on tau, during her first postdoctoral position at the Nathan Kline Institute/New York University in 2001. Wendy returned to the UK in 2004 where at King's College London, she continued to work on tau, helping to elucidate tau biology in health and disease. Most recently, this has included understanding how interactions between glia and neurons affect tau processing and toxic gain of functions. Wendy moved to the University of Exeter in 2023.



### Jackie Casey

Jackie is a postdoctoral Research Fellow in the Schiavo lab at UCL. She studies Frontotemporal dementia using induced pluripotent stem cell (iPSCs) models to examine molecular mechanisms, with a focus on axonal transport and activity-dependant release of tau in iPSC-derived neurons with P301L MAPT mutations. She completed her PhD in Professor Selina Wray's lab at UCL, where she examined the effect of GRN FTD-associated mutations on mitophagy and mitochondrial health in iPSC-derived neurons, astrocytes and microglia.



### Andreas Hermann

Dr. Hermann is senior neurologist and full professor for Translational Neurology, heading the Translational Neurodegeneration Section "Albrecht Kossel" at the Rostock University Medical Center since 2019. He is part of the German Center for Neurodegenerative Diseases (DZNE) and heading the prospective clinical registry of ALS patients within the DZNE.

The main focus of his working group is the bidirectional translation between basic science and clinical patient work, including human iPSC cultures, drug development, biomarker development (wet markers, MRI markers, PET markers and behavioral endophenotypes). To achieve these goals, his group uses the entire spectrum of clinical research from fundamental to patient-oriented research with the overall goal to understand the pathophysiology of neurodegenerative diseases and aging and how much aging contributes to neurodegeneration. This mainly includes the use of patient-specific models and models of artificial aging and the development of individualized therapeutical strategies for neurodegenerative diseases in a bidirectional clinical translation approach.

# Meet the Speakers

## 2D Models



### Sally Cowley

Sally joined the Sir William Dunn School of Pathology as a Wellcome Trust Career Re-Entry Fellow in 2007, engaged in a program of research into the differentiation of human Pluripotent Stem Cell-derived macrophages for disease modelling. Induced Pluripotent Stem cells (iPSC) derived from patients with genetic disease offers a new, hugely exciting opportunity to model human diseases *in vitro*, and iPSC are particularly important for modelling neurodegenerative conditions. To harness this potential, I Head the James and Lillian Martin Centre for Stem Cell Research, with particular interests in the use of iPSC for modelling disease, and expertise in human iPSC derivation, genetic modification, and differentiation to myeloid and neuronal lineages. With the mounting evidence for a role of microglia in the progression of neurodegenerative diseases, I have focussed my research on using our iPSC models to better understand microglial physiology and pathophysiology.



### Tracy Young-Pearse

Tracy Young-Pearse is an Associate Professor in Neurology at Harvard Medical School and the Dennis J. Selkoe Distinguished Chair in Neurology Brigham and Women's Hospital. She received her undergraduate degree from Skidmore College and then entered the Biomedical and Biological Sciences (BBS) program at Harvard Medical School (HMS). There she earned her Ph.D. in Genetics under the mentorship of Connie Cepko. She established her independent lab in 2010 in the Ann Romney Center for Neurological Diseases. She is the co-Director of the Human Nervous System Diseases Program of the Harvard Stem Cell Institute and is the Vice Chair of Neuroscience at BWH. She also is a member of the FNIH funded Accelerating Medicines Partnership Program for Alzheimer's Disease (AMP-AD), beginning from its launch in 2014. The Young-Pearse lab focuses on the identification of the mechanistic causes of neurodegenerative and developmental disorders of the nervous system, with the ultimate goal of identifying novel targets for therapeutic interventions for these diseases. The lab uses human stem cells, rodent models, and primary human tissues to study the impact of genetic risk and resilience factors on the biology of cells in the brain.

# Meet the Speakers

## 2D Models



### Rik van der Kant

Rik van der Kant is an assistant professor with a dual position at the Vrije Universiteit Amsterdam Center for Neurogenomics and Cognitive Research (CNCR) and the Alzheimer Center of the Amsterdam University Medical Center. His lab studies the molecular mechanisms underlying Alzheimer's disease (AD), and other dementias in the elderly. Work in the van der Kant labs focusses on the role of lipid metabolism in these diseases, and the use of human iPSC-derived neuronal models to discover how lipid metabolism contributes to early pathogenesis. Dr. van der Kant is currently co-leading a clinical phase 2a trial (CHOCOLAD) to evaluate the efficacy of low-dose Efavirenz as a brain-cholesterol lowering drug for the treatment of early Alzheimer's disease. In his free time, Dr. van der Kant enjoys ice skating, eating cheese and visiting windmills while wearing clogs.



### Hazel Hall-Roberts

Hazel Hall-Roberts is a postdoctoral researcher at the UK Dementia Research Institute (UKDRI) at Cardiff University, where she leads the iPSC Platform to Model Alzheimer's Disease Risk (IPMAR). Launched in January 2021, IPMAR aims to generate and characterize one of the largest iPSC resources of late-onset AD patients with extremely high polygenic risk, focusing on microglial dysfunction. Hazel completed her PhD at the University of Bath, followed by postdoctoral research at the University of Oxford, where she studied the effects of an AD-associated TREM2 mutation in microglia using human iPSC models. Her research focuses on how genetic risk factors for Alzheimer's disease affect microglial function, and on developing advanced iPSC models that better reflect the complex biology of microglia in brain tissue.

# Meet the Speakers

## 3D Models



### Carles Calatayud Aristoy

Carles Calatayud is a neuroscientist with expertise in neurodegeneration and the molecular mechanisms underlying brain disorders. He earned his Ph.D. from the University of Barcelona under the supervision of Professors Antonella Consiglio and Ángel Raya and has since contributed to understanding key pathways driving neuronal loss in Parkinson's disease. Dr. Calatayud's research integrates advanced methodologies, including stem cell modeling, gene editing, transcriptomics, and cellular imaging, to uncover how genetic and molecular factors contribute to disease phenotypes. His work emphasizes translational approaches and the building of new tools to better study neurodegeneration in the dish.

Currently, he is a postdoctoral researcher in Patrik Verstreken's lab at the VIB-KU Leuven Center for Brain & Disease Research, where he investigates synaptic dysfunction and molecular mechanisms underlying neurodegenerative diseases. His work leverages cutting-edge models to explore how perturbations in synaptic biology contribute to neurodegeneration, with the goal of identifying new therapeutic strategies.



### Florent Ginhoux

Florent graduated in Biochemistry from the University Pierre et Marie CURIE (UPMC), Paris VI, obtained a Masters degree in Immunology from the Pasteur Institute in 2000 and his PhD in 2004 from UPMC, Paris VI. As a postdoctoral fellow, he joined the Laboratory of Miriam Merad in the Mount Sinai School of Medicine (MSSM), New York, where he studied the ontogeny and the homeostasis of cutaneous dendritic cell populations, with a strong focus on Langerhans cells and Microglia. In 2008, he became an Assistant Professor in the Department of Gene and Cell Medicine, MSSM and member of the Immunology Institute of MSSM. He joined the Singapore Immunology Network (SIgN), A\*STAR in May 2009 as a Junior Principal Investigator and became Senior Principal Investigator in 2014. He joined the EMBO Young Investigator (YIP) program in 2013 and is a Web of Science Highly Cited Researcher since 2016. He is also an Adjunct Visiting Associate Professor in the Shanghai Immunology Institute, Jiao Tong University, in Shanghai, China since 2015 and Adjunct Associate Professor in the Translational Immunology Institute, SingHealth and Duke NUS, Singapore since 2016. He is now a Laboratory Director in Gustave Roussy focusing on pediatric cancers and the role of myeloid cells in tumor progression and became an EMBO member in 2022.

# Meet the Speakers

## 3D Models



### Selina Wray

Selina Wray is a Professor of Molecular Neuroscience at UCL Queen Square Institute of Neurology. Her research uses iPSC to model Alzheimer's disease and other forms of dementia, with a particular focus on understanding clinical heterogeneity at the cellular level.



### Anna Williams

Anna is Professor of Regenerative Neurology and runs a research group in the Institute of Regeneration and Repair at the University of Edinburgh. She is also a consultant neurologist, with a busy clinic in the Anne Rowling Regenerative Neurology Clinic. Her research group is interested primarily in understanding how oligodendrocytes and the myelin of the central nervous system work, and how they are maintained and repaired in diseases such as multiple sclerosis and cerebral small vessel disease, with the ultimate aim of trying to improve this and therefore improve patient therapies..



### Taylor Bertucci

Dr. Bertucci's research focuses on building high-quality *in vitro* pluripotent stem cell (PSC)-derived models for neurodegenerative disease modeling, drug screening and regenerative medicine. She advances these test-beds by developing new 3D models, improving scalability, and defining robust quality control metrics. The goal is to recapitulate *in vivo* 3D microenvironments found in different brain regions with an emphasis on vascular-neural and vascular-immune interactions.



### Dries Braeken

Dr. Dries Braeken obtained the MSc degree of Biomedical Sciences in 2003 and the PhD in Medical Sciences from the University of Leuven in 2009. He is Scientific Director and Group Leader at imec, Belgium where he is combining cutting-edge nanotechnology tools with bioware to create novel human models to study disease.

# Meet the Speakers

## 3D Models



### Bhuvaneish Selvaraj

Dr Bhuvaneish T Selvaraj is an engineering graduate from Anna University, India. He began his scientific research career completing a PhD in the lab of Prof Michael Sendtner at University of Wuerzburg, Germany. In 2014, he moved to University of Edinburgh to undertake his postdoctoral research on human stem cell disease modelling of neurodegenerative diseases. In 2020, Dr Selvaraj was awarded the Chancellor's fellowship, at the University of Edinburgh, to undertake a research programme that aims to gain greater understanding of the molecular pathomechanisms neuron-glia crosstalk in neurodegenerative disease using human stem cells and human pathological tissues and leveraging these for high-throughput phenotypic drug screening. In addition, by working closely with clinicians, I am involved in identification and drug selection of repurposed drugs for testing in clinical trials for neurodegenerative diseases such as ALS and FTD.



### Andras Lakatos

Andras is an Associate Professor and Academic Consultant Neurologist at the University of Cambridge. He leads an MRC-funded Human Organoid Neuropathobiology and Genomics group in the Department of Clinical Neurosciences with affiliations to the Cambridge Centre for Brain Repair and the WT-MRC Cambridge Stem Cell Institute. His team has developed complex 3D human stem cell-derived neural organoid models to study how genetic mutations and modifying factors contribute to the initiation of ALS pathogenesis at single cell resolution. He was recently awarded the ARUK David Hague Investigator of the Year Award and the MRC Senior Clinical Fellowship.



### Sally Temple

Dr. Sally Temple is the Scientific Director of the non-profit Neural Stem Cell Institute (NSCI) located in Albany, NY. Her research focuses on the role of stem cells in the development, maintenance, and repair of the central nervous system. Dr. Temple's group leverages patient-derived stem cells to uncover the causes of neurodegenerative diseases and test potential treatments. They have developed human cell models to examine genetic contributions to progression of Alzheimer's disease and related dementias and age-related macular degeneration. In addition, Dr. Temple is deeply involved in translational research and has helped to develop a stem cell-based therapy for age-related macular degeneration, currently in clinical trial. Dr. Temple is honored to have been awarded a MacArthur fellowship, to have served as the President of the International Society for Stem Cell Research (ISSCR), and to have been elected to the National Academy of Medicine.

# Meet the Speakers

## Emerging Technologies



### Linna Zhou

Dr Linna Zhou is in the Nuffield Department of Medicine and the Department of Chemistry at the University of Oxford. She is an investigator and fellow of the Oxford Martin School. Her current research uses stem cell and bioengineering techniques to build 3D tissues.



### Andrea Serio

Andrea is currently Reader in Neural Tissue Engineering at King's College London, as well as Principal Investigator at the UK Dementia Research Institute (UK-DRI) and Group Leader at the Francis Crick Institute. He leads an interdisciplinary group focused on developing next generation platforms for modelling the nervous system in health and disease. He originally trained in Industrial Biotechnology at the University of Padova, and subsequently he obtained a Masters degree in Medical, Cellular and Translational Biotechnologies at the San Raffaele University of Milan. For his PhD he developed novel models on glial neuronal interactions in neurodegenerative diseases at the University of Edinburgh between 2009 and 2013, where he developed novel ways of creating macroglia from patient stem cells and made important discoveries in the molecular pathology of ALS. In 2013 he joined the Stevens group at Imperial where he focused on several project including microfabricated systems for directing neural cell behaviour, scaffolds for tissue engineering and novel spectroscopic imaging methods. In 2017 he established his independent group at King's and subsequently moved to the Crick in 2019. He joined the UK Dementia Research Institute in 2023 where he now leads his research programme.



### Renzo Mancuso

Renzo obtained his PhD in Neuroscience at the Autonomous University of Barcelona in the laboratory of Prof. Xavier Navarro at the group of Neuroplasticity and Regeneration. I performed my postdoctoral trainings in the laboratories of Prof. V. Hugh Perry at the University of Southampton (UK), and Prof. Bart De Strooper at the VIB-KU Leuven Center for Brain & Disease Research in Belgium. His scientific work is focused on the role of microglia in Alzheimer's disease, linking human genetics with microglia function in iPSC-derived microglia and xenotransplantation models. His major findings are elucidating the detrimental role of microglia in the aggregation of tau and tau-induced cell death, the development and application of microglia humanized models for the study of genotype-phenotype interactions and several aspects of human microglial biology, *in vivo*, in neurodegenerative diseases. He is currently Group Leader and Deputy Director at the VIB-Center for Molecular Neurology where he works on multiple basic and translational projects to dissect the role of microglia and neuroinflammation in Alzheimer's and Frontotemporal dementia, using cellular systems to uncover major phenotypic modifications in microglia and building up from there into more complex *in vivo* chimeric models, and human primary samples.

# Meet the Speakers

## Emerging Technologies



### Clotilde Lagier-Tourenne

Clotilde Lagier-Tourenne, MD, PhD is Associate Professor of Neurology at the Massachusetts General Hospital and Harvard Medical School. She is the recipient of the Araminta Broch-Healey Endowed Chair in ALS, and associate member at the Broad Institute of MIT and Harvard. She trained as a medical geneticist and neuroscientist in Strasbourg (France), Columbia University and UC San Diego. Her team investigates the molecular mechanisms driving neurodegeneration in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). She has established collaborations with academic and pharmaceutical partners to develop novel approaches to therapy, including RNA-targeting antisense oligonucleotides and immunotherapies for ALS and FTD. Her group also uses small molecules and genetic screens to identify modifiers of disease-associated phenotypes and new therapeutic targets for neurodegenerative diseases.

# Meet the Speakers

## Improving Diversity in iPSC Models



### Mubeen Goolam

Dr Mubeen Goolam is the Principal Investigator of the Stem Cell Modelling of Development and Disease Group (The BrainStem Lab) in the Department of Human Biology and the Neuroscience Institute at the University of Cape Town. Mubeen received his BSc in Genetics and Microbiology and his Honours and MSc degrees in Medical Cell Biology from the University of Cape Town. In 2012 he was awarded the Mary Gray Fellowship to St John's College, at the University of Cambridge and undertook a PhD in Physiology, Development and Neuroscience in the Zernicka-Goetz lab. He then moved to the University of Oxford as a Junior Research Fellow to Wolfson College and took up a post-doctoral position in the Sir William Dunn School of Pathology in the Robertson Lab. He returned to the University of Cape Town in 2020 to establish his own independent research group. Research in the Goolam lab focuses on using stem cells to model development and disease in culture. They are developing the first African-specific models of the brain and neural tube to investigate early human brain and spinal cord development as well as create a model to study neurodevelopmental disorders in a dish.



### Bradford Casey

Bradford leads the Fox Foundation's Genomics, Computational Biology, and Data Science research portfolios, working with other Foundation scientists to develop and implement the Foundation's research strategy. He collaborates with researchers, clinical leaders, and industry partners to develop new programs, apply new technologies, and ensure that MJFF research priorities reflect and best serve the needs of patients. Bradford serves as a scientific liaison for many of the Foundation's collaborative research partnerships, including the Aligning Science Across Parkinson's Collaborative Research Network, the Global Parkinson's Genetics Program (GP2), and the Accelerating Medicines Partnership (AMP-PD), a public-private consortium focused on leveraging strengths of governmental, academic, and industry partners to develop shared resources for the community. Bradford is passionate about collaborating with experts across disciplines to expand the impact of Parkinson's research on behalf of those affected by the disease, and to advance scientific research in the public interest.

# Meet the Speakers

## Improving Diversity in iPSC Models



### Aleksander Rakovic

Aleksander is a molecular biologist and head the research group "Molecular Pathways in Movement Disorders" at the Institute of Neurogenetics, University of Lübeck, Germany. My aim is to understand the molecular pathways involved in the pathophysiology of Parkinson's disease (PD) and dystonia in order to identify novel therapeutic targets. I use human-derived cellular models such as dermal fibroblasts, induced pluripotent stem cells and zebrafish. These models are further refined using genome editing to either generate more homogeneous cell populations or to generate reporter lines. While my interests range from dystonia and X-linked dystonia-parkinsonism to genetic and sporadic PD, my main research focus is on the role of PINK1 and Parkin in mitochondrial dysfunction and mitophagy.



### Maneesha Inamdar

Prof. Maneesha Inamdar is a cell and developmental biologist with several years of experience in a comparative analysis of cardiovascular development, and is the Director of inStem, India's first stem cell institute. She initiated both teaching and contemporary research in the area of stem cells and developmental biology at the JNCASR. Prof. Inamdar's group has established human embryonic stem cell lines in India and worked extensively on mammalian cardiovascular development. Her laboratory studies cell lineage specification and differentiation during development. They have taken a unique comparative approach using embryonic stem cell models, mouse developmental biology and transgenics, and Drosophila genetics, to decipher the roles of novel genes expressed early in the blood and cardiovascular system. Their current focus is on analyzing mammalian development using stem cell models as well as clinical studies.

# Meet the Speakers

## Selected ECR Speakers



### Rachel Hodgson

Rachel is a postdoctoral researcher in Tatyana Shelkovnikova's lab based at the Sheffield Institute of Translational Neuroscience (SITraN) within the University of Sheffield. Her research uses cellular models to understand the molecular mechanisms of neurodegenerative disease, with a focus on the dysregulation of biomolecular condensates and RNA metabolism.



### Annerieke Sierksma

Dr. Annerieke Sierksma is a staff scientist within the Center for Brain and Disease Research at VIB and the KU Leuven, working in the Laboratory for the Research of Neurodegenerative Diseases (lead by Prof. Bart De Strooper). She obtained a Master in Neuropsychology (2007) and a PhD in Neuroscience (2012; both at Maastricht University, the Netherlands) and subsequently moved to Belgium to continue her career researching the molecular and genetic underpinnings of Alzheimer's Disease (AD). She is currently leading several research projects researching how genetic risk for AD may modulate the response of microglia, the brain's immune cells, to amyloid-beta ( $A\beta$ ) plaques, one of the neuropathological hallmarks of AD. To do so, she is using traditional single gene knock-out human stem cells, but is also looking at the effect of heterogeneous genetic profiles on the microglial transcriptome. To do so, she combines stem cell-derived human microglia, xenotransplantation mouse models, single cell RNA sequencing and proteomics. Her strength lies in building bridges between people, groups and research domains and employing emerging state-of-the-art techniques to answer scientific questions.



### Julien Klimmt

Julien obtained his M.Sc. in Molecular Biosciences at the University of Heidelberg and a PhD in Neuroscience at LMU Munich. His research interest focuses on pioneering and using novel human iPSC-based brain tissue models to study neurodegenerative diseases, with a focus on neuroinflammation and cellular crosstalk, and screen potential therapeutics. He recently developed a reproducible and scalable 3D co-culture system characterized by high maturity and long-term incorporation of homeostatic microglia and used it to model and investigate Alzheimer's disease and test therapeutic compounds.

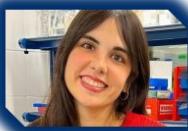
# Meet the Speakers

## Selected ECR Speakers



### Eliona Tsefou

Eliona is a research Fellow at Karen Duff's Lab at UK Dementia Research Institute at UCL and joined the lab in 2022 to establish the iPSC derived neuronal models for tauopathies. Prior to joining the Duff lab, she worked as a Research fellow both at Eisai Pharmaceutical and UCL where she developed disease relevant cellular models in order to study different pathways that are involved in neurodegenerative diseases and identify potential new targets that can be used in drug discovery. She also holds a PhD in Neurobiology from Newcastle University, UK.



### Alba Ortega Gascó

Alba is a biomedical researcher specialized in Neurosciences with a strong background in biotechnology, cell biology, and developmental biology. She completed her PhD in Neuroscience at the University of Barcelona investigating the mechanisms of brain development and adult neuronal plasticity, mainly focusing on the role of the protein NCAM2 in the regulation of adult neurogenesis. She honed her skills in working with stem cells 3D models at the Mass, Eye and Ear, Harvard Medical School, where she developed hiPSCs derived retinal organoids to study retinal development and model degenerative diseases. As a postdoctoral researcher, she is now focused on studying cortico-hippocampal circuits using hiPSC-derived 3D models to investigate the mechanisms underlying memory formation and Alzheimer's disease. Through her research, she aims to bridge stem cell technology and neuroscience to develop advanced models for understanding and treating neurodegenerative diseases.

# Meet the Speakers

## Panel 1 Discussants



### Hilary Carswell

Dr Carswell is an ambassador of the 3Rs and a promoter of the ARRIVE guidelines. As a member of the NC3Rs expert working group, she co-authored the IMPROVE guidelines for minimising severity limits of in vivo stroke models.

Dr Carswell is a preclinical stroke researcher who traditionally has relied on in vivo stroke models. Over more recent years, Dr Carswell has put her research efforts into developing technologies using microfluidics, biomaterials and cerebral organoids that will help replace the use of animals in stroke research. To develop these technologies, she is a recipient of the University of Strathclyde 3Rs Award, a Saudi R & D Grant, a Tenovus Scotland grant and NC3Rs PhD Studentship.

Of relevance to today's session, Dr Carswell has collaborated with Dr Michele Zagnoni, EEE, to develop a microfluidics model for stroke using mouse primary neuronal cultures. Funded by NC3Rs, will aim to develop, validate, and apply this model using human induced pluripotent stem cell (iPS)-derived neuronal cells instead of rodent neuronal cultures to transform it into a human-based stroke model.



### Eunchai Kang

Dr. Kang is a Lecturer at the Institute of Institute of Medical Sciences, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen



### Eva-Maria Surmann

Dr. Eva-Maria Surmann is the Associate Director of Business Portfolio Management at The Jackson Laboratory, where she spearheads the development of advanced cell model platforms. She holds a PhD in Medical Sciences from the University of Cambridge, UK, and has held key roles at GeneAdviser, Horizon Discovery, and Ortho Clinical Diagnostics, contributing to the development and commercialization of innovative clinical and research products. In her current role, Dr. Surmann leads The Jackson Laboratory's commercial stem cell program, collaborating with large-scale engineering initiatives such as the iPSC Neurodegenerative Disease Initiative (INDI) to facilitate global access to human iPSCs.

# Meet the Speakers

## Panel 2 Discussants



### Katerina Gospodinova

Katerina received her PhD in Genomics and Experimental Medicine from The University of Edinburgh. In 2021, she joined the University of Oxford as a post-doctoral researcher within the TREAT-AD consortium. During this time, Katerina developed several Target Enabling Packages and in vitro assay platforms for high-throughput target validation of next generation therapeutic targets in Alzheimer's disease. Katerina joined the Alzheimer's Research UK Oxford Drug Discovery Institute as a Team Leader of the Neuroinflammation group in 2022. Her research focuses on identifying and de-risking neuroimmune and matrisome-associated targets and pathways as potential therapeutic strategies in Alzheimer's disease and related dementias. She has led the development of novel complex human iPSC-derived disease models for probing disease mechanisms and testing compound efficacy.



### Elise Malavasi

Elise obtained a PhD in Cellular and Molecular Basis of Disease from the University of Edinburgh, followed by 9 years of post-doctoral experience in Cellular and Molecular Neuroscience. Her earlier academic research focused on investigating the molecular mechanisms underlying major psychiatric illness, after which she spent several years studying the mechanisms of peripheral myelination. Her research exploited a range of cellular models, including neural cell lines, primary CNS co-cultures and iPSC derived neurons, coupled with advanced microscopy techniques. In 2012, Elise joined Concept Life Sciences, a drug discovery and development Contract Research Organisation, as Principal Scientist for neuroscience, and later progressed to the role of Associate Director. In her current role, she provides scientific leadership to the neuroscience research team and drives the scientific growth and development of the neuroscience service line.



### Emma Jones

Emma holds a PhD from the University of Manchester and then spent several years studying the role of her favourite cell - the astrocyte – in development and disease at McGill University, Canada. Since her postdoctoral studies, she has spent more than 10 years leading translational neuroscience projects in both academic and drug discovery settings. Emma works as a lead scientist at MDC and is responsible for a team of scientists developing complex CNS models for neuroscience medicines discovery projects.

# Meet the Speakers

## Panel 2 Discussants



### Ana Carreras Mascaro

Dr. Carreras Mascaro obtained her Ph.D. from the Erasmus University in Rotterdam, The Netherlands, studying molecular mechanisms of genetic forms of Parkinson's disease. She then worked as a Postdoctoral Fellow at Neurospector, based in the Vrije University in Amsterdam, where she is leading a team of scientists dedicated to accelerating drug development for brain disorders, including genetic neurodevelopmental disorders, frontotemporal dementia, and amyotrophic lateral sclerosis. In collaboration with industry partners, Neurospector uses predictive human iPSC-derived neuron cell models and scalable live microscopy read-outs of neuronal activity to assess drug efficacy.

### Zhi Yao

Dr. Yao is a Principal Scientist at LifeArc, with a focus on translational research and leading several MND therapeutics discovery projects. She is passionate about utilizing a human-first approach and patient iPSC models to facilitate drug development and precision medicine.



### Marc-David Ruepp

Marc-David Ruepp is a Reader in RNA Biology and Molecular Neurodegeneration at the UK Dementia Research Institute at King's College London. His laboratory is interested in RNA metabolism in health and disease with a specific focus on neurodegeneration. He received his PhD in 2009 (Graduate School of Cellular and Biomedical Sciences, University of Bern) and started his independent scientific career as Junior Group Leader in 2014 in the Department of Chemistry and Biochemistry at the University of Bern. He habilitated at the Faculty of Science, University of Bern, Switzerland and received his Venia Docendi in RNA biology in 2018. The same year he joined the UK DRI as Group Leader and King's College London as Senior Lecturer in Neuroscience. In 2022 he was promoted to Reader in RNA Biology and Molecular Neurodegeneration.

# Meet the Speakers

## Panel 3 Discussants



### Chris Morris

Chris Morris is Scientific Director at the Newcastle Brain Tissue Resource, Newcastle University. He has over 30 years of research experience into the neuropathology, genetics, and neurochemistry of neurodegenerative disorders, having been involved with over 200 published scientific research studies. He acts as the University's Designated Individual under the Human Tissue (2004) Act, providing advice and guidance on ethics and tissue banking for medical research. Chris's research looks at why people with different neurological disorders experience different psychiatric symptoms and how we might successfully treat these symptoms. For example, people experiencing Dementia with Lewy Bodies have much higher rates of depression and visual hallucinations than people with Alzheimer's, and the basis of this is unclear. To explore this, brain tissue from clinically well characterised individuals who have donated to the tissue bank are analysed with various methods. For example, quantitative microscopy methods are used to determine if specific cellular changes or pathology underpins key symptoms. Analytical methods including transcriptomics, genetics and biochemical techniques allow a deeper understanding of how symptoms might evolve in these disorders, and how targeted treatments might be of benefit.

As part of these studies, patient specific stem cell models of the different disorders that are found in older populations have been developed. Using the brain bank material, highly characterised cell lines have been developed from older normal donors and also from Dementia with Lewy Bodies and Alzheimer's disease donors. These cell lines are derived from donors entering Newcastle through the Brains for Dementia Research initiative (<https://bdr.alzheimersresearchuk.org/researchers/what-bdr-offers/stem-cells/>) and provide an accessible iPSC model for use by the research community.



### Daniel Paull

Daniel is Senior Vice President, Discovery & Platform Development at The NYSCF Research Institute. Daniel oversees aspects of the NYSCF Global Stem Cell Array, focussing his time on developing novel tools using this robotic platform as well as developing approaches for high-throughput differentiations, drug screening, and phenotyping. During his post-doctoral fellowship at NYSCF in the lab of Dr. Dieter Egli, Daniel helped develop novel approaches to prevent the inheritance of mitochondrial disease, a process that is now in clinical trials in the UK. He received his Ph.D. from University College London, England.

# SCMND 2025 Committee



## Kathryn Bowles

### Committee Chair & Academic Lead

Kathryn completed her PhD in Integrative Neuroscience at Cardiff University with Prof. Lesley Jones, followed by a postdoc position at the Icahn School of Medicine at Mount Sinai in New York, USA, with Prof. Alison Goate. Kathryn started her own lab in 2022, which is based at the UK Dementia Research Institute at the University of Edinburgh, UK. She uses an integrative approach to understand the molecular and genetic mechanisms that underlie risk for Tauopathies and other neurodegenerative diseases. Kathryn primarily works with human iPSC lines differentiated into 3D organoids, as well as 2D mixed and monocultures of neurons, astrocytes and microglia, in order to model how disease-causing MAPT mutations affect cellular function. Computational work includes the generation and analysis of large 'omics datasets, and fine-mapping of human genetic data to understand the consequences of structural variation at the 17q21.31 "MAPT" locus across different genetic ancestries.



## Natalie Connor-Robson

### Scientific Program Committee

Much recent work has highlighted the importance of the endocytic pathway, a cellular mechanism important to recycling and maintenance of normal function, in neurodegeneration. A high proportion of the risk genes for late onset Alzheimer's disease (LOAD) cluster in the endocytic pathway. Dr Connor-Robson's group focuses on understanding how these genes associated to both LOAD and the endocytic pathway contribute to the molecular and cellular changes which cause the disease, and to begin to understand the biological underpinnings of polygenic risk. Her group use novel iPSC models to examine the changes in function of neurons and microglia that occur in disease.



## Rebecca Casterton

### Scientific Program Committee

Rebecca is a postdoctoral research associate based at the UK DRI centre at King's College London, working within the Mizielinska lab to understand how DNA damage pathway dysfunction intersects with nuclear membrane defects in ALS/FTD. Alongside this, Rebecca is also a UK DRI pilot grant awardee, through which she is developing her independent research interest in understanding neuronal cell cycle re-entry as a novel mechanism implicated in neurodegeneration. She is also passionate about equality, diversity & inclusion in STEM and advocating for change towards a research culture that supports everyone to thrive. Throughout her PhD, Rebecca founded and coordinated a staff/student gender equality network within her department to create a space for discussions on these topics. She is currently co-host, producer and editor of The Academinst podcast, which she founded in 2020.

# SCMND 2025 Committee



## Niamh O'Brien

### Scientific Program Committee

Niamh O'Brien is a senior postdoc working in the lab groups of Dr. Marc-David Ruepp and Dr. Sarah Mizielinska at the UK DRI at King's College London. Her research focuses on using iPSC derived neuronal models to investigate molecular mechanisms disrupted in genetic forms of ALS/FTD.



## Carles Calatayud Aristoy

### Scientific Program Committee

Carles Calatayud is a neuroscientist with expertise in neurodegeneration and the molecular mechanisms underlying brain disorders. He earned his Ph.D. from the University of Barcelona under the supervision of Professors Antonella Consiglio and Ángel Raya and has since contributed to understanding key pathways driving neuronal loss in Parkinson's disease. Dr. Calatayud's research integrates advanced methodologies, including stem cell modeling, gene editing, transcriptomics, and cellular imaging, to uncover how genetic and molecular factors contribute to disease phenotypes. His work emphasizes translational approaches and the building of new tools to better study neurodegeneration in the dish.

Currently, he is a postdoctoral researcher in Patrik Verstreken's lab at the VIB-KU Leuven Center for Brain & Disease Research, where he investigates synaptic dysfunction and molecular mechanisms underlying neurodegenerative diseases. His work leverages cutting-edge models to explore how perturbations in synaptic biology contribute to neurodegeneration, with the goal of identifying new therapeutic strategies.



## Stefan Hauser

### Scientific Program Committee

Dr. Hauser is a Group Leader at the German Center for Neurodegenerative Diseases (DZNE) and the Hertie Institute for Clinical Brain Research, Tübingen. His group focuses on the development of iPSC-based models for monogenetic neurodegenerative diseases with the aim of identifying novel genetic treatment strategies.



## Alex Colcutt

### UK DRI Central Team Research Communications Manager

Dr Alex Colcutt is Research Communications Manager at the UK Dementia Research Institute, where he combines his neuroscience background with communications expertise to share the Institute's groundbreaking research. After completing a PhD in Neuroimmunology at the University of Southampton, Alex developed educational programs at the British Neuroscience Association before joining UK DRI in 2019. He leads content strategy and internal communications, working closely with researchers to translate complex science for diverse audiences while overseeing the Institute's brand and engagement initiatives.

# SCMND 2025 Committee



**Beverly Roberts**

Financial Manager & UK DRI Edinburgh Centre Manager



**Sam Jackson**

UK DRI Central Team Tools & Technology Platform Manager



**Ana Atunes-Martins**

UK DRI Central Team Science Manager

# Posters

## Resources pgs 36-39

Poster Sessions I - V

**R1:** Suleiman Hamidu Kwairanga. \*\*\*SCMND travel award\*\*\*

Modelling Alzheimer's Disease in Northern Nigeria: Establishing a Cohort and iPSC Resource

**R2:** Eva-Maria Surmann: The JAX iPSC Repository: Facilitating Access to High-Quality Isogenic iPSCs for Advanced Disease Modeling and Drug Discovery

**R3:** Richard V. Pearse II. Molecular analyses of human iPSC-derived brain cells and matched brains from over 100 genetic backgrounds highlight systemic variations in response to genetic ancestry, sex and disease risk

**R4:** Chris Morris. The BDR Stem Cell and Fibroblast Cell Bank for Dementia Research

**R5:** Hazel Hall-Roberts. IPMAR: iPSC Platform to Model Alzheimer's disease Risk

**R6:** Erin Hedges. An iPSC platform for drug discovery in motor neuron disease

**R7:** Daniel Paull. Automating iPSC research for biobank development and high-throughput phenotyping

## Group 1 pgs 40-73

Poster Sessions I - III

**#1:** Kim de Kleijn. TREM-family receptor-ligand discovery to fine-tune microglia function in Alzheimer's disease

**#2:** Sam Washer. Knockout CRISPR screens identify key regulators of phagocytosis and endolysosomal function in human induced pluripotent stem cell derived microglia

**#3:** Zhizhong Yang. Generating in vitro platforms for testing blood-brain barrier penetrance of novel anti-complement drugs for therapy of dementia

**#4:** Dominik Paquet. A human iPSC model of Tauopathies engineered for 4R Tau isoform expression endogenously develops late-stage neuronal Tau pathology

**#5:** Tania Atienzar. Rapid Oligodendrocyte Generation for TDP-43 Disease Modelling: A Novel Stem Cell Approach

**#6:** Dafni Kampitsi. Impact of MAPT mutation on ER-phagy in iPSC-derived neurons

**#7:** Rafaela Hasse e Silva Policarpo. Modelling FTLD-FET using patient-derived induced pluripotent stem cell models

**#8:** Ruxandra Dafinca. Mitochondrial dysfunction in iPS-derived motor neurons from patients with Amyotrophic Lateral Sclerosis

**#9:** Shikha Kataria. Investigating the link between neuronal function and the autolysosomal pathway (ALP) in Parkinson's using mutant LRRK2 iPSC derived neuronal models

**#10:** Tony Oosterveen. An in vitro toolkit to study the cell-specific roles of glutamatergic neurons and glia in Alzheimer's Disease

**#11:** Sophie Goldsmith. A cerebral organoid model of progranulin-associated frontotemporal dementia

**#12:** Filipa Henderson Sousa. The role of ELAVL4 in neuronal vulnerability in MAPT mutation iPSC-derived neurons

**#13:** Freya Cracknell. Development of a human iPSC-derived triculture system to study late onset Alzheimer's disease associated microglial TREM2 variants

**#14:** Chloe Kan. Developing an enhanced glioblastoma model using in vivo xenografts of hPSC-derived assembloids with cerebral tissue, glioblastoma cells and immune cells

**#15:** Preethi Sheshadri. Modelling Human iPSC-derived AGTR1+ Dopaminergic Neurons for Parkinson's Disease

**#16:** Gizem Onal. Using CRISPRi Screens in Human iPSC-Derived Dopamine Neurons to Probe Endolysosomal Pathway Dysfunction in Parkinson's Disease

**#17:** Dianne Lopez. Investigating the role of LRRC37A2 gene in iPSC-astrocytes and Parkinson's

**#18:** Ella Nightingale. Investigating homeostatic nuclear mRNA retention in iPSC-derived neurons

**#19:** Emma Jones. Global identification of integral membrane proteins that require Retromer for their sorting in neuronal systems

# Posters

## Group 1

Poster Sessions I - III

- #20: Joseph Stone. Using iPSCs and neurons to model modifiers of somatic CAG repeat expansion in Huntington's disease
- #21: Charlie Arber. Mutations in PSEN1 predispose inflammation in an astrocyte model of familial Alzheimer's disease through disrupted regulated intramembrane proteolysis
- #22: Kathryn Knowles. Investigating the Role of Neurofilament Heavy Chain (NfH) in iPSC-Derived Neuronal Models of ALS
- #23: Sabino Mendez Pastor. Using iPSC models to investigate the impact of TREM2 variants on the microglial response to amyloid-beta
- #24: Aiko Robert. \*\*\*Qkine travel award\*\*\*  
Lipidomic characterization of iPSC-derived microglia models
- #25: Katerina Gospodinova. A novel, rapid human iPSC-derived triculture system enhances target validation and drug discovery for neurodegeneration
- #26: Karolina Nowak. Investigating how LOAD risk genes BIN1 and APOE4 impact neuronal function
- #27: Tushar Shah. Enhanced, high-throughput single-cell DNA methylation analysis using massively paralleled indexing
- #28: Gabriella Crawford. Development of Neuroimmune-Vascular Organoids
- #29: Kimberly Cheam Ai Xian. Investigating cellular mechanisms of tau uptake in glial cells and in co-culture with neurons.
- #30: Diogo Dias. Harnessing the power of iPSCs to accelerate the discovery and development of autophagy modulators for the treatment of Parkinson's Disease
- #31: Nirmal Sampathkumar. Using iPSC-derived motor neuron models of ALS to evaluate the efficacy and therapeutic potential of autophagy modulator SAM001
- #32: Yujing Gao. Vectorised U7 snRNAs as gene therapy approach for ALS/FTD.
- #33: Eliška Waloschková. \*\*\*FLASH TALK SPEAKER\*\*\*  
Electrophysiological characterization of genetically modified iPSC neurons as a Frontotemporal Dementia model using automated patch clamp – differences in ion channel and action potential properties.
- #34: Ajantha Abey. Modelling Lewy Pathology in Differentially Vulnerable Cortical and Dopaminergic Neurons from Patients with Alzheimer's and Parkinson's Disease
- #35: Alexander Fröhlich. Establishing cell type-specific CRISPRi tools in 3D organoid models to study tauopathies
- #36: Joseph Thayer Jin Robin. Utilization of GENtoniK to mature cellular models of Parkinson's Disease
- #37: Celia Gomez Sanchez. The interplay between Wnt and FGFR1-FGF8 signaling in GnRH neuron formation
- #38: Srilakshmi Goberdhan. Investigating the role of Alzheimer's disease risk gene PICALM in iPSC-derived microglia
- #39: Ilka Rinke-Weiβ. Characterization of ion channels recorded from hiPSC-derived neurons after culturing in different conditions: an automated patch clamp study
- #40: Carlos Anton-Plagaro. Mapping the endosomal proximity proteome reveals Retromer as a hub for RAB GTPase regulation
- #41: Alya Masoud Abdelhafid. Investigating Dysregulated Axonal Transport in Human Motor Neurons Expressing ALS-Causing Mutations
- #42: Stan Majewski. Investigating astrocyte transcriptomic and proteomic heterogeneity in ALS
- #43: Arkoprovo Paul. \*\*\*FLASH TALK SPEAKER\*\*\*  
Reprogramming iPSC-derived Microglia for modelling Aging and Alzheimer's Disease
- #44: Anna Klingseisen. iPSC derived vasculature on a chip deciphering disease mechanisms in RVCL

# Posters

## Group 1

### Poster Sessions I - III

**#45:** Muhammad Zaman Khan Assir. A Novel 3D Ex Vivo Culture Model of Human Cortical Tissue Unveils Extracellular Matrix Remodelling in Neuroinflammation

**#46:** Nina-Lydia Kazakou. \*\*\*FLASH TALK SPEAKER\*\*\*

Development of a functional hPSC-derived neuron-glia tri-culture model of sporadic Parkinson's Disease (PD) for phenotypic screening

**#47:** Uroosa Chughtai. Using stem cell-derived models to investigate cell autonomous microglial dysfunction in TBK1-associated ALS-FTD

**#48:** Silvia Oldani. Next-Generation Electrophysiology for Functional Characterization of Human Neural Organoids and Assembloids

**#49:** Hyunjin Kim. Chronic non-cytotoxic manganese exposure disrupts glutamate homeostasis and pathways involved in the integrated stress response in hiPSC cortical model of Alzheimer's disease.

**#50:** Ana Cristina Rios Mejia. Axonal elongation in vitro- The characterization and engineering of outgrowth mechanisms in different neuronal subtypes

**#51:** Lena Erlebach. \*\*\*TCS bio Travel Award\*\*\*

Chimeric brain slice cultures to study human microglia in vitro

**#52:** Patricia Lopez Garcia. Investigating LRRK2-G2019S PD pathogenesis using iPSC-derived neuronal and glial models

**#53:** Cathleen Hagemann. \*\*\*FLASH TALK SPEAKER\*\*\*

In depth multi-omic analysis of axonal homeostasis across scale using a bioengineered platform and human IPSC-derived neurons

**#54:** Giulia Sofia Marcotto. Multiple system atrophy dopaminergic neurons differentiated from induced pluripotent stem cells exhibit distinct structural and functional abnormalities

**#55:** Marina Shiryaeva. Lipid metabolism in neuronal and glial iPSC-derived models of familial Alzheimer's disease.

**#56:** Dasa Bohaciakova. Modeling of SORL1-Associated Alzheimer's Disease in iPSC-Derived Neurons and Cerebral Organoids

**#57:** Zedeng Yang. Ser-Arg Protein Kinase (SRPK) controls MAP1S processing and acquisition of microtubule binding activity during neuronal development.

**#58:** James Crowe. \*\*\*FLASH TALK SPEAKER\*\*\*

Modelling neuronopathic Gaucher disease using human iPSC-derived brain organoids

**#59:** Alexis Penverne. Ferroptosis Drives Neuronal and Glial Vulnerability in Parkinson's Disease via the A53T SNCA Mutation

**#60:** Linus Wiora. Efficient AAV-mediated gene delivery in iPSC-derived neurons

**#61:** Soňa Česnáriková. Decoding Alzheimer's: 3D Cerebral Organoids as a Window into Early Disease Progression

**#62:** Alicia González Díaz. Building a Panel of Stem Cell Models to Capture the Molecular Heterogeneity of Alzheimer's Disease through AI-Guided Multiplexed Functional Genomics

**#63:** Brent Ryan. Integrating iPSC approaches to understand the role of PINK1 in neurons

**#64:** Katherine White. miRNA motif analysis supports a role for ALS-associated FUS protein in astrocyte extracellular vesicle-mediated sorting and loading

**#65:** Hugo Fernandes. Elucidating Lipid Droplets in Neuronal Models of Dementia

**#66:** Aswathy Chandran. Rethinking  $\alpha$ -Synuclein: How SNCA disease variants affect phase separation

**#67:** Miranda Lastra Osua. TMEM106B loss-of-function leads to impaired pre-synaptic protein machinery in human iPSC-derived cortical neurons

**#68:** Carol Geukens. Neural Stem Cells harboring POLG mutation mimic mitochondrial disease phenotypes which can be rescued by novel POLy activator

# Posters

## Group 2 pgs 74-107

Poster Sessions III - V

- #1: **Zaid Muhammad.** Generation of an induced pluripotent stem cell line (BIORTCi001-A) from a healthy adult indigenous Nigerian participant
- #2: **Athanasia Kalogirou.** Predicting Cognitive Ability in Patients with Frontotemporal Dementia Using Brain Measures Volumetric Data
- #3: **Yiyun Zhang.** \*\*\*FLASH TALK SPEAKER\*\*\*  
Investigating Pathologies of TDP-43 Mislocalization by In Vitro Neuromuscular Junction Models
- #4: **Éanna Ryan.** Exploiting Oligogenic Models of ALS to Identify Pathogenic Mechanisms
- #5: **Laura R. Rodríguez.** Human 5xFAD iPSC models with inducible and tunable pathology
- #6: **Melissa Barber.** RELN-DAB1: a novel pathway that interacts with APOE to modulate microglial activation and Alzheimer's disease-risk
- #7: **Ben Clarke.** Multi-omic analysis reveals ERAP2-dependent dysregulation in VCP-ALS astrocytes
- #8: **Hilary Carswell.** A 'stroke-on-a-chip' microfluidic model using mouse primary neuronal cultures- a pathway for the future development of a human iPS stroke model.
- #9: **Rana Fetit.** Can ES-derived human oligodendrocytes recapitulate known regional and functional transcriptional heterogeneity?
- #10: **James Evans.** \*\*\*FLASH TALK SPEAKER\*\*\*  
Modelling oligodendrocyte dysfunction in Parkinson's disease
- #11: **Lizzie Glennon.** Complex modelling of neurodegeneration using iPSC derived cells and applications to drug discovery.
- #12: **Poulomi Banerjee.** \*\*\*FLASH TALK SPEAKER\*\*\*  
Hyperphosphorylated tau aggregates drives microglial activation and synapse loss in microglia-containing cortical organoids
- #13: **Tom Cremer.** Understanding mitochondrial control of tau hyperphosphorylation
- #14: **Miguel Minaya.** \*\*\*SCMND travel award\*\*\*  
4R-tau disrupts molecular networks, including SALL1, which promotes tau aggregation
- #15: **Lois Keavey.** Investigating non-cell autonomous effects of MAPT mutations through mixed genotype neuron-astrocyte co-cultures
- #16: **Francesco Paonessa.** Targeting the acetyltransferase NAT10 corrects pathologies in human frontotemporal neurons and extends lifespan in an *in vivo* *Drosophila* tauopathy model
- #17: **Olivia Soper.** \*\*\*FLASH TALK SPEAKER\*\*\*  
Generation of Human Hippocampal Organoids
- #18: **Áine Heffernan.** Understanding the consequence of LRRK2 dysregulation in human stem cell-derived astrocytes'
- #19: **Orjona Stella Taso,** Novel TDP-43 aptamers identify early aggregation events in C9orf72 mutant human motor neurons
- #20: **Mark Gurney.** Transcriptomic profiling and comparative analysis of human iPSC-derived reactive astrocytes
- #21: **Sheikh Shahzabe Mukhtar.** Investigating the Effect of PD-Linked GBA1 Mutations on Neuronal Activity and the Autophagy Lysosomal Pathway Using iPSC-Derived Neurons
- #22: **Rebeka Popovic.** PINK1 mDA human neurons display mitochondrial dysfunction not due to mitophagy impairments.
- #23: **Alice Sartini.** Electrophysiological and Morphological Characterization of human-derived neurons carrying ACTG1 actin mutation
- #24: **Shreya Das Sharma.** Loss of TDP-43 causes AMPAR current dysfunction in iPSC derived motor neurons
- #25: **Nazli Eskici.** Dual role of DLK1 in GNRH neuron ontogeny
- #26: **Ana Carreras Mascaró.** Functional assays for ALS drug screening using predictive human cell models

# Posters

## Group 2

Poster Sessions III - V

#27: Eilish Mackinnon. \*\*\*FLASH TALK SPEAKER\*\*\*

A standardised framework for evaluating human iPSC-derived microglial cultures

#28: Mario Yanakiev. APOE-Dependent Mechanisms of the Response to Amyloid- $\beta$  in iPSC-derived Microglia

#29: Tom Campbell. A phenotypic screen for novel small molecules that suppress tau-mediated pathologies in human frontotemporal dementia neurons

#30: Niamh O'Brien. Generation of a de novo isogenic ALS iPSC cell bank to investigate converging pathomechanisms in ALS and FTD

#31: Paolo Marchi. A multimodal screening platform for endogenous dipeptide repeat proteins in C9orf72 patient iPSC-neurons

#32: Georgia Boothe. Optogenetic modelling of the C9orf72 dipeptide-repeat protein (DPR) pathology in ALS/FTD

#33: Sara Tacconelli. Identifying novel synaptic interactors of FUS in different experimental models.

#34: Sascha Koppes-den Hertog. Cholesterol as a regulator of astrocyte reactivity impaired by ApoE4

#35: Xu Zhang. A novel chimeric model investigating human neurological features in Alzheimer's disease

#36: Timothy Birkle. Isogenic MAPT S305N/10+3 iPSCs to study 4R tauopathy using i3Neurons

#37: Ropafado Mzezewa. Inducing pathological insults in hIPSC-derived neural cultures to model Alzheimer disease in vitro

#38: Rebecca Gabriele & Marieta Vassileva. From Bench to High-Throughput: Optimising iPSC-based assays for Drug Discovery

#39: Matthew Reid. A Drug Discovery Pipeline for Inhibitors of Seeded Aggregation of Wildtype Tau

#40: Antonio Rocco Fuciardi. Investigating the interaction of APOE4 with TDP43 proteinopathy in driving neurodegeneration

#41: Hannah Clarke. Comparison of hiPSC-derived dopaminergic neurons

#42: Johanna-Katharina Maninger. Unravelling the Role of APOE and BIN1 in Shaping Microglial Function in Late-Onset Alzheimer's Disease

#43: Luise Schlotterrose. Traumatic Brain Injury: Insights into Astrocyte and Neuronal Interactions

#44: Aleksandar Rakovic. Selective vulnerability of dopaminergic neurons in Parkinson's disease links PRKN and differential expression of CHCHD2 and GPNMB

#45: Mosi Li. Investigating Microglia's Role in Alzheimer's Disease Progression with a Microglia-Deficient Mouse Model

#46: Björn Vahsen. Microglia-dependent synaptic dysregulation and complement activation in C9orf72-ALS iPSC-derived motor neuron-microglia co-cultures

#47: Anastasiia Tourbier. The importance of high-density microelectrode arrays for recording multi-scale extracellular potential and label-free characterization of network dynamics in iPSC-derived neurons

#48: Owen Gwydion James. Investigating the role of Staufen as a modifier of ALS-associated pathology

#49: Magda Liczmanska. Investigating the role of Ser-Arg Protein Kinases (SRPK) in neurodegeneration using human stem cell-derived neurons.

#50: Stefanie Fruhwürth. Investigation of microglia-amyloid beta interactions using an in vitro Alzheimer's disease model.

#51: Nathasia Mudiwa Muwanigwa. Dysregulation of RNA metabolism in an iPSC derived Neuronal Model of Tauopathy

#52: Victoria Lievens. Elucidating Lipid Droplets in Neuronal Models of Dementia

# Posters

## Group 2

Poster Sessions III - V

- #53:** *Josefine Rågård Christiansen.* Development of a neuronal-glial tri-culture-based in vitro model of Alzheimer's disease for phenotypic drug screening
- #54:** *Rachel O'Donoghue..* Exploring the influence of the protective Apolipoprotein E (APOE) variant, R251G, on the function of APOE-e4 iPSC-derived microglia
- #55:** *Ahmad Jibai.* Investigating Tripartite Synapse pathology in ALS utilizing a hiPSC-derived organoid model
- #56:** *Annika Wagener.* Astrocyte-Neuron Crosstalk in Parkinson's disease
- #57:** *Mizuki Morisaki.* Understanding the role of Alzheimer's endocytic risks genes in disease pathogenesis
- #58:** *Sonia Yiakoumi.* 3D Human iPSC Neurosphere Models Reveal Tau-Dependent Microtubule Dysregulation in Tauopathies
- #59:** *Jamie Toombs.* Multiplexed transcriptome profiling of small molecule perturbations in stem cell-derived neuron models.
- #60:** *Tatiana A. Giovannucci.* Neurofilament light protein (NfL) as a dynamic biomarker: insights from stable isotope labelling kinetics
- #61:** *Pragati Thakur.* Role of micro-RNA dysregulation to study TDP-43 mislocalisation in Amyotrophic Lateral Sclerosis
- #62:** *Iris Kruijff.* Understanding the effect of LXR-treatments on Amyloid production in iPSC-derived neurons.
- #63:** *Lukas van den Heuvel.* The making of a Lewy Body: An ultrastructural comparison of aSyn pathology in post-mortem human brain and iPSC-derived human dopaminergic neurons seeded with pre-formed fibrils.
- #64:** *Natalia Garcia Perez.* Live-cell label-free imaging of microtubules to assess neuronal development and degeneration
- #65:** *Emma Knowling.* The role of SFPQ liquid-liquid phase separation in amyotrophic lateral sclerosis
- #66:** *Esther Muñoz Pedrazo.* Dissecting the genetic interactions controlling α-Synuclein accumulation in Parkinson's Disease
- #67:** *Ines Ferreira.* Driving experimental reproducibility and lot-to-lot biological consistency at scale in human iPSC-derived cells enabled by opti-ox technology
- #68:** *Ines Ferreira.* A versatile toolbox of human iPSC-derived microglia for disease modelling, CRISPR screens, and multicellular in vitro models for neurodegeneration drug discovery.

# Posters

## Resources

Poster Sessions I - V

**R1:** Suleiman Hamidu Kwairanga; \*\*\*SCMND travel award\*\*\*

Modelling Alzheimer's Disease in Northern Nigeria: Establishing a Cohort and iPSC Resource

Dementia cases are rising globally, with the burden projected to rise in low- and middle-income countries (LMICs). Despite Africa's high genetic diversity, dementia remains poorly studied in the region, leaving significant gaps in understanding its epidemiology and underlying mechanisms. This is especially true in Nigeria, particularly in the North, where research on Alzheimer's Disease (AD) and other dementias is scarce.

To address this, we have established Northern Nigeria's first dementia registry, utilizing the CERAD and other Neuropsychology Assessment Battery NAB, integrated into a digital data entry application (KoboToolbox) to facilitate easy data collection. Using this, we have commenced northern Nigeria's first detailed AD cohort, from which we collect blood samples and skin biopsies from participants to generate induced pluripotent stem cells (iPSCs) to model AD in this unique genetic background. These iPSC lines will undergo rigorous characterization for genomic stability, pluripotency, and differentiation potential, providing data for studying population-specific disease mechanisms in AD. By bridging clinical neuropsychological assessments with advanced molecular research, our work project aims to increase Africa's representation in global dementia studies, enabling better-targeted interventions and advancing precision medicine for neurodegenerative diseases.

**R2:** Eva-Maria Surmann: The JAX iPSC Repository: Facilitating Access to High-Quality Isogenic iPSCs for Advanced Disease Modeling and Drug Discovery

In 2022, The Jackson Laboratory, the global leader in high-quality mouse model distribution, launched a repository for human induced pluripotent stem cells (iPSCs) with support of the National Institute on Aging (NIA), the Chan Zuckerberg Initiative (CZI), and the Aligning Science Across Parkinson's (ASAP) initiative. This repository aims to remove barriers and facilitate easy access to iPSCs for scientists in academia, biotechnology, and the pharmaceutical industry. By providing high-quality, readily available research tools, the repository strives to accelerate the pace at which the global scientific community can study and develop treatments for various diseases.

The catalog now features over 500 human iPSC lines, all derived from a well-characterized parental iPSC line (KOLF2.1J). These lines have been engineered to carry disease-relevant single nucleotide variants (SNVs) in both heterozygous and homozygous forms, along with a revertant line, forming trio sets for each variant. Additionally, gene deletions and protein-tagged lines have been incorporated. Detailed information on design and quality control for each line is readily available, ensuring researchers can rely on the quality of these lines and the validity of their results. To advance diversity in research, additional parental and derived mutant lines will be released into the repository in the future.

---

**R3:** Richard V. Pearse II. Molecular analyses of human iPSC-derived brain cells and matched brains from over 100 genetic backgrounds highlight systemic variations in response to genetic ancestry, sex and disease risk

The goal of this study was to increase the genetic complexity of existing human cellular models of Alzheimer's disease (AD), and to interrogate the impact of sex and genetic ancestry on molecular pathways relevant to AD. To this end, we have generated over 100 induced pluripotent stem cell (iPSC) lines from participants in the Religious Order Study and Memory and Aging Project (ROS, MAP) cohorts that span the clinical and neuropathological spectrum of aging. We then differentiated these iPSC lines to neuronal, astrocyte and microglia fates and used single cell and bulk RNA sequencing as well as proteomic profiling to define the molecular signature of these cells. These data were then compared to the same measurements from matched brain tissue of the same individuals to validate associations of physiological relevance. Associations were observed between molecular profiles and polygenic risk score for AD and neuropathological and clinical progression to AD at the gene and protein level that are concordant between the cellular models and brain tissue. Early analyses show evidence of dysregulated amyloid beta, synaptic vulnerability, glial activation and dysregulated proteostasis in distinct subsets of individuals. Candidate genetic variants that could potentially drive these altered signatures were identified through QTL analyses. These signatures inform new methods for subtyping those at risk for AD in a manner that facilitates precision medicine approaches.

**R4:** Chris Morris. The BDR Stem Cell and Fibroblast Cell Bank for Dementia Research

Neurodegenerative disorders represent a significant healthcare challenge with current treatments being limited and palliative. Modelling disorders such as Alzheimer's disease using human induced pluripotent stem cells (hiPSC) is possible, particularly where disease causing mutations are known. However, genetic forms of neurodegenerative disease are rare and the predominant late onset forms of neurodegenerative disorders are seldom associated with single gene defects. hiPSC models derived from rare autosomal dominant cases may therefore not represent the reality of neurodegenerative disorders in the older individual where these disorders are prevalent. To address this gap, we have utilised a simple method to isolate proliferating fibroblast-like cells from human post-mortem leptomeningeal tissue with high success rates (>85%) even at extended post mortem intervals. These fibroblast-like cells (HSP47- and vimentin-positive) due to post mortem selection come from clinically and importantly pathologically verified donors including Alzheimer's Disease, Dementia with Lewy Bodies, Vascular Dementia, Frontotemporal Lobar Degeneration, Parkinson's Disease, Progressive Supranuclear Palsy, Motor Neuron Disease, and, importantly, clinically and pathologically older normal donors. Fibroblast-like cells can be converted to hiPSC using established approaches, allowing conversion into neural cells for further study. The isolation of viable cells from post-mortem human brain tissues and subsequent reprogramming to hiPSC provides an invaluable resource for late onset typical forms of neurodegenerative disorders, with extensive medical history, pathological confirmation, and corresponding banked tissue samples, and should allow the study of the biology of typical neurodegenerative disorders, and identification of greatly needed therapeutic targets. Using this approach we have generated over 50 cell lines from specific disorders and also clinically and pathologically normal individuals over 100 years of age available through open access.

---

**R5:** Hazel Hall-Roberts. IPMAR: iPSC Platform to Model Alzheimer's disease Risk

**R6:** Erin Hedges. An iPSC platform for drug discovery in motor neuron disease

---

**R7:** Daniel Paull. Automating iPSC research for biobank development and high-throughput phenotyping

### #1: Kim de Kleijn. TREM-family receptor-ligand discovery to fine-tune microglia function in Alzheimer's disease

Microglia cells play a prominent but disparate role in Alzheimer's disease (AD), and many genetic risk factors for AD control microglia function. Microglia express a number of receptors of the TREM-receptor family (TREM1, TREM2, TREML1, TREML2 and TREML4), of which TREM2 and TREML2 are genetically linked to AD risk and soluble (s)TREM1 and sTREM2 are biomarkers for disease progression. Though the mechanism of TREM2 activation and signaling in microglia activation in AD has been studied, whether and how microglia can be activated by the other TREM-receptors is not clear, because their function and receptor-ligand interactions are largely unknown. We performed a TREM-interactome discovery proteomics approach in (non-)AD brain tissues, to identify receptor-ligand pairs for all five TREM-receptors and their soluble extracellular domains. This method identified the already reported interactors for TREM1, TREM2 and sTREM1, substantiating this new approach for receptor-ligand identification. In addition, we identified novel binding partners for (s)TREML1 related to platelet degranulation, complement and coagulation, a binding partner for (s)TREML4 related to APP processing and binding partners for (s)TREML2 related to pre-synaptic function and lipid transport in neurons and glia. We will functionally validate these novel binding partners in human iPSC-derived microglia, in which we have correlated the expression of TREM-receptors to specific microglia states relevant to AD. These new TREM-receptor binding partners serve as trigger points to fine-tune microglial activation states in AD, and provide possible new strategies for AD therapy development.

### #2: Sam Washer. Knockout CRISPR screens identify key regulators of phagocytosis and endolysosomal function in human induced pluripotent stem cell derived microglia

#### Background:

Linking disease associated genes to function is the next step in the genomic revolution of Alzheimer's disease (AD). By utilising pooled knockout CRISPR screen in human induced pluripotent stem cell (hiPSC) derived microglia, we can start to elucidate function at scale in an unbiased way. Phagocytosis and clearance of neuronal debris is a critical function of microglia and can be perturbed in disease brains. We thus undertook a CRISPR knockout screen of candidate genes at AD risk loci to identify if any are regulators of neuronal phagocytosis and autophagy using a novel dual colour reporter system.

#### Method:

hiPSC derived microglia precursor cells were transduced with a CRISPR/Cas9 knockout library and differentiated for two weeks to microglia. After two weeks hiPSC-microglia were fed dead double GFP/mCherry fluorescent labelled SH-SY5Y cells to act as a phagocytic cargo. Phagocytosis occurred for 6 hours before washing non-phagocytosed cargo, harvesting, and fixing hiPSC-microglia. hiPSC-microglia were sorted into high and low levels of phagocytosis by fluorescent activated cell sorting for single mCherry positive cells. Genomic DNA was extracted from sorted cell populations and sequenced by Illumina Novaseq and data analysed using MAGeCK.

#### Results:

We identified several genes of interest which resulted in increased autophagy, including TREM2, VAMP4, CD11c, SLC26A1, PRKN, PLCG2, and several which decreased phagocytosis such as HAVCR2, RAB7a, HMGCR, SORT1, INPP5D, LRP1, DCAF7 when knocked out in hiPSC-microglia. We have validated the TREM2 finding through arrayed CRISPR screening and antisense oligonucleotide knockdown in three different hiPSC backgrounds using flow cytometry and high-content microscopy. Further work is ongoing to validate other targets.

#### Conclusion:

We have developed a successful pipeline for linking genes to function in hiPSC-microglia through unbiased CRISPR screening. We have identified potential roles of AD risk genes in phagocytosis and endolysosomal function and are undertaking further validation of identified targets.

---

**#3:** [Zhizhong Yang](#). Generating in vitro platforms for testing blood-brain barrier penetrance of novel anti-complement drugs for therapy of dementia

**Background:** In vitro blood-brain barrier (BBB) models enable for screening of drug penetration across the BBB. These models consist of brain microvascular endothelial cells (BMECs) in confluent monolayer and co-cultured with astrocytes and/or pericytes. Barrier integrity is evaluated by quantifying the permeability of tracers or measuring transendothelial electrical resistance (TEER). These platforms were used to assess anti-complement drugs' ability to penetrate across BBB reducing dependence on animal studies while accelerating the discovery of drugs for the treatment of dementia.

**Methods:** Initially, a transwell-based BBB model utilising the human BMEC cell line (hCMEC/D3) has been generated. This prototype model served as a basis for developing a precise close ex vivo human model comprising induced pluripotent stem cell (iPSC)-derived BMECs. TEER measurements, permeability to fluorescein isothiocyanate (FITC)-dextran, and formation of tight junction (TJ) were used to assess the barrier integrity. Finally, the barrier was subjected to evaluate the penetrance of anti-complement drugs optimised for BBB permeability.

**Results:** The hCMEC/D3 and iPSC-derived BBB models were successfully established, with the iPSC model exhibiting markedly enhanced barrier integrity with higher TEER ( $30\text{--}50 \Omega\cdot\text{cm}^2$ , hCMEC/D3;  $3000 \Omega\cdot\text{cm}^2$ , iPSC-derived BMECs), lower permeability to FITC-dextran (70 kDa), and increased TJ formation (claudin-5, ZO-1, and occludin). The anti-complement BBB penetrating drugs demonstrated the ability to traverse the artificial barrier, whereas the non-BBB penetrating controls did not.

**Conclusions:** The in vitro transwell-based BBB models established align with existing literature. The iPSC-derived model more accurately replicated the BBB, exhibited enhanced reproducibility and integrity, and demonstrated superior performance in drug penetration tests, making it a valuable tool for precisely evaluating BBB permeability.

**#4:** [Dominik Paquet](#). A human iPSC model of Tauopathies engineered for 4R Tau isoform expression endogenously develops late-stage neuronal Tau pathology

Tauopathies, such as Alzheimer's disease and Frontotemporal Dementia, are common neurodegenerative diseases characterized by misfolding, hyperphosphorylation, and aggregation of Tau. Molecular mechanisms underlying Tauopathies are still poorly understood, in part due to a lack of human models endogenously developing major disease hallmarks. Adult Tau isoform expression contributes to Tau pathogenesis but is challenging to replicate in human stem-cell-derived systems, which impedes formation of late-stage disease phenotypes and hence research on underlying mechanisms and drug development. We show that induction of adult human brain-like 4R Tau isoform expression enables endogenous formation of late-stage Tauopathy hallmarks in iPSC-derived neurons engineered to contain synergistic Tau mutations. Neurons accumulated seeding-competent, hyper-phosphorylated, fibrillar Tau in tangle-like structures. Furthermore, exclusive expression of mutant 4R in the absence of the 3R Tau isoform disproportionately intensified pathology, resulting in highly abundant Tau misfolding and aggregation. Finally, we provide proof-of-principle that our model can be translationally applied both to test chemical disease modulators and evaluate a human Tau PET tracer. Collectively, our model enables novel investigations on endogenous mechanisms of human Tauopathy formation, suggesting a central role of 4R Tau isoform expression for pathogenesis in human neurons. Moreover, it may also serve as a platform supporting urgently needed development of disease-modifying drugs.

---

## #5: Tania Atienzar. Rapid Oligodendrocyte Generation for TDP-43 Disease Modelling: A Novel Stem Cell Approach

**Background:** Despite the clinical and genetic heterogeneity of amyotrophic lateral sclerosis (ALS), up to 97% of cases share a common denominator: nuclear depletion and cytoplasmic aggregation of the RNA-binding protein TDP-43. Importantly, this pathology is observed not only in motor neurons, but also in glial cells such as oligodendrocytes. This suggests that TDP-43-mediated neurodegeneration can occur without neuronal involvement and highlights the need to deepen our understanding of oligodendroglial TDP-43 pathology. However, current methods to study cell-specific mechanisms in oligodendrocytes are limited.

**Objectives:** To establish a protocol for the rapid generation of oligodendrocytes from human iPSCs and subsequently model TDP-43 pathology in these cells.

**Methods:** iPSC-derived neural progenitor cells were transduced with a lentiviral vector encoding oligodendrocyte-specific transcription factors to promote their maturation. Differentiation efficiency was monitored via immunostaining for relevant markers. Our group has developed an innovative system that enables TDP-43 mislocalisation without artificial interventions. Accordingly, healthy iPSCs with GFP-tagged TDP-43 were differentiated into oligodendrocytes and transduced with adeno-associated viruses expressing anti-GFP nanobodies. These nanobodies are fused to a nuclear export signal that can trigger the relocation of TDP-43-GFP to the cytoplasm over time.

**Results:** By day 23, 70% of cells express the early oligodendrocyte marker O4, with 25% also expressing the myelin-associated marker MBP. By day 30, clear TDP-43 mislocalisation can be observed in MBP+ oligodendrocytes. By day 43, MBP+ cells substantially increase in number and branching complexity, with their processes contacting neuronal axons in co-culture, indicating potential myelination.

**Discussion:** The transcription factor-based protocol for rapid oligodendrocyte generation, coupled with the TDP-43 mislocalisation model, provides a strong basis for exploring the effects of TDP-43 alterations on oligodendrocyte development and function. Future research will use this approach to assess how TDP-43 mislocalisation differentially impacts oligodendrocyte morphology, survival, and gene expression, aiming to uncover potential therapeutic targets for ALS treatment.

## #6: Dafni Kampitsi. Impact of MAPT mutation on ER-phagy in iPSC-derived neurons

**Background:** To maintain their homeostatic environment, cells will degrade part of their endoplasmic reticulum (ER) through a highly specialised intracellular degradation pathway, called ER-phagy. This process occurs when there is increased cellular stress, such as lack of nutrition or proteotoxicity. Aggregation of hyperphosphorylated tau in the cytoplasm of neurons has been shown to cause increased ER stress, and changes in cells' autophagy.

**Hypothesis and Aims:** This project's hypothesised there is a direct link between the aberrant presence of hyperphosphorylated tau and ER-phagy flux, leading to differences in the ER-phagy rate, protein presence, and tau and ER colocalization.

**Methodology:** To monitor ER-phagy flux, we transduced MAPT-mutation and isogenic control iPSC-derived neurons with a lentivirus expressing ss-SRAI-KDEL reporter. ER-phagy under basal, nutrient-starvation conditions and nutrient-starvation conditions with bafilomycin was observed via live imaging for 154 hours and at specific time points. To investigate the relationship between pathogenic tau and ER, cell pellets were collected at 6 and 21 hours under full nutrient and starvation conditions for western blot to quantify total tau, phosphorylated tau, and TEX264, an ER-phagy reporter, in mutation and isogenic control iPSC-derived neurons. Co-localisation between tau and ER protein calnexin was validated and visualised by Proximity Ligation Assay.

**Results:** We observed consistently increased ER-phagy in the MAPT-mutation neurons, compared to the isogenic ones at all time points, although there were no significant differences depending on the conditions. In MAPT-mutation neurons 22 hours after conditioning, relatively increased ER-phagy in nutrient-starvation condition was detected, and decreased ER-phagy in the presence of bafilomycin was found, compared to controls. We further confirmed colocalization of total tau and calnexin in MAPT-mutation neurons, but not in isogenic ones, that was further increased in starvation conditions. Finally, decreased TEX264 and total tau were detected in MAPT-mutation neurons compared to control.

---

**#7: Rafaela Hasse e Silva Policarpo.** Modelling FTLD-FET using patient-derived induced pluripotent stem cell models

State of the art: Frontotemporal lobar degeneration with cytoplasmic inclusions containing FET proteins (FTLD-FET), including FUS, EWS and TAF15, is estimated to represent 10% of FTLD patients. The etiology of atypical FTLD-U (aFTLD-U), the most common FTLD-FET subgroup, is still largely unknown, hampering the development of effective therapies.

**Methodology:** We generated the first human induced pluripotent stem cell (iPSC)-derived models from patients diagnosed with aFTLD-U. For this, we leveraged our unique patient cohort from the aFTLD-U International Consortium to reprogram four independent lymphoblastic lines into iPSCs (3 aFTLD-U, 1 control). We further differentiated these lines into neuronal progenitor cells and cortical neurons. One line containing a genetically engineered mutation in FUS (p.Arg495X) that causes amyotrophic lateral sclerosis (ALS) and the corresponding isogenic control line were included to compare pathological hallmarks in aFTLD-U with ALS-FUS.

**Results:** All 6 iPSC lines (3 aFTLD-U, 1 control, 1 ALS-FUS, 1 isogenic control) robustly express distinct pluripotency markers. Neural induction and cortical identity of the neural tissue was confirmed using well established markers. We observed no obvious cytoplasmic accumulation of FET proteins nor loss of FUS methylation in 30-day old neurons derived from the aFTLD-U or ALS-FUS patients. We next will focus on assessing whether patient-derived cortical neurons manifest pathological features associated with FTLD-FET at later timepoints (60 and 90-days old).

**Conclusion:** By developing and characterizing a human iPSC model of aFTLD-U using patient-derived cells, we aim to create a platform for targeted therapies to be developed, ultimately leading to new treatment options for patients.

**#8: Ruxandra Dafinca.** Mitochondrial dysfunction in iPS-derived motor neurons from patients with Amyotrophic Lateral Sclerosis

Mitochondrial dysfunction has emerged as a significant molecular mechanism in the pathogenesis of amyotrophic lateral sclerosis (ALS). Mitochondrial abnormalities associated with ALS include impaired oxidative phosphorylation, increased production of reactive oxygen species (ROS) and disrupted calcium handling. The aim of the study presented here is to identify the effects of ALS mutations in TDP-43 on mitochondrial function and downstream dynamics of axonal transport in patient-derived motor neurons.

Mitochondria provide the ATP necessary to actively transport mRNAs, proteins and organelles throughout the cells. In ALS patient iPS-MNs, we identified significant reductions in ATP production and basal respiration, which correlated with an increased interaction between ALS-TDP-43M337V with ATP synthase (ATPB) and COX5A, compared to healthy and CRISPR/Cas9 isogenic controls. Electron microscopy also revealed morphological differences in patient mitochondria, which had reduced area size compared to healthy controls. While we detected no differences in the percentage of anterograde and retrograde running mitochondria, mitochondria from TDP-43 patients travelled retrogradely at significantly lower speeds compared to healthy controls. Analysis of mean velocity of endosomal transport also showed a significant reduction in retrograde speed in patient iPS-MNs compared to healthy controls, indicating a general impairment in the motor protein complexes coupled with low ATP availability. Indeed, these deficits correlated with a decrease in the expression of dynactin-1 (DCTN1) and dynein. Lentiviral overexpression of DCTN1 in patient iPS-neurons significantly improved dynamics of retrograde transport, reducing the number of stationary mitochondria and doubling the percentage of retrograde running mitochondria. Our results show that ALS mutations contribute to mitochondrial dysfunction through both direct and indirect interactions with members of the electron transport chain complex in iPS-derived neurons from patients. Furthermore, mutations in TDP-43 contribute to the reduction of retrograde transport through a downregulation of the motor protein DCTN1 and these impairments can be partially rescued by overexpression of DCTN1.

---

**#9:** Shikha Kataria. Investigating the link between neuronal function and the autolysosomal pathway (ALP) in Parkinson's using mutant LRRK2 iPSC derived neuronal models

Mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene represent the most prevalent genetic cause of familial Parkinson's disease (PD). LRRK2 dysfunction has been implicated in the autolysosomal pathway (ALP) impairment seen in Parkinson's. Furthermore, studies demonstrate robust neurotransmission and electrophysiological abnormalities in LRRK2 mutant PD models critical in the clinical phenotypes observed. Recent evidence alludes to a direct physiological relationship between these two pathways.

Specifically, heightened neuronal activity triggers lysosomal localisation to dendritic spines, thereby facilitating synaptic plasticity. Conversely, lysosomal calcium release may initiate neurotransmitter release. This process appears to be associated with glutamatergic activity, which exhibits abnormalities in LRRK2 mutant lines.

Whilst the mechanisms linking ALP and synaptic function is not fully elucidated, this connection presents a novel avenue for investigating PD pathogenesis.

Our research utilises isogenic induced pluripotent stem cells (iPSCs) carrying the most prevalent LRRK2 disease-associated mutations to derive cortical neuronal cultures. Through multielectrode array (MEA) analysis, we have documented alterations in neural network activity between 35 and 100 days in vitro, comparing control and isogenic LRRK2-PD lines. Our preliminary MEA data reveals distinct electrophysiological signatures among different mutations. Additionally, we are employing DQ-BSA analysis to identify ALP disruptions, enabling investigation of temporal variations in the interplay between these critical cellular processes. Understanding the relationship between these processes will establish a foundation for developing more dynamic and effective therapeutic interventions.

**#10:** Tony Oosterveen. An in vitro toolkit to study the cell-specific roles of glutamatergic neurons and glia in Alzheimer's Disease

In neurodegenerative diseases, such as Alzheimer's Disease (AD), the impairment of neuronal functions leads to the loss of cognition and memory. Consequently, a neuron-centric view has prevailed in AD research, and there has been limited research into the role of glial cells. Recent evidence shows that astrocytes, microglia, and oligodendrocytes are impacted by amyloid beta peptides, thereby playing a critical role in Alzheimer's disease progression. In particular, amyloid beta peptides can induce inflammatory markers in astrocytes, a destructive inflammation in microglia, possibly resulting in excessive synapse pruning and neuronal death, as well as as a focal demyelination of axons that are ensheathed by oligodendrocytes. Using deterministic cell programming technology, opti-ox, we generated the key cell types involved in AD progression, from iPSCs, and engineered genetically matched disease models and CRISPR-ready cells. We generated heterozygous and homozygous PSEN1 M146L, APP KM670/671NL or APP V717I mutations – associated with early-onset AD – in our excitatory ioGlutamatergic Neurons. These mutations recapitulate the increase in amyloid beta 42 secretion as observed in Alzheimer's patient material and show the expected change in ratio of the amyloid beta peptide variants. In addition, we introduced TREM2 and APOE mutations into ioMicroglia, which are associated with an increased risk of late-onset Alzheimer's disease. Our protocol for the co-culture of ioMicroglia and ioGlutamatergic Neurons enables the creation of complex cellular model systems to study the role of glial cells in AD progression. The generation of CRISPR knockout-ready derivatives of ioGlutamatergic Neurons and ioMicroglia allows the identification and characterisation of novel genes involved in AD progression. Furthermore, biological complexity can be further increased with the addition of ioAstrocytes and ioOligodendrocyte-like cells. Overall, our in vitro neuroscience toolkit provides opportunities for disease mechanism elucidation and therapeutic discovery, in the human context.

---

**#11: Sophie Goldsmith.** A cerebral organoid model of progranulin-associated frontotemporal dementia

Heterozygous mutations in the progranulin gene (GRN) are the cause of 5-10% of frontotemporal dementia (FTD) cases, with patients consistently showing TDP-43 pathology at post-mortem. There is evidence to suggest that progranulin (PGRN) plays an important role in lysosomal function. PGRN is localised to the lysosome and has been shown to regulate lysosomal enzyme activity. Additionally, homozygous GRN mutations lead to neuronal ceroid lipofuscinosis (NCL), a group of lysosomal storage disorders, further highlighting PGRN's role in lysosomal function. PGRN is expressed throughout the brain with the highest expression seen in microglia. Recent evidence suggests lysosomal function is particularly impaired in PGRN deficient microglia despite TDP-43 pathology being predominantly localised to neurons. Therefore, further studies looking at the interactions between microglia and neurons are essential to assess the potential non-cell autonomous mechanisms at play. The aim of this current project is to establish forebrain organoids, iPSC derived microglia and a co-culture model of the two, using INDI initiative stem cell lines with R493X mutations. Using western blots, immunocytochemistry, and ELISA, we will characterise our model and assess FTD associated pathology, specifically looking at progranulin levels, TDP-43 aggregates, and lysosomal functional markers. We aim to present preliminary results on model development and characterisation as well as TDP-43 pathology, lysosomal dysfunction and progranulin levels within these models.

**#12: Filipa Henderson Sousa.** The role of ELAVL4 in neuronal vulnerability in MAPT mutation iPSC-derived neurons

Frontotemporal dementia (FTD) can be sporadic or inherited due to MAPT gene mutations encoding tau protein. Understanding FTD-tau pathology is limited, with no effective therapies. We identified ELAVL4, a neuronal-specific RNA-binding protein, as a regulator of glutamatergic dysfunction and splicing in tau-V337M iPSC-organoids. ELAVL4's role in disease mechanisms is not well-understood, but its aberrant expression is linked to neurodegenerative diseases. This project investigates how ELAVL4 contributes to neuronal dysfunction and death in MAPT-mutation neurons.

We knockdown (KD) ELAVL4 in V337M-tau NGN2-induced glutamatergic neurons using shRNA. We assessed the impact of ELAVL4-KD on glutamate toxicity, tau levels, and neuronal excitability. V337M-tau neurons showed disrupted neurite morphology and increased excitability but no significant difference in glutamate-induced cell death compared to isogenic control neurons. ELAVL4-KD in V337M-tau neurons did not influence neuronal death in response to glutamate exposure and showed similar network burst frequency as the isogenic control. However, glutamate treatment upregulated ELAVL4 expression, whereas ELAVL4 knockdown increased tau protein levels.

Neuronal hyperexcitability is linked to increased ELAVL4 expression, which regulates glutamatergic signalling. Although ELAVL4-KD did not protect against excitotoxicity, it reduced excitability, suggesting its role in modulating glutamatergic signalling in V337M-tau pathology. This indicates a functional interaction between ELAVL4, tau, and excitotoxicity, warranting further investigation.

---

**#13: Freya Cracknell.** Development of a human iPSC-derived triculture system to study late onset Alzheimer's disease associated microglial TREM2 variants

Microglia, as the innate immune cells of the brain, are the sole expressors of the membrane receptor, triggering receptor expressed on myeloid cells-2 (TREM2) in the CNS. As surveyors of the brain, microglia have a pivotal role in recognising and responding to pathogen and damage-associated molecular stimuli. In the healthy brain, TREM2 has a role in mediating downstream signalling controlling normal microglial processes, including metabolism, survival and phagocytosis. Genome wide association studies have highlighted functional variants in TREM2, including the heterozygous R47H variant, which have been linked to late-onset Alzheimer's disease (LOAD). In vitro models using patient-derived iPSC microglia expressing the R47H TREM2 variant demonstrate functional deficits; aberrations in metabolism and clearance of the pathogenic oligomeric amyloid beta (A) associated with AD, as well as enhanced phagocytosis of synapses have all been noted. Microglial dysfunction has also been identified as a central mechanism of pathogenesis in a number of other diseases of the brain such as Huntington's and Parkinson's diseases. Enhancing microglial function via TREM2 could therefore offer a potential target for therapies in several neurodegenerative conditions. Agonistic anti-TREM2 antibodies (TREM2 Abs) demonstrate promising results in preclinical mouse models of familial Alzheimer's disease (FAD), supporting a protective role for microglial TREM2 against AD-associated amyloid pathology. Modified anti-TREM2 antibodies with an increased capacity for blood brain barrier uptake, also enhanced mitochondrial metabolism in human iPSC-derived microglia. It is not yet clear, however, how TREM2-activating antibodies may affect microglia in the context of a human LOAD 'disease-in-a-dish' model. The current project aims to address this by antibody-mediated targeting of microglial TREM2 in an iPSC LOAD-associated triculture of microglia, neurons and astrocytes. In doing so, we hope to understand whether enhancing microglial function via TREM2 could confer protection to neurons and astrocytes in an Alzheimer's disease context.

**#14: Chloe Kan.** Developing an enhanced glioblastoma model using in vivo xenografts of hPSC-derived assembloids with cerebral tissue, glioblastoma cells and immune cells

Glioblastoma (GBM) is a complex and aggressive brain cancer with a poor prognosis. Currently, there are limited treatments for GBM, and patients often require surgery followed by radiation or chemotherapy that can compromise their quality of life. Experimental models fully recapitulating the GBM microenvironment are essential for understanding its mechanisms and developing effective therapeutic strategies. Recent advancements in 3D organoids enable better characterisation of GBM tumours. However, these organoids lack normal brain tissue, immune cells and vessels which may be inadequate to model the complete GBM tumour microenvironment. Therefore, the present study aims to create a novel multi-lineage assembloid model by all human iPSC-derived GBM, cerebral tissue, innate immune cells and vessels. This strategy facilitates the immunisation and vascularisation of GBM tumours within a human cerebral organoid context, thereby establishing a more physiologically relevant model. For subsequent in vivo modelling, the assembloid will be xenografted into immunodeficient NSG mice to observe its integration and interaction with the brains of the host mice. This approach can provide a more comprehensive GBM model by closely mimicking the tumour microenvironment within living organisms. Continuous refinement of 3D organoid and assembloid systems is critical to enhancing their applications in preclinical testing and understanding GBM mechanisms. By integrating both in vitro and in vivo methods, this study aims to develop an improved GBM model to develop more effective therapeutic strategies for patients.

---

**#15: Preethi Sheshadri.** Modelling Human iPSC-derived AGTR1+ Dopaminergic Neurons for Parkinson's Disease

Parkinson's Disease (PD) is a debilitating condition characterised by the degeneration of dopaminergic neurons (DA neurons) in the substantia nigra. While the differential vulnerability of ventral midbrain DA neurons compared to dorsal ones is well established, deep single-cell sequencing performed on human Braak stage 5/6 PD brain tissue has identified a specific susceptibility of Angiotensin II Receptor (AGTR1+) DA neurons, predominantly distributed in the ventral region of the midbrain at the onset of PD. AGTR1 is a component of the Renin-Angiotensin System (RAS), which plays a crucial role in maintaining redox homeostasis across cells and tissues in the human body. Additionally, RAS is essential for regulating the production of free radical species, thereby influencing mitochondrial function. Utilising human patient-derived induced pluripotent stem cells (iPSCs) as a model system, we aim to further investigate the specific vulnerability of AGTR1+ DA neurons. We have established a differentiation protocol in the laboratory to generate AGTR1+ DA neurons from human iPSCs, representing the ventral midbrain DA neurons of the substantia nigra, which have been characterised through immunofluorescence and deep single-cell RNA sequencing. Seahorse studies indicate a reduced oxygen consumption rate in these DA neurons when treated with 1-Methyl-4-phenyl pyridinium (MPP+) Iodide. Further investigations in the laboratory are currently ongoing to examine the role of mitochondria in the specific susceptibility of AGTR1+ human iPSC-derived DA neurons

**#16: Gizem Onal.** Using CRISPRi Screens in Human iPSC-Derived Dopamine Neurons to Probe Endolysosomal Pathway Dysfunction in Parkinson's Disease

Parkinson's disease (PD) remains a challenging neurodegenerative disorder characterized by the progressive loss of dopamine neurons in the substantia nigra, leading to motor and cognitive impairments. Despite significant advancements, understanding the precise molecular mechanisms underlying PD pathogenesis and identifying effective therapeutic targets remain elusive. Here, we present a high-throughput approach utilizing CRISPR-interference (CRISPRi) gene knockdown technology in induced pluripotent stem cell (iPSC)-derived dopamine neurons (DaNs) to elucidate the role of endolysosomal biology in PD, with the ultimate aim of identifying potential therapeutic targets.

Our methodology employs integrated catalytically inactive Cas9 (dCas9) machinery in control and PD patient-derived DaNs, enabling precise modulation of gene expression. Utilizing a 384-well automated format, we deliver a dual single-guide RNA(sgRNA) lentiviral library targeting over 100 individual lysosomal pathway-related genes of interest. By systematically perturbing gene expression, we aim to identify genes critical for endolysosomal function in DaNs. To assess lysosomal function, we use the DQ-BSA assay as the primary readout, allowing us to evaluate lysosomal activity, morphology, and endolysosomal trafficking dynamics as a multiparameter phenotypic readout.

We achieve a high efficiency of DaN transduction, with transduction rates reaching up to 90% by Day35 of DaN maturation. Through qRT-PCR analysis, we assessed the knockdown efficiency of selected guides in pilot studies, revealing significantly decreased gene expression with knockdown levels ranging from 50% to 95%. Our multiparameter DQ-BSA phenotypic assay identified candidate genes that either enhance or suppress lysosomal number, size, and activity, offering valuable insights into potential targets for modulating endolysosomal pathways in PD.

Next, we plan to validate the candidate results obtained from the CRISPRi screen using complementary functional assays (e.g., GCase activity, lysosomal pH) to further unravel the molecular mechanisms of specific candidate genes in neuronal homeostasis and/or pathogenesis. These efforts in control and patient-derived DaNs aim to uncover new avenues for therapeutic intervention.

---

**#17:** Dianne Lopez. Investigating the role of LRRC37A2 gene in iPSC-astrocytes and Parkinson's

Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by a combination of motor and non-motor dysfunctions. Emerging evidence, including findings from our own research, highlights the potential role of the LRRC37A2 gene in influencing PD risk. Despite its promising implications, LRRC37A2 has traditionally been excluded from genetic studies, leaving its cellular function largely unexplored.

LRRC37A2 is situated at the inversion breakpoint of the 17q21.31 'MAPT' locus, a region prone to genomic instability that results in diverse copy number variations (CNVs) across haplotypes. These CNVs differ based on an individual's haplotype and ancestry, with increased LRRC37A2 expression and copy number being associated with a reduced risk of PD.

In our previous work, we demonstrated that LRRC37A2 expression in astrocytes plays a pivotal role in regulating cellular migration and inflammation in response to alpha-synuclein. Remarkably, we also found that LRRC37A2 co-localises with alpha-synuclein in Lewy bodies, providing the first direct link between the 17q21.31 locus and the pathological mechanisms of PD.

Given its critical role in astrocyte migration and inflammation, our current research aims to investigate the non-cell-autonomous mechanisms modulated by LRRC37A2. To explore these mechanisms further, we are employing transcriptomic analyses and whole-cell patch-clamp electrophysiology. Our research focuses on astrocytes and neurons derived from induced pluripotent stem cells representing diverse ancestral backgrounds. This approach allows us to examine how variations in ancestry and LRRC37A2 expression influence glutamatergic processing, ion channel conductance, and neuronal health.

Ultimately, this study seeks to uncover the molecular and cellular mechanisms by which LRRC37A2 modulates PD risk and to better understand its role in the variability of PD susceptibility across different ancestral populations.

**#18:** Ella Nightingale. Investigating homeostatic nuclear mRNA retention in iPSC-derived neurons

Protein abundance and localisation must be dynamically regulated by cells in order to respond to acute changes in their environment or adapt to chronic conditions such as advanced age or disease. In particular, cells need to precisely control the dosage of condensation-prone proteins, many of which tend to mislocalise and aggregate in several neurodegenerative conditions. We discovered a new mechanism, 'interstasis', which is induced by accumulation of condensation-prone proteins in nuclear speckles, enabling negative feedback regulation of condensation-prone proteins through the selective retention of their mRNAs in nuclear speckles (Faraway et al. 2023). Leading on from this we established iPSC-derived neuron models of interstasis that induce selective nuclear mRNA retention. We validated our model by smFISH before then optimising the fractionation of nuclear and cytoplasmic compartments and performing 3' RNA sequencing to identify novel mRNA targets of interstasis in iNeurons. Furthermore, in order to screen condensation-prone peptides for their capacity to induce interstasis in iNeurons, we are developing a Bxb1-expressing iPSC line that facilitates inducible expression of integrated genes. Together, our work investigates the mechanism of selective nuclear retention in iNeuron models.

---

**#19: Emma Jones.** Global identification of integral membrane proteins that require Retromer for their sorting in neuronal systems

The efficient transport of membrane proteins through the endosomal network is essential for maintaining cellular and organellar function. The role of this system in neuronal cells and other supporting brain cells appears especially critical, with proper function shown to be neuroprotective. Functional deficiency of the evolutionarily conserved Retromer complex, a key regulator of sorting through the endosomal network, has been implicated in a number of neurodegenerative diseases including Alzheimer's and Parkinson's diseases. However, to understand the role of Retromer in neuroprotection, it is essential that we obtain a detailed understanding of the global array of cargo proteins that rely on this complex for efficient transport.

To address this question and gain a global and unbiased view of the role of Retromer in brain function, we have used a proteomics-based approach to identify integral membrane proteins whose recycling to the plasma membrane is mediated by Retromer and two associated cargo adaptors, sorting nexin 3 (SNX3) and sorting nexin 27 (SNX27), in primary rat cortical neurons, astrocytes and immortalised human H4 neuroglioma cells. From these approaches we have identified a number of cargo that require the SNX3- and SNX27-Retromer complexes for endosomal recycling, including proteins involved in synapse organisation (e.g. adhesion molecule LRFN2, neuroligin and neurexin family members), chemical synaptic signalling (e.g. GABA and glutamate receptor subunits) and solute transport (e.g. copper transporter SLC31A1). Many of the identified Retromer cargo proteins also have defined links to neuroprotection and dysregulation in neurodegenerative disease.

Our study highlights multiple novel Retromer cargoes across two distinct sub-complexes, providing further insight into the mechanistic basis of Retromer deficiency in neurodegeneration. We are now looking to apply these methodologies and questions to a human neuronal cell model to account for species and cell-type specific effects of Retromer dysfunction, through genetic modification of Retromer components in i3 neurons.

**#20: Joseph Stone.** Using iPSCs and neurons to model modifiers of somatic CAG repeat expansion in Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder that typically onsets in mid-life, resulting in progressive motor, cognitive and neuropsychiatric symptoms. HD is caused by an expanded CAG repeat in the HTT gene, inherited in an autosomal dominant manner. This CAG repeat further expands in some somatic cells, particularly in the medium spiny neurons of the striatum—an area that undergoes early atrophy—but also in glutamatergic cortical neurons. One leading hypothesis in the field is that these somatic expansions drive disease, thus representing a novel therapeutic avenue.

We utilise a patient-derived induced pluripotent stem cell (iPSC) model of HD that exhibits somatic CAG repeat expansion in vitro. Using CRISPR Cas9, we have knocked out the MLH1, PMS1, and MLH3 genes, demonstrating that the proteins they encode are required for somatic CAG repeat expansion in cultured iPSCs. To create a more disease-relevant model, we introduced an inducible NGN2 expression cassette into our iPSC line, enabling rapid differentiation of the iPSCs into glutamatergic cortical neurons. These neurons demonstrate somatic CAG repeat expansion in culture, so can be used to assay genetic and therapeutic modifiers of somatic expansion in a disease relevant cell type. Using this system, we are further investigating the role played by PMS1 in somatic CAG repeat expansion in HD.

---

**#21: Charlie Arber.** Mutations in PSEN1 predispose inflammation in an astrocyte model of familial Alzheimer's disease through disrupted regulated intramembrane proteolysis

Mutations in PSEN1 cause familial Alzheimer's disease with almost complete penetrance. Age at onset is highly variable between different PSEN1 mutations and even within families with the same mutation. PSEN1 is the catalytic subunit of g-secretase, responsible for regulated intramembrane proteolysis of numerous substrates that include cytokine receptors. For this reason, we tested the hypothesis that mutations in PSEN1 impact inflammatory responses in astrocytes, thereby contributing to disease progression.

Here, using patient-derived iPSC-astrocytes, we show that PSEN1 is upregulated in response to inflammatory stimuli, and this upregulation is disrupted by pathological PSEN1 mutations.

Using transcriptomic analyses, we demonstrate that PSEN1 mutant astrocytes have an augmented inflammatory profile in their basal state, concomitant with an upregulation of genes coding for regulated intramembrane proteolysis, and robust activation of JAK-STAT signalling. Using JAK-STAT2 as an example signalling pathway, we show altered phosphorylation cascades in PSEN1 mutant astrocytes, reinforcing the notion of altered cytokine signalling cascades. Finally, we use small molecule modulators of g-secretase to confirm a role for PSEN1/g-secretase in regulating the astrocytic response to inflammatory stimuli.

Together, these data suggest that mutations in PSEN1 enhance cytokine signalling via impaired regulated intramembrane proteolysis, thereby predisposing astrocytic inflammatory profiles. These findings support a two-hit contribution of PSEN1 mutations to FAD pathogenesis, not only impacting APP and Abeta processing but also altering the cellular response to inflammation.

**#22: Kathryn Knowles.** Investigating the Role of Neurofilament Heavy Chain (NfH) in iPSC-Derived Neuronal Models of ALS

**Project Overview:**

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder characterised by the degeneration of motor neurons. Current biomarkers for ALS remain limited, highlighting the critical need for reliable diagnostic and disease progression markers. Neurofilament heavy chain (NfH) has emerged as a promising biomarker for ALS, due to its essential role in neuronal structure and function.

**Project Aims:**

The aim of my PhD project is to investigate the role of NfH in ALS pathogenesis by exploring its downregulation in neuronal models and characterising its proteomic profile in patient-derived samples. Additionally, the project will examine the effects of inducing a synthetic disease model using cell stressors on neurofilament release and aggregation within the cell.

**Methods:**

Cell stressors, including B27 without antioxidants and glutamate, will be optimised in i3 cortical and lower motor neuron (LMNs) models using iNDI cell lines with familial ALS (fALS) TDP-43, SOD1, C9orf72, and FUS mutations and CRISPRed with NGN2 cassettes at the CLYBYL safe harbour locus. Cell stress conditions will be optimised, and the outcomes will be measured using lactate dehydrogenase (LDH) and neurofilament light chain (NfL) assays, which will help assess cell death and neuronal degeneration. Introducing cell stressors into iPSC-derived neuronal models with fALS mutations will generate a more complex disease phenotype, providing a robust model for studying ALS pathogenesis.

**Outcomes and Significance:**

Once the synthetic disease models are established, direct comparisons of the secretome can be made between healthy parental cell lines and fALS mutation lines. This will enable investigation of the effects of downregulated NfH on the cellular and molecular dynamics in both cortical and LMN models.

Ultimately, the project will culminate in proteomic profiling of the iPSCs and their neuronal derivatives, offering a comprehensive exploration of the impact of NfH knockdown on the proteome in vitro. This work will contribute to a deeper understanding of ALS pathogenesis and the role of NfH as a potential biomarker for the disease.

---

**#23: Sabino Mendez Pastor.** Using iPSC models to investigate the impact of TREM2 variants on the microglial response to amyloid-beta

The TREM2 variants R62H and R47H are linked to increased risks of Alzheimer's disease (AD). TREM2 is a microglial receptor for endosomal uptake of amyloid- $\beta$  (A $\beta$ ) aggregates and inflammatory modulator. Our group previously showed that AD patients carrying R62H or R47H display greater brain A $\beta$  deposition and increased expression of proinflammatory genes in microglia. Here, we aimed to test for impaired uptake of oligomeric A $\beta$  (oA $\beta$ ) and dysregulated proinflammatory cytokine release in R62H and R47H microglia in vitro.

IL-6 release was quantified in cultures of human iPSC-derived microglia (iMG) carrying the TREM2 common variant (CV), R62H, R47H, or a TREM2 KO after treatment with oA $\beta$  or LPS. iMG were also incubated with oA $\beta$  conjugated to the fluorescent dye pHrodo-Red (pHR) or with pHR-labelled zymosan and the percentage of iMG phagocytosing these cargoes was determined via flow cytometry.

oA $\beta$  induced a dose-dependent increase in IL-6 production in all iMG lines, but the TREM2 KO enhanced IL-6 secretion by >3-fold in cultures incubated with 0.1; 0.5 or 1.5  $\mu$ M oA $\beta$  relative to TREM2-CV cells. All iMG lines secreted IL-6 following stimulation with LPS, and this release was greater in R47H iMG compared to TREM2-CV cultures. >90% of iMG incubated with pHR-labelled oA $\beta$  or zymosan emitted fluorescence regardless of the TREM2 variant carried. However, TREM2 KO iMG showed higher median fluorescence intensity (MFI) than TREM2-CV cells after treatment with oA $\beta$ -pHR. R62H and R47H also increased the MFI of iMG incubated with zymosan-pHR relative to TREM2-CV.

The findings indicate that loss of TREM2 function leads to greater oA $\beta$  uptake by microglia and exacerbates the proinflammatory cytokine response of these cells to oA $\beta$ . They also suggest that R47H impairs the modulation of cytokine release in microglia exposed to LPS and that R47H and R62H increase the amount of zymosan internalised by these cells.

**#24: Aiko Robert.** Lipidomic characterization of iPSC-derived microglia models

Over the last decade, numerous protocols have been developed to generate induced pluripotent stem cell (iPSC)-derived microglia-like cells (iMGLs). Most methods aim to mimic microglial ontogeny through formation of embryoid bodies (EB microglia). These iMGLs exhibit core characteristics of primary fetal and adult human microglia, including expression of microglial signature genes and key functional properties. Recently, a rapid simplified protocol leveraging doxycycline-inducible expression of microglial transcription factors (6TF microglia) was developed. While 6TF microglia have been shown to resemble EB microglia and primary human microglia at the transcriptional level, it is unclear how these protocols affect microglial lipid metabolism.

To understand how the two differentiation protocols affect the microglial lipidome, we performed unbiased lipidomic analysis on microglia generated from EB and 6TF differentiation protocols in two independent iPSC lines (WTC11 and KOLF2.1J) and measured >1000 lipid species spanning 16 lipid classes. We further validate our lipidomic findings by immunofluorescence and western blot analysis.

We observed striking differences in the lipid composition of EB and 6TF microglia generated from the same iPSC lines. Notably, fatty acid storage products diglycerides (DGs) and triglycerides (TGs), which form core components of lipid droplets (LDs), were considerably more abundant in 6TF microglia. We assessed the effect of different microglia maturation media components on the resulting microglial lipidome and identified B-27 supplement as a suppressor of TG accumulation. Our study highlights notable differences in LD accumulation between EB and 6TF microglia, whereby 6TF microglia bear a high load of TG-rich LDs. Such alterations in microglial lipid metabolism have previously been linked to aging and disease models such as 5xFAD mice, where they have been associated with enhanced pro-inflammatory cytokine secretion and impaired phagocytic functions. Our findings underscore the influence of culture conditions on the metabolic profiles of iMGL models, with potential implications for their functional outcomes.

---

**#25:** Katerina Gospodinova. A novel, rapid human iPSC-derived triculture system enhances target validation and drug discovery for neurodegeneration

A novel, rapid human iPSC-derived triculture system enhances target validation and drug discovery for neurodegeneration

The Alzheimer's Research UK Oxford Drug Discovery Institute has developed a novel, 2D brain triculture system comprising human iPSC (hiPSC)-derived neurons, astrocytes, and microglia. This system achieves an accelerated set-up time of 28 days from cell thaw to mature triculture, allowing for agility and versatility in the early stages of drug discovery projects, whilst providing physiological and disease relevance of complex *in vitro* systems.

Our strategy employs doxycycline-inducible hiPSC lines to drive forward programming of astrocytes and neurons in 12 and 14 days, respectively. Microglial precursors are derived using a direct differentiation protocol and added 14 days from the start of the triculture set-up, achieving full maturation to microglia in 14 days. Using microelectrode arrays, we have shown that neurons grown in this system display similar firing patterns but sustain their activity for longer compared to those in monoculture. Image-based analysis demonstrated ramified glial morphology and high expression of microglial and astrocytic markers. Proof-of-concept experiments have evidenced the ability of the system to recapitulate complex cellular crosstalk. We have shown that perturbing TREM2 signalling in microglia impairs neuronal signalling and induces astrogliosis. Moreover, introducing mutations associated with neurodegenerative diseases (e.g., APP SWE and Tau P301L) models key disease phenotypes including altered neuronal firing, enhanced tau phosphorylation, A $\beta$ 40/42 and APOE secretion. We are currently performing an in-depth proteomic analysis to assess the global proteome and levels of disease associated post-translational modifications in the tricultures.

In summary, we have developed a rapid hiPSC-derived brain triculture model amenable to both target validation and profiling compound efficacy prior to *in vivo* testing. We are developing this system further by generating fluorescently tagged cell lines to enable multiplexed live cell imaging and cell sorting for downstream multi-omic analyses.

**#26:** Karolina Nowak. Investigating how LOAD risk genes BIN1 and APOE4 impact neuronal function

Late onset Alzheimer's disease (LOAD) has a strong genetic component with over 75 genetic risk factors. However, there is still a lack of understanding into how the combined presence of multiple common risk loci and their potential interactions cause disease. Genome wide association studies have identified APOE4 and BIN1 as the two most significantly associated risk loci, respectively. Assessment of our LOAD cohort in Cardiff has shown that approximately one-third of individuals with AD carry both an APOE4 allele and BIN1 risk SNP. BIN1 is a canonical player in endocytosis, a pathway facilitating internalization, trafficking and recycling of molecules by cells which contributes to their overall health. BIN1 is also involved in specialised endocytosis utilised by neurons to recycle synaptic vesicle membranes for maintained synaptic transmission. APOE has also been implicated in phenotypes of neuronal endocytic dysfunction including early endosome enlargement and impaired receptor recycling. In this project, novel iPSC lines have been generated using CRISPR-Cas9 to knock-out BIN1 on APOE33 and APOE44 backgrounds and differentiated to cortical neurons. Functional phenotyping of the neurons was performed using high content fixed and live imaging of the endolysosomal system and markers of its compartments including early and late endosomes and lysosomes. Our data suggests the iPSC-derived cortical neurons have alterations in size, number and distribution of these organelles between genotypes. Further experiments are currently underway to examine the neuronal network activity of these lines.

---

**#27:** [Tushar Shah](#). Enhanced, high-throughput single-cell DNA methylation analysis using massively parallel indexing

The epigenetic landscape of the human brain undergoes extensive changes during tumorigenesis. DNA methylation-based epigenetic modifications have been studied using bulk techniques like bisulfite sequencing and enzymatic methyl sequencing. However, these methods lack the granularity of single-cell analysis, which is crucial for understanding cellular heterogeneity and functional diversity within complex biological systems like tumors. While single-cell analysis has revolutionized our understanding, traditional methods remain costly, low throughput, and laborious.

Scale Bio utilizes massively parallel indexing where the cell itself acts as a compartment for sequential barcoding in a plate-based workflow, eliminating the need for complex instrumentation, while boosting throughput. This technology has been adapted to assess DNA methylation at the single-cell level, offering a robust, affordable, high-throughput protocol.

Here, we used ScaleBio's single-cell RNA-seq and methylation kits to investigate gene expression and DNA methylation patterns during oncogenesis in human isocitrate dehydrogenase (IDH) mutant glioma cells. IDH1/2 mutations are present in over 80% of low-grade gliomas and about 1/5th of all adult diffuse gliomas, making them attractive therapeutic targets. By uncovering DNA methylation patterns at the single-cell level, we provide an epigenetic map to better understand this complex disease and inform clinical discoveries.

We achieved high cell recovery and robust cytosine coverage throughout our analysis of single-cell methylomes isolated from human glioma tumor tissue. We generated a ranked list of the top hypo- and hypermethylated genomic regions and identified cell type-specific clusters by analyzing Differentially Methylated Regions (DMRs). This uncovered unique single-cell methylation profiles that may be obscured by bulk or pseudo-bulk analysis.

These data show that the ScaleBio single-cell methylation workflow offers increased sensitivity and accuracy in identifying DNA methylation sites. It provides a comprehensive view of methylomes, offering insights into cellular heterogeneity and tumor evolution, ultimately contributing to a deeper understanding of glioma development and potential therapeutic strategies.

**#28:** [Gabriella Crawford](#). Development of Neuroimmune-Vascular Organoids

Multiple regions of the human brain are highly susceptible to neurodegenerative diseases, making brain organoids valuable tools for studying disease pathogenesis, including in conditions such as Alzheimer's disease. While brain organoids effectively model forebrain features and neurogenic niches, they lack the full array of cell types present in the human brain—most notably, microglia and blood vessel cells, which are important mediators of neuroinflammation and neurosurveillance during pathogenic conditions.

To address this gap, we have developed a novel protocol for the generation of human induced pluripotent stem cell-derived and vascularised cortical and hippocampal organoids integrated with microglia. These novel systems will be validated utilising our access to human foetal tissue. Increasing the cellular diversity of forebrain organoids enhances their physiological relevance, improves their accuracy in replicating the human forebrain, and modelling neuroinflammation and dysregulation of the blood brain barrier. These vascularised organoids with microglia offer a powerful platform for investigating neuro-immune-vascular interaction under neurodegenerative conditions.

---

**#29:** Kimberly Cheam Ai Xian. Investigating cellular mechanisms of tau uptake in glial cells and in co-culture with neurons.

Tau accumulation in intracellular aggregates is a major hallmark of tauopathies. The contribution of glial cells to tau uptake and spread is poorly understood. We aim to understand the capacity of tau uptake in glial cells and the molecular machinery mediating tau uptake. Additionally, we aim to investigate the transcriptional changes that occur to different cells when exposed to different sources of tau seeds. Microglia and astrocytes were differentiated from human induced pluripotent stem cells (iPSCs). iNeurons were generated through the simultaneous inducible expression of NGN2. Full length recombinant ON3R and ON4R tau was labelled with Alexa Fluor 488 and 594 respectively. Both microglia and astrocytes were treated with different drugs and incubated with both labelled monomeric and heparin-aggregated tau. The amount of tau taken up was measured using flow cytometry and live cell imaging. Single-cell RNA-seq was performed in mono- and co-cultures of microglia and neurons using tau extracted from human disease patient, immortalised biosensor cells and recombinant protein. Both astrocytes and microglia readily internalised recombinant monomeric and aggregated ON3R and ON4R tau with different rates of tau uptake between the two isoforms in astrocytes. Blockage of clathrin-mediated endocytosis (CME) and phagocytosis significantly reduced tau uptake. Additionally, inhibition of the main receptor of tau, LRP1, significantly reduced tau uptake in astrocytes. In co-culture models, microglia showed increased sensitivity to tau exposure, with distinct responses depending on the tau species, compared to neurons.

**#30:** Diogo Dias. Harnessing the power of iPSCs to accelerate the discovery and development of autophagy modulators for the treatment of Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide and is characterised by the progressive loss of dopaminergic neurons (DaNs) in the substantia nigra. The pathological hallmark of PD is the accumulation of toxic aggregates consisting of phosphorylated (pS129)  $\alpha$ -synuclein. Increasing evidence suggests that dysfunction in the autophagy-lysosomal pathway, which is responsible for removing misfolded protein aggregates and damaged organelles, is a critical component in the pathogenesis of PD. Samsara Therapeutics is a preclinical-stage biotech company focussed on identifying novel therapeutics for neurodegenerative diseases. We employ high-throughput phenotypic screens to identify small molecule autophagy modulators that can rescue disease-relevant pathology and improve cell health. The advent of induced pluripotent stem cells (iPSCs) has provided a paradigm shift in the ability to physiologically model complex diseases such as PD. At Samsara, we have established iPSC-DaN models of PD from patient lines carrying mutations in the lysosomal glucocerebrosidase 1 gene (GBA1), the most common genetic risk factor for PD. Importantly, GBA1-DaNs show an impairment in autophagic flux, decreased lysosomal activity and an increase in pS129  $\alpha$ -synuclein. Therefore, this model system provides an excellent tool to assess the therapeutic efficacy of our compounds at ameliorating disease-relevant pathology. SAM001, our most advanced series to date, potentiates autophagy in a TRPML1-TFEB/TFE3-dependent manner. Critically, we observe that SAM001 compounds can increase the biogenesis of key autophagy vesicles, boost autophagic flux, and restore lysosomal proteolytic activity in GBA1-DaNs. Furthermore, we observe a clearance of toxic pS129  $\alpha$ -synuclein. Finally, we also demonstrate SAM001 has therapeutic effects in an in vivo mouse model of PD, underscoring the translational potential of iPSCs in accelerating and improving drug discovery platforms. These models can ultimately act as a bridge between preclinical compound evaluation and progression of candidates for clinical development.

---

**#31:** *Nirmal Sampathkumar*. Using iPSC-derived motor neuron models of ALS to evaluate the efficacy and therapeutic potential of autophagy modulator SAM001

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss motor neurons, severe muscle atrophy and death usually within 2-5 years of symptom onset. Currently, there are no effective treatments that slow or halt the progression of this devastating disease. While this is likely attributable to an incomplete understanding of complex disease etiology and lack of efficient clinical biomarkers, the translatability of most *in vitro* and *in vivo* models has also been called into question. Human induced pluripotent stem cells (iPSCs) offer the promise of building better *in-vitro* disease models for increased translational relevance. Despite significant disease heterogeneity, ~97% of all ALS cases share the pathological hallmark of mislocalisation and aggregation of TDP-43. Therefore, the discovery of therapeutics that can prevent or alleviate TDP-43 pathology could be clinically beneficial to nearly all ALS patients. Autophagy is a highly conserved intracellular degradative process by which misfolded proteins and aggregates are marked for clearance via degradation or extracellular release. At Samsara Therapeutics, our mission is to identify and develop novel small molecules that target and restore autophagy pathways for the treatment of neurodegenerative diseases, such as ALS. We have established our own iPSC motor neuron (iPSC-MN) models from patient lines carrying mutations in C9ORF72 and TARDBP, given the strong links to impaired autophagy and proteostasis. Interestingly, iPSC-MNs from C9-ALS and TDP-ALS patients display hallmark pathological features including TDP-43 mislocalisation, aberrant stress granules, and mis-splicing/ altered expression of the TDP-43 target, STMN2. Importantly, we show that SAM001, our most advanced series to date, can potentiate autophagic flux, clear cytoplasmic phosphorylated TDP-43, partially restore STMN2 expression and improve the survival of ALS-MNs. Finally, we demonstrate the striking therapeutic potential of SAM001 in an *in vivo* mouse model of ALS, highlighting the effectiveness and translational potential of incorporating iPSC-derived models into drug discovery platforms.

**#32:** *Yujing Gao*. Vectorised U7 snRNAs as gene therapy approach for ALS/FTD.

ALS is a devastating neurodegenerative disease without cure. Over 90% of all cases are sporadic with no clear genetic linkage, however in >95% of cases there is loss of the nuclear DNA binding protein TDP-43. Loss of TDP-43 can induce a multitude of splicing changes, including the derepression of intronic sequences that are aberrantly incorporated in mature RNA – called cryptic exons (CE). Specifically, inclusion of a single TDP-43 dependent CE in the UNC13A gene can impact disease progression and shorten survival by up to 30%, and in the STMN2 gene can result in dramatic defects in axonal growth.

Whilst the development of targeting ASOs remain a continued effort, an alternative approach capable of incorporating multiple targets simultaneously in a gene therapy, is the use of vectorised modified uridine rich-7 (tU7) small nuclear RNAs. To test the functionality of tU7s targeting cryptic splice sites, we generated human iPSCs harbouring homozygous knock-in of c-terminal degron-tagged TDP-43 cDNA by CRISPR-Cas9 for inducible TDP-43 knockdown. Depletion of TDP-43 is achieved following removal of a small molecule ligand that stabilises the degron. Further, to investigate potential deficits in neuronal function, the iPSCs express Neurogenin 2 under a tetracycline-inducible promoter for neuronal differentiation (*i3Neurons*). In the presence of the c-terminal degron tagged TDP-43 STMN2 and UNC13A, CEs remain repressed. However, upon degradation of TDP-43, we observe increased expression of STMN2 and UNC13A CEs, and reductions in correctly spliced transcripts and protein. Finally, transduction with lentiviral expressing vectors containing tU7s targeting cryptic splice sites of STMN2 can repress CE expression and restore STMN2 expression.

Taken together, we have developed an inducible TDP-43 degradation iPSC line that is compatible with doxycycline induced transcription factor mediated differentiation systems. This is a valuable tool for investigating interventions to suppress TDP-43 associated CE splicing.

---

**#33: Eliška Waloschková. \*\*\*FLASH TALK SPEAKER\*\*\***

Electrophysiological characterization of genetically modified iPSC neurons as a Frontotemporal Dementia model using automated patch clamp – differences in ion channel and action potential properties.

Induced pluripotent stem cells (iPSCs) show great potential for the generation and characterization of neuronal subtypes as well as the investigation of neurological disease models. However, in practice the intercellular variability in a population of iPSC derived neurons in combination with the low-throughput nature of manual electrophysiological experiments, have made such studies challenging.

Here we use automated patch clamp (APC) for high-throughput characterization and comparison of commercially available healthy (WT) and frontotemporal dementia (FTD; genetically engineered Granulin R493X heterozygous knockout) iPSC-derived excitatory neurons. The results include an optimization of the cell suspension for APC and an analysis of voltage-gated (K<sub>v</sub> and N<sub>a</sub>) and ligand-gated (AMPA) ion channel currents. We also analyze action potential parameters (such as spike frequency, spike threshold and action potential amplitude) in WT and FTD model iPSC derived excitatory neurons. We see a clear correlation between the functionality of N<sub>a</sub> channels and the ability to fire action potentials, with the FTD neurons showing more immature properties.

The study demonstrates the usability of high-throughput APC for the electrophysiological characterization of iPSC derived neurons and statistically robust identification of disease cell phenotypes.

**#34: Ajantha Abey. Modelling Lewy Pathology in Differentially Vulnerable Cortical and Dopaminergic Neurons from Patients with Alzheimer's and Parkinson's Disease**

Alzheimer's (AD) and Parkinson's disease (PD) feature progressive aggregate pathology and neurodegeneration in a regionally selective manner. Neuropathological studies show that alpha-synuclein deposits, while typically associated with PD, are also remarkably common in AD. However, their origin and role in AD is poorly understood and underappreciated. Therefore, we modelled synucleinopathy in induced pluripotent stem cell (iPSC)-derived neurons. We aimed to determine cell-type and disease specific determinants of cellular vulnerability, and the effects of pathological aggregates on live cells.

iPSCs from patients with AD-related presenilin-1 mutations (n=6), PD-related leucine rich repeat kinase 2 mutations (n=6), and isogenic corrected (n=4) and healthy controls (n=4) were differentiated into both cortical and midbrain dopaminergic neurons. This enabled comparison of pathology in different neuronal subtypes from the same patient. We utilised a pre-formed fibril (PFF) model to induce Lewy pathology in neurons, and multielectrode arrays, high-content live imaging, and immuno-labelling to assess the drivers and effects of pathology.

PFF insult seeded time and dose-dependent synucleinopathy in iPSC-derived neurons which was phospho-alpha-synuclein+, TOM20+, P62+, and ubiquitin+, and strikingly morphologically akin to brain pathology, including Lewy body structures. TH+ cells in midbrain dopaminergic cultures were highly susceptible to aggregate formation, while cortical neurons were relatively resilient, except with PSEN1-Intron-4-deletion mutations. This differential vulnerability pattern was reversed for tau PFFs. We found cell-intrinsic deficits in calcium and autophagic flux that led to heightened vulnerability to aggregation in a cell-type and genotype-dependent manner. Furthermore, PFFs led to disease-relevant, time-dependent functional perturbations, including on neurite outgrowth, synaptic density, and nuclear morphology.

These results are the first demonstration of synucleinopathy in an AD cell model, an overlooked aspect of the disease. These also demonstrate that iPSC-derived neurons exhibit cell-type and pathology-dependent differences in vulnerability that reflect selective vulnerability patterns in the brain, allowing for insights into disease mechanisms and therapeutic targets.

---

**#35:** Alexander Fröhlich. Establishing cell type-specific CRISPRi tools in 3D organoid models to study tauopathies

Large scale CRISPRi screens represent a powerful and adaptable tool for the discovery of novel pathways of cellular dysfunction in neurodegenerative diseases, including tauopathies. However, CRISPRi screens and gene manipulations have primarily been performed in directly differentiated monocultures. While these studies provide valuable insights, they overlook the complexity of cellular interactions in multicellular environments, leaving the effects of gene manipulation on neighbouring cell types unclear. In addition, systems designed to regulate Cas9 activity or gRNA expression often face challenges, including off-target effects, reduced expression or unintentional background activity. As advanced 3D organoid models and insights into neuronal-glial interactions expand, the need for platforms enabling precise, cell type-specific genetic manipulation becomes critical. Such tools would ensure that the complexity of 3D brain modelling is harnessed effectively while maintaining cellular specificity, advancing our ability to investigate and manipulate intricate brain functions and diseases. Here, we engineered an accurately controlled sgRNA expression construct by integrating a loxP-stop-loxP cassette into the human U6 promoter to facilitate spatial and temporal gRNA expression in a Cre recombinase-dependent manner. One loxP site was strategically placed at the U6 promoter's terminus, with the spacer region modified to incorporate essential regulatory elements, including the TATA box. The incorporation of this specific sequence arrangement only allows transcription of the gRNA in the presence of a cell-type specific promoter driving Cre expression. This work establishes a foundation for CRISPRi targeting in human 3D organoid models, addressing concerns about unintended effects of gene knockdown across cell types. We are also generating novel MAPT mutation CRISPRi lines to modify cell-specific targets which, in combination, can facilitate complex CRISPRi screens and uncover tau-related pathways, advancing our understanding of tauopathies. Ultimately, this approach offers new avenues for dissecting disease-specific targets in complex brain-like structures, contributing to therapeutic development tailored to tauopathies and other neurodegenerative disorders.

**#36:** Joseph Thayer Jin Robin. Utilization of GENtoniK to mature cellular models of Parkinson's Disease

Ageing is the largest risk factor for Parkinson's Disease, the second most common neurodegenerative disease that impacts ~10 million people worldwide. However, the relationship between the ageing process and the pathology of Parkinson's Disease is poorly understood. Previously published models for studying the role of ageing in stem cell models of Parkinson's Disease have not managed to recapitulate ageing phenotypes *in vitro*. Therefore, we examined the effect of GENtoniK, a recently published small molecule treatment, to determine if it is possible to replicate ageing characteristics and whether ageing can modulate the pathological phenotypes found in cellular models of Parkinson's Disease. The effect of this small molecule treatment was analysed in midbrain dopaminergic and cortical neurons derived from human pluripotent stem cells through measurements of key hallmarks of ageing and synaptic density. We found that small molecule treatment resulted in transcriptomic alterations in pathways associated with ion channel and structural functions, and an upregulation of synaptic-associated genes. However, there were no changes in ageing signatures or synaptic density. Based on these results, we concluded that GENtoniK is not suitable for inducing age related phenotypes and has limited ability to accelerate neuronal maturity.

---

**#37: Celia Gomez Sanchez.** The interplay between Wnt and FGFR1-FGF8 signaling in GnRH neuron formation

Defects in gonadotropin-releasing hormone (GnRH) signaling impair puberty and fertility. Mutations in the fibroblast growth factor receptor 1 (FGFR1) are the most frequent monogenic (~10%) cause of congenital hypogonadotropic hypogonadism. We have previously studied the role of FGFR1 in human GnRH neuron development using our well-established GnRH neuron differentiation protocol consisting of dual SMAD inhibition (dSMADI), FGF8b treatment, and Notch inhibition. Here, we demonstrate that GnRH neurons can be formed through concomitant dSMADI and WNT inhibition (WNTi) in the absence of exogenous FGF8b. GnRH neurons generated by WNTi and FGF8b-treatment showed a similar transcriptomic profile, but the WNTi protocol had a higher efficiency in producing GnRH neurons. WNTi promoted the expression of ventral forebrain markers in neural progenitors and accelerated neurogenesis, likely enhancing GnRH neuron development. WNTi also upregulated endogenous expression of FGF8. We further studied the role of FGFR1 in different stages of the GnRH neuron differentiation by temporally depleting FGFR1 via the auxin-inducible degron system. FGFR1 depletion during the FGF8b and Notch inhibition phases decreased the GnRH output, an effect that was exacerbated in the WNTi protocol. Moreover, FGFR1 depletion in neural progenitors derived from the WNTi protocol downregulated the expression of FGF8 as well as members of its synexpression group. Put together, these results indicate that the levels of WNT and FGFR1-FGF8 signaling are tightly regulated during GnRH neuron development, and that imbalance in these levels can prevent GnRH neuron formation.

**#38: Srilakshmi Goberdhan.** Investigating the role of Alzheimer's disease risk gene PICALM in iPSC-derived microglia

Alzheimer's Disease (AD) is the most common form of dementia, accounting for 60-70% of cases. In late-onset AD (LOAD), studies indicate that the pathophysiological progression begins more than a decade prior to the onset of clinical symptoms, therefore early detection and intervention is critical in the discovery of potential therapies. Dysfunction in the endocytic pathway is one of the earliest identified pathological hallmarks of AD. Many of the AD risk genes identified through genome wide association studies (GWAS) and pathway analysis highlight the importance of the endocytic pathway including PICALM ( $p=9.3 \times 10^{-26}$ ), one of the most significant risk genes after APOE.

PICALM encodes a key protein for clathrin mediated endocytosis. Risk associated single-nucleotide polymorphisms (SNPs) in PICALM reside in non-coding regions and suggesting a role in the regulation of PICALM expression. Notably, a recent study looking at the microglia regulome found that the LOAD PICALM risk variant (rs10792832) is associated with reduced expression specifically in microglia. Post-mortem and eQTL studies further support decreased PICALM expression in LOAD brains whereas protective PICALM SNPs are able to increase its expression.

To investigate disease mechanisms driven by changes in PICALM expression we have developed novel induced pluripotent stem cell (iPSC) PICALM $^{+/-}$  and differentiated them to microglia to investigate cellular phenotypes. We aimed to phenotypically assess these cells through live cell imaging including transferrin, zymosan, amyloid beta and myelin uptake as well as fixed imaging to assess endocytic compartments. We have also further investigated the effect of PICALM on both lipidomics and immune profiles.

Our findings reveal that loss of PICALM impairs endocytic activity and amyloid beta clearance whilst also causing alterations in phagocytic capabilities, lipid homeostasis and immune responses. These results highlight the broader consequences of PICALM depletion in microglia beyond clathrin mediated endocytosis, providing insights into its role in LOAD pathogenesis.

---

**#39:** Ilka Rinke-Weiß. Characterization of ion channels recorded from hiPSC-derived neurons after culturing in different conditions: an automated patch clamp study

The use of human induced pluripotent stem cells (hiPSCs) is becoming commonplace in biomedical research. Culture conditions for maturation of hiPSC-derived neurons are continuously being optimized, but these can be long in duration, and are not yet standardized. We have used hiPSC-neurons cultured under different conditions and compared the presence of different ion channels, along with their biophysical properties such as current amplitude and  $V_{half}$  using high throughput automated patch clamp (APC). We found that hiPSC-derived neurons could be used on APC with success rates exceeding 75% in some conditions and culture conditions had little effect on seal resistances and success rate. However, when hiPSC-neurons were co-cultured with astrocytes, NaV currents were detected in more neurons (100% when co-cultured, 59% when cultured alone) and amplitudes were significantly larger compared with those cultured alone ( $-3.3 \pm 0.29$  nA ( $n = 96$ ) versus  $-1.0 \pm 0.2$  nA ( $n = 37$ )), whereas the  $V_{half}$  of activation and inactivation remained the same regardless of culture conditions. Similarly, KV currents were detected in more cells (85% when cultured with astrocytes versus 57% when cultured alone) and amplitudes were also larger in hiPSC-neurons co-cultured with astrocytes compared with neurons cultured alone ( $1.5 \pm 0.1$  nA ( $n = 81$ ) versus  $0.85 \pm 0.08$  nA ( $n = 56$ )). We also investigated the presence of different ligand-gated ion channels and could detect responses to acetylcholine, GABA, glycine and glutamate in neurons cultured in all conditions. A concentration response curve to GABA revealed an EC<sub>50</sub> of around 20  $\mu$ M.

In summary, we show that co-culturing hiPSC-neurons with astrocytes does not affect sealing properties of cells on APC, but does affect the expression of NaV and KV channels. Co-culturing hiPSC-neurons with astrocytes, therefore, may be a reliable method for maturation of hiPSC-derived neurons for biomedical research and drug discovery.

**#40:** Carlos Anton-Plagara. Mapping the endosomal proximity proteome reveals Retromer as a hub for RAB GTPase regulation

Endosomal retrieval and recycling of integral cargo proteins is essential for cell, tissue and organism-level development and homeostasis and is orchestrated through a specialised retrieval sub-domain on the endosomal vacuole. However, although sub-domain dysfunction is associated with human disease our appreciation of the molecular details and functional components of the retrieval sub-domain(s) remains poorly described. Here, using comparative proximity proteomics of critical retrieval sub-domain components Retromer and Retriever, their cargo adaptors, and a component of the opposing ESCRT-degradative sub-domain, we provide a data-rich resource that identifies new molecular details of retrieval sub-domain composition and organization, including an unrecognised complexity in the interface of Retromer with RAB GTPases. Combining X-ray crystallography and *in silico* predictions with extensive biochemical and cellular analysis, we dissect the direct association of Retromer with RAB10 regulators DENND4A, DENND4C, TBC1D1, and TBC1D4, and the RAB35 regulator TBC1D13. Interestingly, Retromer complex has been linked to multiple neurodegenerative diseases including Parkinson's Disease (PD), as Retromer mutation VPS35(D620N) causes autosomal dominant PD, potentially through the hyperactivation of the PD-linked kinase LRRK2. Some RABs recruit LRRK2 to membranes, where LRRK2 phosphorylates a subset of RABs, including RAB10 and RAB35, modifying their activity and localization. Therefore, we conclude that the Retromer retrieval sub-domain constitutes a major hub for the regulated switching of selected RAB GTPases and we propose that this constitutes a major component of the role of Retromer in neuroprotection. However, this data was obtained in non-neuronal cells. To overcome this limitation, we are adapting this methodology in more neurological cellular context, including i3Neurons, which will allow us to have a neuronal inducible and isogenic platform, the later being a highly desirable value for proteomic approaches.

---

**#41:** [Alya Masoud Abdelhafid](#). Investigating Dysregulated Axonal Transport in Human Motor Neurons Expressing ALS-Causing Mutations

Motor neuron disease (MND) and other neurodegenerative conditions, pose a great health burden on our society and currently lack effective therapies directed to halt or significantly slow down progression of the disease. Our knowledge of how MND develops and affects the survival of specific nerve cells located in the spinal cord and brain, termed motor neurons, is currently incomplete. Crucially, we do not understand which processes are important for the appearance of the symptoms and their progression, ultimately resulting in the death of the patient.

Our findings indicate that a process called axonal transport is impaired at the very beginning of disease. Axonal transport ensures long-range transfer of information and nutrients between the neuromuscular junction – the contact between the nerve cell and muscles - and its cell body located within the spinal cord. Our hypothesis is that by restoring a healthy, normal level of axonal transport we will block the process leading to nerve cell death and therefore halt disease progression.

**#42:** [Stan Majewski](#). Investigating astrocyte transcriptomic and proteomic heterogeneity in ALS

Astrocytes perform numerous critical homeostatic functions to support neurons in the brain. In neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), astrocytes become dysfunctional, ultimately contributing to motor neuron death. Previous studies utilising single cell transcriptomics have shown disease-associated astrocyte heterogeneity in ALS patients and models, however, the functional implications of this on disease progression and neurodegeneration are unknown. Similarly, we have shown that a proportion of VCP-ALS astrocytes derived from human induced pluripotent stem cells (hiPSCs) become dysfunctional independently of their surrounding environment. To study the disease relevance of a potentially heterogeneous astrocyte population, we optimised and performed single cell transcriptomics and mass spectroscopy using highly enriched cultures of hiPSC derived astrocytes from control and VCP-ALS lines. We identified several perturbations in VCP mutant astrocytes, including up-regulation of numerous proteins involved in RNA splicing and ribosome formation.

---

**#43: Arkoprov Paul. \*\*\*FLASH TALK SPEAKER\*\*\*****Reprogramming iPSC-derived Microglia for modelling Aging and Alzheimer's Disease**

Microglia, the primary immune cells of the brain, play a crucial role in maintaining neural homeostasis and responding to neurodegeneration. Dysregulated microglial function is strongly implicated in both aging-associated neuroinflammation and Alzheimer's disease (AD). However, the lack of physiologically relevant human models has limited the understanding of microglial aging and disease mechanisms. Here, we establish a novel human iPSC-derived microglia model incorporating both a progerin-based aging system and a TREM2 R47H genetic variant, enabling the study of aging and AD-associated microglial dysfunction. Using an optimised cellular reprogramming protocol, we generated iPSC-derived microglia that closely resemble primary human microglia in transcriptional profile, morphology, and function. The progerin-induced accelerated aging model recapitulates age-related microglial phenotypes, including increased expression of senescence-associated markers, heightened basal inflammation, and impaired homeostatic functions. In parallel, we derived TREM2 R47H mutant microglia, which exhibit upregulation of pathways associated with cytokine signaling, interferon response, and extracellular matrix remodeling, reduced amyloid- $\beta$  phagocytosis, and altered metabolic reprogramming. Transcriptomic analysis of both models reveals distinct yet overlapping inflammatory pathways, providing insights into how aging and genetic risk factors converge to exacerbate neuroinflammation in AD. Furthermore, co-culture experiments with iPSC-derived neurons demonstrate aging- and TREM2-driven microglial dysfunction in synaptic pruning and neuronal viability, highlighting the role of microglia in neurodegenerative progression. Our approach establishes a physiologically relevant and scalable platform for studying microglial aging and AD, with potential applications in drug discovery and therapeutic development targeting neuroinflammation.

**#44: Anna Klingseisen. IPSC derived vasculature on a chip deciphering disease mechanisms in RVCL**

RVCL (Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations) is a disease which affects the microvascular beds of multiple organs, including the brain where it leads to progressive neurocognitive deterioration. Therapeutic progress depends on identifying key disease mechanisms and understanding the cell-specific nature of this endotheliopathy. RVCL is caused by a truncating mutation in the Trex1 gene encoding three prime repair exonuclease 1, the function of which has been implicated in cytosolic DNA sensing and the regulation of innate immunity. RVCL-causing mutations abolish the C-term domain of the enzyme that tethers it to the ER. Trex1RVCL is thus found not restricted to the ER but throughout the cell, including the nucleus.

A key pathophysiological step is exonuclease-dependent DNA damage in endothelial cells. Analysing UK Biobank data, we have shown that TREX1 frameshift mutations lose pathogenicity if nuclease function is compromised, and we have performed a series of human cellular studies which show that the toxic TREX1RVCL allele causes exonuclease-dependent DNA damage in endothelial cells. This is associated with cell cycle disruption, specifically G2/M accumulation, and increased chromatin abnormalities, most noticeably increased chromatin bridges in cells expressing TREX1RVCL.

We now want to use iPSC-derived endothelial cells (viECs) from patients carrying the TREX1RVCL allele to investigate the mechanism of disease caused by a 'gain of function' exonuclease activity gone rogue. We will use viECs to establish a microvasculature-on-a-chip model to investigate the genotoxic impact of active Trex1 exonuclease that lost its specific cellular localisation. Patient iPSC-derived microvessels will be a new iPSC model of vasculopathy leading to myelin loss and neurodegeneration.

---

**#45: Muhammad Zaman Khan Assir. A Novel 3D Ex Vivo Culture Model of Human Cortical Tissue Unveils Extracellular Matrix Remodelling in Neuroinflammation**

Neuroinflammation is a key contributor to the neurodevelopmental disorders and progression of neurodegenerative diseases. This complex process involves not only neurons and immune cells such as microglia, but also vascular endothelial cells, pericytes, glial populations, and the extracellular matrix (ECM). To model human neuroinflammation in a system that incorporates these critical components, we established a novel high-throughput 3D ex vivo culture of human fetal brain tissue, termed cerebroids. These cerebroids preserve the cytoarchitectural organization and cellular complexity of in vivo brain tissue.

To induce neuroinflammation, we treated cerebroids with interleukin-17A (IL-17A), a key inflammatory cytokine. Microglia isolated from IL-17A-treated cerebroids exhibited a distinct inflammatory gene expression signature, including upregulation of genes involved in cytokine and chemokine signalling, Tumor Necrosis Factor (TNF), Nuclear Factor kappa B (NF- $\kappa$ B), NOD-like receptor, and Toll-like receptor pathways. Additionally, there was increased expression of genes associated with phagosomal activity and ECM remodelling. Transcriptomic analysis of neuronal populations also revealed significant dysregulation of ECM-related genes.

Proteomic analysis confirmed extensive ECM remodelling, with upregulation of chondroitin sulfate proteoglycans Brevican (BCAN) and Versican (VCAN). These molecular alterations were regulated by NF- $\kappa$ B signalling and histone deacetylase complex 1 (HDAC1). Notably, pharmacological inhibition of NF- $\kappa$ B and HDAC1 with Parthenolide effectively reversed IL-17A-induced changes, highlighting potential therapeutic targets for neuroinflammation-associated disorders.

Our findings establish cerebroids as a powerful model for studying human neuroinflammation and uncover key molecular mechanisms linking inflammation to ECM remodelling in the brain.

**#46: Nina-Lydia Kazakou. \*\*\*FLASH TALK SPEAKER\*\*\***

**Development of a functional hPSC-derived neuron-glia tri-culture model of sporadic Parkinson's Disease (PD) for phenotypic screening**

Sporadic Parkinson's disease (PD) is a chronic neurodegenerative disorder marked by severe motor symptoms and characterized by progressive loss of dopamine-(DA)-producing neurons in the substantia nigra, with recent studies underscoring complex interactions among CNS cells—particularly astrocytes and microglia—that significantly influence disease progression. Genetic PD often arises from separate individual pathways converging on specific pathology, while sporadic PD likely results from multiple distinct pathways, which combined lead to brain-tissue inflammation and DA cell death. While disease-modifying treatments are urgently needed, drug discovery remains challenging due to poorly representative animal models and an overemphasis on single-target screening, which may not address PD's complex pathology.

We aim to create a physiologically relevant model of sporadic PD by combining hiPSC-derived neuronal and glial cells with multiple PD-related insults, such as ageing, alpha-synuclein aggregation/phosphorylation, inflammation, mitochondrial and lysosomal dysfunction. Our model will be used in a high-throughput phenotypic screen to test hundreds of small molecules for functional DA release, measured through sniffer cells.

Using both WT and alpha-synuclein-overexpressing hiPSCs, we generated high-purity cultures of FOXA2+/TH+ caudal ventral midbrain (VM) neurons, SOX9+/GFAP+ VM astrocytes, and CD45+/IBA1+ yolk sac-like microglia. We then combined them in a functional human tri-culture system. By introducing pre-formed alpha-synuclein fibrils (PFFs), we successfully modeled PD pathology, observing both aggregated and phosphorylated alpha-synuclein, along with impaired DA release. To further examine PD-related insults, we induced mitochondrial dysfunction with rotenone, resulting in a marked reduction in DA release, while L-DOPA treatment served as a positive control, restoring DA levels. Inhibition of the neddylation pathway induced cellular hallmarks of ageing, such as increased cell death, decreased proliferation and age-associated DNA damage. Finally, treatment with IFN $\gamma$  led to a generalized inflammatory response at transcript and proteome levels. Thus, our model captures essential disease features, creating a powerful platform for target-agnostic functional drug screen.

---

#### **#47: Uroosa Chughtai. Using stem cell-derived models to investigate cell autonomous microglial dysfunction in TBK1-associated ALS-FTD**

Heterozygous loss-of-function mutations in TANK-binding kinase 1 (TBK1) are a common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Whilst TBK1 haploinsufficiency has been shown to contribute to cell autonomous neuronal dysfunction, the impact of TBK1 loss-of-function in microglia is less well understood. We aimed to investigate the microglial function of TBK1 and understand how ALS-FTD-associated TBK1 mutations may intrinsically impair microglial function to contribute to disease pathophysiology.

First, we aimed to establish an *in vitro*, human model of microglial TBK1 loss-of-function. Using a CRISPR-Cas9 gene-editing approach, we generated and characterised an isogenic allelic series of TBK1 knockout human pluripotent stem cells (hPSCs). Subsequently, using well-established differentiation protocols, we generated microglia-like cells (hPSC-MG) from homozygous TBK1 knockout hPSCs along with their wild-type controls. Next, using unbiased quantitative proteomics and targeted functional assays, we investigated the cellular and molecular consequences of microglial TBK1 loss. We found that homozygous TBK1 deletion results in widespread cell autonomous microglial dysfunction, including global dysregulation of the microglial proteome and phosphoproteome, endocytic dysfunction, and immune dysregulation. Notably we observed that this dysfunction is exacerbated upon immune stimulation.

In conclusion, our data suggests that TBK1 plays a vital role in the physiological function of microglia and that TBK1 loss-of-function mutations may contribute to ALS-FTD pathogenesis via cell autonomous microglial dysfunction. Ongoing work aims to validate and further explore our findings across our panel of TBK1 knockout hPSC-MG and investigate how cell autonomous microglial dysfunction may contribute to neuronal dysfunction in TBK1-associated ALS-FTD.

#### **#48: Silvia Oldani. Next-Generation Electrophysiology for Functional Characterization of Human Neural Organoids and Assembloids**

Three-dimensional neural models derived from human-induced pluripotent stem cells (hiPSCs), such as organoids and assembloids, are essential tools for replicating key features of human brain development. They play a vital role in advancing research on neurodegenerative disorders, such as Alzheimer's and Parkinson's disease. Real-time, label-free measurement of electrical activity is needed for unraveling the intricate dynamics of the neuronal networks formed within these self-organizing *in-vitro* systems.

High-density microelectrode arrays (HD-MEAs) provide a powerful, non-invasive platform for high-resolution electrical imaging, enabling real-time recordings of electrical signals from a wide range of electrogenic samples, including neural organoids, assembloids, and brain or retinal tissue slices. In this study, we employed the MaxOne and MaxTwo HD-MEA platforms, featuring 26.400 electrodes per well, to record extracellular action potentials from 3D neural models across multiple levels of resolution, spanning entire networks to individual cells and subcellular structures. We demonstrated the systems' flexible electrode selection, leading to enhanced reproducibility and statistical power of the collected data. Extracted and analyzed parameters included firing rate, spike amplitude, and network burst profile.

To further explore subcellular dynamics, the Axon Tracking Assay was used to trace the propagation of action potentials along axonal branches, enabling precise investigation of axonal properties, such as conduction velocity, latency, axonal length and branching patterns. This revolutionary assay offers a high-resolution approach for studying disease models focusing on axon initial segment, axonal development, branching and conduction.

The capacity of these HD-MEA platforms to selectively target specific electrodes enhances the quality of the collected data while ensuring higher reproducibility. Together with the integrated tools for automated data visualization and metric extraction, the here presented systems offer a user-friendly and robust platform for disease modelling and drug testing, supporting both acute and long-term electrophysiological studies.

---

**#49:** [Hyunjin Kim](#). Chronic non-cytotoxic manganese exposure disrupts glutamate homeostasis and pathways involved in the integrated stress response in hiPSC cortical model of Alzheimer's disease.

Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder. Most AD cases involve contributions from both genetic and environmental risk factors. Manganese (Mn) is an essential metal widespread in the environment that in excess can cause neurotoxicity. Acute exposure to high Mn can inhibit extracellular glutamate uptake and trigger the integrated stress response (ISR), both of which are molecular pathologies implicated in AD. Additional mechanisms of Mn neurotoxicity in AD etiology remain unclear. We hypothesized that chronic exposure to environmentally relevant Mn levels drives dysregulated ISR that manifests as transcriptional/functional maladaptation. Further, we propose that severity of response to Mn is dictated by individual genetic risk to AD. To address our hypothesis, we utilize cortical neurons and astrocytes from induced pluripotent stem cells differentiated from neurotypical control and AD patients. Cells were cultured for  $\approx$ 100 days and subsequently exposed to Mn (vehicle, 0.5, or 5.0  $\mu$ M) for 4-6 weeks. Net glutamate uptake was quantified using  $^{14}\text{C}$ -glutamate. We observed a significant decrease in glutamate uptake only in AD neuron-astrocyte co-cultures. Gene expression and immunocytochemical analyses indicated absence of astrocyte reactivity, suggesting impaired glutamate uptake is likely a direct Mn effect rather than an epiphenomenon caused by neuroinflammation. scRNA-sequencing and bioinformatic analyses identified alterations in pathways associated with AD and Mn neurotoxicity. These included pathways involved in energy metabolism, glutamate neurotransmission, and ISR-related pathways such as EIF2 signaling. Consistently, gene ontology analysis revealed enrichment of terms associated with mRNA translation, ribosome homeostasis, and cellular energy, suggesting dysregulated ISR. In summary, we provide an initial framework for Mn  $\times$  AD interaction model wherein chronic Mn exposure leads to differential ISR activation and transcriptional/functional maladaptation as a function of AD genetic risk.

**#50:** [Ana Cristina Rios Mejia](#). Axonal elongation *in vitro*- The characterization and engineering of outgrowth mechanisms in different neuronal subtypes

Neurons navigate complex tissues during development to reach targets in the central nervous system (CNS) or further away in the peripheral nervous system (PNS), environments with distinct structures and cues. Precise mechanisms of axonal pathfinding and elongation across different neuronal subtypes have been mostly studied in animal models and revealed fundamental differences at the growth cone of the axon to navigate these two different environments. How these processes differ in humans remains speculative yet with the emergence of human induced pluripotent stem cells (iPSCs) and advanced bioengineering techniques, we can now investigate axonal elongation in humans systematically and controlled. Long motor arrays (LAMs) are an ideal system for studying axonal length in a novel open top microfluidic device where the entire axon is accessible. We used LAM devices to study motor neurons (MNs) and cortical neurons (CNs) derived from human iPSCs using a doxycycline-inducible transcription factor cassette, enabling rapid and large-scale production of neurons with distinct identities. We identified that CNs respond vastly different to topography of the arrays than MNs, indicating that their path finding behavior is strongly influenced by given features. We then further modified the cell culture medium, to optimize elongation behaviors. Here we found that both cell types respond differently to the compositions in the medium, especially to a lipid containing component further solidifying the evidence that distinct cell types have alternative responses to their co-environment. Lastly, LAMs are permissive for live imaging studies, thus we dissected outgrowth behaviors using a live axonal dye. We show that CNs grow faster and pause less frequently than MNs, who stop constantly and slowly elongate. Taken together this is the first of its kind study, showing differences between CNs and MNs *in vitro* for axonal elongation, illuminating pathfinding behaviors and strategies to cross different microenvironments.

---

**#51: Lena Erlebach. \*\*\*TCS bio Travel Award\*\*\*****Chimeric brain slice cultures to study human microglia in vitro**

As the primary myeloid cells of the brain microglia are crucial for maintaining brain homeostasis but have also been implicated in various neuropathological processes. Microglial identity and function are determined by ontogeny and brain environment and differ significantly between species. Therefore, model systems which faithfully reproduce mature human microglial molecular and functional phenotypes are crucial for understanding their role in brain health and disease. Recent advances in the fields of induced pluripotent stem cells (iPSC) and organoids have allowed significant progress in the study of human microglial function. However, in vitro models with a more mature environment that allow for easy experimental manipulation and the study of complex neuropathological processes are still lacking. Here, we present chimeric brain slice cultures (cBSC) as a novel model that combines murine organotypic brain slice cultures with human iPSC-derived microglia (iMic). We demonstrate that iMic precursor cells integrate and differentiate in cBSCs with a near-complete replacement of murine with human microglia that is stable for at least 12 weeks. In this model, the precursor maturation is solely driven by the tissue environment. iMics in cBSCs display age- and disease-related transcriptional profiles and functional responses reminiscent of adult human microglia which we studied in the context of alpha-synuclein lesions.

Our findings establish cBSCs as a new 3D in vitro tool for investigating human microglia in a physiologically relevant environment. Concurrently, the model is highly flexible for experimental manipulation and allows examining microglial contribution and responses to neuropathology over several months

---

**#52: Patricia Lopez Garcia. Investigating LRRK2-G2019S PD pathogenesis using iPSC-derived neuronal and glial models**

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by both motor and non-motor neurological symptoms, including bradykinesia, rigidity, cognitive impairment, and autonomic dysfunction. One of the most common genetic causes of PD is a mutation in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene, the G2019S variant, which accounts for 1-6% of sporadic and 3-19% of familial PD cases. This mutation leads to increased LRRK2 kinase activity, contributing to neuronal dysfunction and degeneration. However, the exact molecular and cellular mechanisms underlying LRRK2-PD remain unclear.

Patient-derived induced pluripotent stem cells (iPSCs) emerged as a powerful tool to study LRRK2-PD, offering a human-based, physiologically relevant system to investigate disease mechanisms and test potential therapeutics. Here, we utilized iPSC-derived neuronal and glial cells, including midbrain dopaminergic neurons, cortical neurons, astrocytes and microglia, from LRRK2-PD patients to assess disease-associated phenotypes compared to iPSC-derived cells from healthy individuals. Our findings revealed significant differences between these cohorts, including impaired endolysosomal trafficking, decreased autophagy, blockage in ciliogenesis and aberrant inflammatory responses.

Furthermore, iPSC-derived models serve as a valuable platform not only to understand PD but also for drug screening and biomarker discovery. Targeting LRRK2 kinase activity with small-molecule inhibitors like MLi-2 has shown promise in rescuing some of these disease-associated phenotypes helping to expand our understanding of LRRK2-PD and accelerating the development of personalized, disease-modifying therapies for PD patients carrying LRRK2 mutations.

---

**#53: Cathleen Hagemann. \*\*\*FLASH TALK SPEAKER\*\*\***

In depth multi-omic analysis of axonal homeostasis across scale using a bioengineered platform and human IPSC-derived neurons

Motor neurons are among the largest cells in the human body, with their axons spanning up to 1m in length. Despite their size, the majority of resources are located in the cell body. Managing the fundamental components of the cell—RNA, proteins, and lipids—across this length becomes crucial for maintaining axonal integrity and connecting to the motor neuron target in the periphery. Previously, we developed a novel bioengineered platform that allowed us to demonstrate how axons adapt their biological processes across their length. These adaptations are evident in changes to mitochondria, local protein synthesis, and the positioning of RNA binding proteins. However, our understanding of molecular synergies has been limited by examining isolated phenotypes. To address this gap, we integrated lipidomic, proteomic and transcriptomics and enhanced our bioengineered platform, enabling us to isolate significant amounts of material from distinct fractions of axons. This yields a comprehensive axonal atlas with millimetre and even micrometre spatial resolution. We found changes in lipid composition across the axons, with enrichments in the distal part. Proteomics of both motor and cortical neurons highlights clear cut differences between these neuronal subtypes for axonal proteomes. Taken together, our study offers unprecedented insight into axonal biology and homeostasis across varying axonal lengths. Moreover, it correlates molecular phenotypes with length-dependent axonal characteristics for the first time, these insights also build a fundamental brick to understand motoneuron vulnerability and dying back phenotypes in Amyotrophic lateral sclerosis and other neurodegenerative diseases.

**#54: Giulia Sofia Marcotto. Multiple system atrophy dopaminergic neurons differentiated from induced pluripotent stem cells exhibit distinct structural and functional abnormalities**

**Background:** Multiple system atrophy with predominant parkinsonism (MSA-P) is a sporadic, adult-onset, fatal neurodegenerative disease that affects several neuronal pathways, including the mesencephalic district of the nigrostriatal system. Cellular pathology of MSA-P is marked by cell death and reduction of mesencephalic dopaminergic (mDA) neurons within the nigrostriatal system and other monoaminergic systems of the brainstem. Damage of DA neurons is associated with impairment of motor control resulting in Parkinsonian symptoms. To date, the pathogenetic mechanism underlying MSA-P remains unclear [1]. In this study, we investigated the cellular pathology of mesencephalic floorplate (mFP)-derived DA neurons differentiated from induced pluripotent stem cells (iPSCs) of MSA-P patients.

**Materials and Methods:** iPSC lines were established by reprogramming peripheral blood mononuclear cells from three MSA-P patients who signed the Informed Consent. Selected clones were studied for their pluripotency, trilineage differentiation capacity, and karyotype integrity. iPSCs were subsequently differentiated in FP-derived DA neurons [2]. For morphological studies, TH+ DA neurons were evaluated by computer-assisted morphometry [3]. Functional features were investigated by performing the Mito Stress Test with the Seahorse XFe24 analyzer and by analyzing electrophysiological parameters with the patch-clamp technique.

**Results:** Immunofluorescence and quantitative PCR analysis confirmed the pluripotency and trilineage differentiation potential of iPSCs derived from patients affected by MSA-P. Immunofluorescence validated the differentiation of iPSCs into FP-derived midbrain DA neurons at day 35 and day 45, showing the presence of TH+ neurons forming a network with GABA+ and vGlut+ neurons. Morphological analysis showed a significant reduction of dendritic length, number of primary dendrites, and soma area in DA neurons from MSA-P patients compared to controls. Functional studies revealed differences in mitochondrial respiration and electrophysiological properties of DA neurons between MSA-P patients and controls.

**Conclusion:** Our results indicate that DA neurons of MSA-P showed defective morphology associated with alterations in oxygen consumption and neuronal excitability.

---

**#55: Marina Shiryaeva.** Lipid metabolism in neuronal and glial iPSC-derived models of familial Alzheimer's disease.

Alzheimer's disease (AD) is characterized by significant metabolic disturbances, particularly in lipid metabolism, which may contribute to disease progression. Although alterations in various lipid species have been observed, their precise role in AD pathophysiology remains unclear. This study investigates lipid metabolism in iPSC-derived neurons and glial cells carrying Familial Alzheimer's Disease (FAD)-associated mutations in APP, PSEN1, and PSEN2.

For neuronal differentiation, we employed the well-established NGN2 overexpression protocol, known for generating mature and functional neurons. For astrocyte differentiation we performed a systematic comparison of existing strategies. We identified SOX9-NFIA overexpression as the most effective approach, resulting in the generation of mature astrocytes with robust expression of key astrocitic markers. We find that, as expected, APP, PSEN1 and PSEN2 mutations affect APP processing and Abeta secretion. Lipidomic analysis indicated that the lipid profiles of monocultured neurons were not affected by FAD mutations. However, the astrocytes harboring FAD-associated mutations displayed lipidomic alterations that may be mutation-dependent, although these findings require further validation. Additionally, we explored the effects of  $\gamma$ -secretase blockade on neural cell lipid metabolism and found that it modifies cholesterol metabolism in both neurons and astrocytes.

These findings highlight the complex role of lipid metabolism in AD, suggesting that lipid disturbances contribute to disease progression. Further research is needed to unravel the mechanisms behind these alterations and their implications for disease development.

**#56: Dasa Bohaciakova.** Modeling of SORL1-Associated Alzheimer's Disease in iPSC-Derived Neurons and Cerebral Organoids

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting over 55 million individuals worldwide, with an incidence of ten million cases each year. The disease is characterized by the gradual loss of memory and the presence of distinct neuropathological features, including amyloid- $\beta$  plaques and Tau neurofibrillary tangles. Recent advancements have identified genetic variations in the SORL1 gene, encoding the SORLA protein, as a significant contributor to AD pathogenesis. Designated as one of the causal genes for AD, the involvement of SORL1 in neuronal biology and its mechanistic contributions to the development of AD-specific pathological hallmarks remain understudied.

To elucidate the molecular mechanisms underlying SORL1-associated AD, we employed CRISPR/Cas9 to introduce the pathogenic p.Y1816C mutation within a 3Fn-domain of SORLA in induced pluripotent stem cells (iPSCs). These iPSCs were differentiated into 2D induced neurons and 3D cerebral organoids for in-vitro disease modeling. Our studies demonstrated a decrease in intracellular SORLA levels and reduced sSORLA shedding for the p.Y1816C mutants, suggesting abnormalities in protein maturation and trafficking. Endosomal enlargement, accumulation of APP, and increased secretion of Ab40 and Ab42 peptides were observed for both 2D and 3D models, highlighting key AD-related features. Notably, this study provides the first evidence that the SORL1 pathogenic variant significantly disrupts axonal transport of both Rab5+ endosomes and of APP itself. Finally, functional experiments also revealed altered electrophysiological properties of human iPSC-derived neurons.

Our findings thus not only provide further evidence for why the p.Y1816C mutation is considered a causal variant for AD but also reveal a novel aspect of SORL1 pathology activity, impairing neuronal axonal cargo-trafficking. Our experiments also validate the use of stem cell-derived models for studying SORLA dysfunction and prove that these models offer a valuable platform for dissecting the unknown molecular pathways underlying neurodegeneration and may also aid in developing targeted therapeutic strategies.

---

**#57: Zedeng Yang. Ser-Arg Protein Kinase (SRPK) controls MAP1S processing and acquisition of microtubule binding activity during neuronal development.**

Neurological diseases are frequently characterised by dysregulation of the microtubule cytoskeleton, which is critical for neuronal integrity and functioning. However, the precise mechanisms by which microtubule dynamics are regulated during the development of the nervous system remain poorly understood. Here, we use global phosphoproteomic screening in mouse embryonic stem cells (mESCs) to identify cytoskeletal substrates of Ser-Arg Protein Kinase (SRPK), which is implicated in several neurodevelopmental and neurodegenerative conditions. We show that SRPK directly phosphorylates microtubule-associated protein 1S (MAP1S) at multiple sites in a C-terminal region involved in proteolytic maturation and microtubule binding. We demonstrate that MAP1S associates with microtubules both in vitro and in mESCs-derived neurons, with SRPK-mediated phosphorylation modulating its microtubule-binding affinity. Besides, SRPK regulates the efficient proteolytic processing of MAP1S by the Calpain-10 protease, a process that is progressively induced during neuronal differentiation from human-induced pluripotent stem cells (hiPSCs). AlphaFold modelling indicates that SRPK phosphorylation changes the association of MAP1S and Calpain 10, providing a potential underlying mechanistic basis. Our results demonstrate a key role for SRPK in controlling the processing and function of a key microtubule regulatory protein during neurodevelopment and provide insight into mechanisms by which the microtubule cytoskeleton may be dysregulated in neurological diseases.

**#58: James Crowe. \*\*\*FLASH TALK SPEAKER\*\*\***

Modelling neuronopathic Gaucher disease using human iPSC-derived brain organoids

Gaucher disease is a rare lysosomal storage disorder with an incidence rate of 0.39 - 5.80 in 100,000 live births. GBA1-variant mutations encode defective lysosomal enzyme glucocerebrosidase, and in 5-10% of cases results in fatal early-onset neuronopathic variants exhibiting developmental delays, seizures and death. Interestingly, GBA1 mutations are the biggest known risk factor for Parkinson's disease. Existing studies investigating disease mechanisms of neuronopathic Gaucher disease are based mostly on animal models and focus predominantly on late neuronal progression phenotypes. Thus, little is known as to how disruption impacts human early neurodevelopment, or different neural cell subtypes are involved. Altogether, new human models of neuronopathic Gaucher disease are essential to unveil missing aetiology and provide therapeutic targets.

Our primary aim was to generate relevant, novel human models of brain development using rare disease patient-derived isogenic lines. Second, we aimed to discover key knowledge of human Gaucher disease onset/progression, including investigating cellular subtype deficits in network development and maintenance to provide targets for translational research.

We engineered novel isogenic Gaucher disease and healthy patient stem cell lines using genome editing , then generated cortical and subpallial brain organoids. Early (75 days) and mature (150 days) organoids were assessed for aspects of the disease including: enzymatic activity, lysosomal load by Western blot, FACS and immunocytochemistry, gene expression levels and altered population dynamics by single cell RNA sequencing, altered ganglioside content, migrational impairment by viral labelling and assembloid formation, and network function by multi-electrode array. In our study, we have identified a putative new phenotype consisting on a deficit for interneuron fate determination. This is significant as many patients exhibit an excitation/inhibition imbalance leading to treatment-resistant seizures. Furthermore, a defined human model of Gaucher disease with a clear phenotype can be used to aide in development of new therapeutic strategies to ameliorate Gaucher disease.

---

**#59: Alexis Penverne. Ferroptosis Drives Neuronal and Glial Vulnerability in Parkinson's Disease via the A53T SNCA Mutation**

Parkinson's Disease (PD) is characterised by the progressive loss of midbrain dopaminergic neurons (mDA) and is associated with iron accumulation,  $\alpha$ -synuclein ( $\alpha$ -syn) aggregation, altered redox state and neuroinflammation. Mutations in the  $\alpha$ -syn-encoding SNCA gene such as the A53T mutation have been shown to cause early onset, rapidly progressive PD. Ferroptosis is a form of cell death resulting from iron accumulation, lipid peroxidation and loss of antioxidant defences. While it has emerged as a potential contributor to neurodegeneration, the precise mechanisms linking ferroptosis to PD pathogenesis remain elusive. We employed an -omics based approach and human-induced pluripotent stem cells (hiPSC) models to investigate the links between PD and Ferroptosis. Transcriptomic analyses of postmortem PD brains and hiPSC-derived midbrain neurons revealed significant enrichment of ferroptosis-related genes in PD patients. To further validate these findings, we generated a rapid hiPSC-derived neuronal model with the A53T SNCA mutation and assessed oxidative stress, lipid peroxidation, and vulnerability to ferroptosis. A53T SNCA mutant neurons exhibited increased cytosolic and mitochondrial reactive oxygen species (ROS) and glutathione (GSH) depletion, hallmarks of ferroptotic stress. Pharmacological inhibition of ALOX15, a key lipid peroxidation enzyme, rescued oxidative stress phenotypes and restored GSH levels. Moreover, A53T neurons displayed heightened sensitivity to ferroptosis-induced cell death, which was mitigated by ALOX15 inhibitors. We extended our findings to microglia, where the A53T mutation rendered microglia more susceptible to ferroptotic lipid peroxidation, while astrocytes remained resistant. Knockdown and pharmacological inhibition of Glutathione Peroxidase 4 (GPX4), an enzyme protecting from ferroptosis, confirmed that A53T neurons and microglia were significantly more susceptible to ferroptotic cell death.

Our findings establish ferroptosis as a key driver of neuronal and glial vulnerability in PD, linking  $\alpha$ -syn to ferroptotic mechanisms. Targeting ferroptosis, particularly through ALOX15 inhibition, represents a promising neuroprotective strategy in PD. Further exploration of ferroptotic pathways in distinct CNS cell types may reveal novel therapeutic opportunities.

**#60: Linus Wiora. Efficient AAV-mediated gene delivery in iPSC-derived neurons**

Adeno-associated viruses (AAVs) have emerged as a promising tool for gene delivery due to their diverse tissue preferences, robust transduction and low immunogenicity. While extensively studied in animal models, the translation of AAV tropism from tissues to primary cells and human induced pluripotent stem cell (hiPSC)-derived models remains challenging. The development of AAVs with high tropism for these cultured cell types would be a valuable tool, as hiPSC-derived neural cell types offer the unique opportunity to study the pathogenesis of neurodegenerative diseases in a human model system and in the affected cell type. This is underlined by the fact that transfection and/or transduction of fully differentiated cells, especially of the CNS, using classical methods still poses major obstacles.

The goal is to identify capsids with high tropism for hiPSC-derived neural cells, which could serve as a versatile tool to study disease pathology and genetic intervention in a standardised, scalable human model system. We have analysed the transduction efficacy of 18 AAV serotypes in iPSC-derived cortical, dopaminergic and NGN2 neurons. Several serotypes were identified that have a high transduction efficacy throughout all neuronal cell types tested without neurotoxic effects. The expansion to more cell types and complex models will further extend the impact of this work.

The objective of this study is to identify highly efficient and selective capsid variants for iPSC-based applications. These could serve as a versatile tool to study disease pathology and genetic interventions in a human model system. The results from 2D monocultures lay the foundation for the implementation of AAV in complex model systems such as brain organoids. Ultimately, this platform has the potential to identify AAV serotypes that are optimal for delivering genomic cargo to hiPSC-based disease models, thereby advancing our understanding of neurodegenerative diseases in the human context and facilitating potential therapeutic interventions.

---

**#61:** Soňa Cesnáriková. Decoding Alzheimer's: 3D Cerebral Organoids as a Window into Early Disease Progression

Alzheimer's disease (AD) is a neurodegenerative disorder that remains incurable despite extensive therapeutic efforts. Induced pluripotent stem cells (iPSCs) play a significant role in studying human neurodevelopment and neurodegenerative diseases like AD. iPSCs have been used to create 3D cerebral organoids (COs) that replicate key aspects of AD *in vitro*, offering insights into the disease's molecular mechanisms. Studies have suggested that altered neurodifferentiation may play a role in AD pathogenesis. Our project leverages these advanced 3D stem cell models to explore AD development in detail.

In our laboratory, we recently generated iPSC lines from patients with familial form of AD. These patient-specific cell lines carry mutations in PSEN1(A246E) and PSEN2(N141I) genes and are sex- and age-matched to non-demented controls (NDC). Our preliminary data, obtained from mature organoids aged 60-130 days, demonstrate that AD-COs successfully mimic AD pathology. Single-cell sequencing of 60-day-old COs indicated premature neural differentiation in AD-COs, suggesting disruptions in early development. These alterations affect organization, structure, and key signaling pathway activity in younger organoids (D4–D25). Using CRISPR-Cas9 technology, we introduced mutation in PSEN1(A246E) into iPSCs derived from healthy donors. This allowed us to create isogenic mutant lines that can help confirm whether this particular mutation causes the observed developmental changes in AD organoids. For morphology analysis, we use whole-mount staining, while for investigating signaling pathways crucial for neurodevelopment, we use Western blot at the protein level and single-cell sequencing to assess gene expression. Our findings now support the idea that developmental abnormalities and disrupted neurogenesis may significantly contribute to AD. Investigating these developmental changes in AD organoids provides valuable insights into the mechanisms driving disease progression.

**#62:** Alicia González Díaz. Building a Panel of Stem Cell Models to Capture the Molecular Heterogeneity of Alzheimer's Disease through AI-Guided Multiplexed Functional Genomics

Alzheimer's disease (AD) comprises a wide spectrum of heterogeneous disorders rather than a single pathological entity. Several longitudinal studies revealed a striking variability in clinical progression and neuropathological features among patients sharing an AD diagnosis. This heterogeneity prompts the discovery and integration of patient-specific molecular signatures into preclinical models to advance precision medicine.

We hypothesise that the heterogeneity of AD emerges from distinct interactions between genetic vulnerability and environmental factors, driving cells into disease states that vary across cell types, brain regions, and patient subtypes. By combining artificial intelligence (AI) tools and patient-derived omics data, we then predict the molecular nature of these disease states.

We propose here a method of generating panels of stem cell models that represent computationally-predicted disease states by perturbing gene expression networks (GENs) to recapitulate patient-specific transcriptomic signatures. This approach complements current AD stem cell models based solely on genetic mutations, focusing on transcriptomics as the functional convergence point of genetic and environmental disease triggers.

As proof-of-concept, we demonstrate that the transient downregulation of FBXO2, a ubiquitin protein ligase complex component predicted to be an early driver of sporadic AD induces pathological hallmarks, including A $\beta$  aggregation, tau hyperphosphorylation and transcriptomic signatures of mitochondrial dysfunction in wild-type human iPSC-derived cortical neurons.

Moving from single-gene to network-based modelling, we are currently developing a PiggyBac-based screening platform with inducible gRNA arrays for multiplexed CRISPRi-mediated validation of disease state-specific GENs. In parallel, we are engineering a system based on serine integrases for rapid integration of GEN modulators, which will enable efficient generation of panels of stable AD stem cell models.

In conclusion, by using AI-guided multiplexed functional genomics we expect to be able to build a panel of stem cell models to capture the molecular heterogeneity of Alzheimer's disease.

---

### **#63:** Brent Ryan. Integrating iPSC approaches to understand the role of PINK1 in neurons

Parkinson's disease (PD) is characterised by the aggregation of the pre-synaptic protein  $\alpha$ -synuclein into Lewy bodies and Lewy neurites. This process leads to the death of neurons primarily in the substantia nigra pars compacta. A large body of evidence implicates mitochondrial dysfunction in contributing to neuronal death – however, the mechanisms underlying the interplay of  $\alpha$ -synuclein aggregation and mitochondrial dysfunction are not completely understood.

Most cases of PD do not have a monogenic cause, yet recent genome-wide association studies have identified 90 loci that have been linked to PD genetic risk to which they contribute by unknown mechanisms. We hypothesise that these genes may modulate key Parkinson's-linked phenotypes including  $\alpha$ -synuclein aggregation and PINK1/PRKN-dependent mitophagy. To investigate this, we are combining the tractability of human iPSC-derived neurons with CRISPR interference (CRISPRi) screening, delivering a library of 71 candidate genes sourced from identified risk loci. Using this platform, we have found candidate modulators of PFF-induced  $\alpha$ -synuclein aggregation and PINK1 activation using high-content imaging.

This screen is expected to uncover target genes that contribute to PD genetic risk via modulation of  $\alpha$ -synuclein aggregation and the PINK1/PRKN-dependent mitophagy, which have been strongly implicated in PD aetiology. Understanding the mechanism of action of sporadic risk genes may allow for patient stratification based on their genetic risk profiles, thus enabling more targeted and personalised therapeutic approaches.

### **#64:** Katherine White. miRNA motif analysis supports a role for ALS-associated FUS protein in astrocyte extracellular vesicle-mediated sorting and loading

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by the progressive loss of upper and lower motor neurones. Mutations in Fused in Sarcoma (FUS) are most often associated with the juvenile form of the disease. FUS is an RNA-binding protein predominantly found within the nucleus and involved in various cellular processes, including RNA transport and the processing of micro RNAs (miRNAs). Mutations in FUS can lead to mislocalisation into the cytoplasm where it forms aggregates, resulting in neuronal death. More recent work has identified FUS as a major regulator of extracellular vesicle (EV)-mediated export of selected miRNAs [Garcia-Martin (2022) Nature]. However, this fundamental process has not been analysed in human disease-relevant cells such as astrocytes and neurones. Astrocytes and neurones communicate bidirectionally, with astrocyte-derived EVs thought to be involved in the transport of neuroprotective molecules to neurones. In ALS, reactive astrocytes can promote neurotoxicity exacerbating motor neurone death. We hypothesise that FUS dysfunction contributes to ALS pathology through abnormal EV-mediated miRNA signalling. ncRNA-sequencing analysis of wild-type quiescent human primary astrocytes and their purified EVs identified an enrichment of miRNAs with known FUS-binding motifs in the EVs, relative to whole-cell miRNAs. This enrichment was maintained in a model of chronic astrocyte reactivity, and was replicated in a meta-analysis of astrocyte-derived EV miRNA sequencing data from other published studies. These observations support a role for FUS in sorting and loading of EV miRNAs in astrocytes. Extending to human iPSC-derived astrocytes and motor neurones from isogenic control and ALS-mutant FUS lines, producing EV miRNA atlas will identify 'inclusion vs exclusion' miRNA motifs and determine if/how EV contents are altered by different FUS mutations.

---

## #65: Hugo Fernandes. Elucidating Lipid Droplets in Neuronal Models of Dementia

Lipid homeostasis is essential for cellular function. Lipids regulate a wide range of biological processes and represent over 50% of the adult human brain's dry weight. A growing body of evidence places lipid dysregulation as a central theme in dementia and neurodegeneration, from genetic risk factors to altered lipid profiles. However, the mechanisms linking lipid metabolism to neuronal dysfunction remain unclear. We have developed advanced in-vitro neuronal and glial disease models of AD and PD and identified alterations in lipid metabolism.

Lipid Droplets (LDs) are cellular organelles that are critical for lipid homeostasis and recent evidence supports a potential pathological role for LDs in dementia and neurodegenerative disorders. In animal models of PD, accumulation of LDs precedes degeneration and reduction of LDs delays the onset of neurodegeneration. In AD, risk genes can impact LD homeostasis and LD accumulation preceded the formation of pathogenic protein inclusions. These findings suggest a possible new therapeutic opportunity for disease intervention targeting LDs. However, the functional significance of LDs in human neurons remains elusive.

We found that human dopamine and cortical neurons contain LDs that can be induced in response to stressors. We will present novel findings highlighting cell-type differences in LDs and describe our ongoing work characterizing the dynamics of LDs production and usage. Furthermore, we are exploring the functional interplay between LDs with other organelles in these cell models. We will show that mitochondrial dysregulation can regulate LDs and that LDs manipulation disrupts the endolysosomal pathway in neurons.

To explore underlying mechanisms, we have optimized CRISPR lentiviral-based tools for high-throughput genetic-screens in human cortical and dopamine neurons (~90% transduction efficiency) and are building-up CRISPR libraries to elucidate neuronal dependency on lipid homeostasis that could lead to the identification of novel targets for disease intervention.

## #66: Aswathy Chandran. Rethinking $\alpha$ -Synuclein: How SNCA disease variants affect phase separation

### Objectives:

Recent work from our lab has revealed that VAMP2 regulates the formation of alpha-synuclein ( $\alpha$ SYN) biomolecular condensates through liquid-liquid phase separation. Here, we aim to investigate how this process is affected by Parkinson's disease (PD)-associated  $\alpha$ SYN mutations.

### Methods:

$\alpha$ SYN-YFP variants and VAMP2 were co-expressed in HeLa cells and the resulting condensates were quantified using microscopy. Subsequently, the extent of  $\alpha$ SYN phase separation in iPSC-derived cortical neurons was evaluated by measuring levels of synaptic enrichment and FRAP profiles.

### Results:

We find that  $\alpha$ SYN variants greatly differ in their ability to form condensates. Only a few variants namely, E46K and E83Q, show higher levels of condensate formation compared to wild-type, coupled with increased levels of S129 phosphorylation. However, several  $\alpha$ SYN disease variants are unable to form condensates in HeLa cells upon co-expression of VAMP2. In addition, using CLEM, we show that  $\alpha$ SYN condensates induce clustering of intracellular vesicles. Finally, experiments in iPSC-derived neurons suggest that synaptic enrichment of  $\alpha$ SYN variants correlates with their ability to form condensates.

### Conclusions:

We find that PD-related mutations differentially affect the ability of  $\alpha$ SYN to form biomolecular condensates which points to diverse  $\alpha$ SYN-related pathological mechanisms in familial PD. We also show condensates are immunopositive for pS129 and cluster intracellular vesicles, two features which are associated with Lewy bodies. In addition, we find a correlation between the levels of  $\alpha$ SYN variants at the synapse and their inherent ability to form condensates. Collectively, these findings suggest that dysregulated condensate formation may have a pathological role in synucleinopathies and future research efforts will focus on evaluating how this process affects cellular pathways, particularly at the synapse.

---

**#67: Miranda Lastra Osua.** TMEM106B loss-of-function leads to impaired pre-synaptic protein machinery in human iPSC-derived cortical neurons

### Introduction

TMEM106B haplotypes modulate the risk for several neurodegenerative diseases and impact healthy aging and neuronal reserve, suggesting that they determine neuronal vulnerability. These haplotypes are thought to regulate the expression levels of TMEM106B, a lysosomal type-II transmembrane protein, with a slight increase in expression associated with the risk haplotype. However, the mechanisms through which TMEM106B exerts its pathogenicity remain unclear.

### Methods

We generated TMEM106B knockout (TMEM106B<sup>-/-</sup>) iPSC-derived cortical neurons by using CRISPR/Cas9. We characterized the lines and performed whole cell mass spectrometry of these neurons at DIV80. We also analyzed lysosomal trafficking with live cell imaging and lysosomal enzymatic activity. In order to have fully mature, spine-bearing neurons, we xenografted neuronal precursor cells in immunodeficient (*Rag2<sup>-/-</sup>*) mice. Neurons from these mice will be analyzed at 9 months to determine cell ramification, spine density and synaptic content.

### Results

We observed a downregulation of proteins involved in synaptic vesicular metabolism and transport (SYT1, logFC [TMEM106B<sup>-/-</sup> vs. WT]=-1,27, -log [p-value]=1.8; GAD2, logFC = -1,82, -log [p-value]=2,97) and an upregulation of galectin-3 (logFC=2, -log[p-value]=1,47), a marker for lysosomal damage, suggesting alterations of the endolysosomal pathway. We confirmed a significant loss on presynaptic markers (SYT, p-value= 0,0141, SYT1-2, p-value= 0,0103) and increase on lysosomal markers (LAMP1, p-value=0,0294) in the TMEM106B<sup>-/-</sup> neurons by western blot. Moreover, functional characterization of endolysosomal fitness showed reduced lysosomal trafficking, lysosomal accumulations in the soma and dysregulated cathepsin D activity.

### Conclusions

Our results show that loss of TMEM106B leads to a dysregulation of the presynaptic terminal and the endolysosomal system, pointing towards a dysfunction in the recycling or docking of these vesicles. This would indicate a direct role of TMEM106B in the maintenance of healthy presynaptic compartments, and could explain how TMEM106B dysregulations affect neuronal vulnerability.

**#68: Carol Geukens.** Neural Stem Cells harboring POLG mutation mimic mitochondrial disease phenotypes which can be rescued by novel POLy activator

Mitochondrial dysfunction is implicated in over 50 known diseases including mitochondrial DNA depletion syndromes (MDDS), a group of rare genetic disorders classified as primary mitochondrial diseases (PMDs). These conditions, which affect individuals across all age groups, currently lack effective treatment options. Among the genes responsible for MDDS, DNA polymerase γ (POLy) mutations have emerged as one of the most common causes of MDDS given the critical role of POLy in mitochondrial DNA (mtDNA) replication. Mutations in POLy lead to mtDNA depletion, primarily affecting energy-demanding tissues such as the brain, liver, and muscles. Given the brain's reliance on ATP production via oxidative phosphorylation (OXPHOS), POLy mutations often result in severe neurological symptoms, including epileptic seizures that can arise early in neuronal development. To date, over 300 POLy mutations have been identified in MDDS patients, underscoring the complexity of disease mechanisms and the urgent need for targeted therapeutic interventions. Our work focused on developing a treatment to improve POLy function and increasing mtDNA levels in tissues linked to disease pathophysiology. Pretzel Therapeutics is investigating a first in class, small molecule drug, PZL-A, for its therapeutic potential in neural stem cells (NSCs) carrying the severe homozygous G848S POLy mutation. Our study showed that PZL-A increases mtDNA levels and improves OXPHOS function in a concentration -dependent fashion in this disease-relevant neuronal model.

#1: Zaid Muhammad. Generation of an induced pluripotent stem cell line (BIORTCi001-A) from a healthy adult indigenous Nigerian participant

Genetic backgrounds influence cellular phenotypes, drug responses, and health outcomes, yet most human iPSC lines are derived from individuals of European descent, with lines from indigenous Africans particularly scarce. Addressing this gap, we generated iPSCs from dermal fibroblasts of a healthy 60-year-old indigenous Nigerian male of the Babur ethnic group using Sendai virus. The iPSC line displayed a normal karyotype, was characterized for pluripotency markers and differentiated into neural progenitor cells and astrocytes. To enhance African representation in research, this iPSC line will be available to the scientific community, with ongoing efforts focused on creating an open-access African iPSC biobank.

#2: Athanasia Kalogirou. Predicting Cognitive Ability in Patients with Frontotemporal Dementia Using Brain Measures Volumetric Data

Dementia is a devastating disease. It is heterogeneous not only because there are different underlying causes of it, but also high variation in patients' experience and prognoses. This variability is influenced by factors such as the type of dementia, the individual's overall health, as well as the brain regions affected. Even within the same type of dementia there is variability in the rate of progression and symptoms. Understanding this heterogeneity is crucial for accurate diagnosis. Especially nowadays with new treatments being explored, predicting the dementia type and cognitive impairment progression is an invaluable tool for examining patients' eligibility for clinical trials and planning personalized treatment strategies. In our research, we used brain imaging measures data and explored a range of different methods to select variables for predicting the cognitive impairment rate in patients suffering from frontotemporal dementia. We mostly explored Bayesian Variable Selection methods and compared them with some classical Statistical methods. The resulting promising models combine good predictive ability in predicting patients' cognitive ability, but also interpretability which is essential when working with patients and clinicians

Transactive response DNA-binding protein 43 (TDP43) cytoplasmic aggregation and loss-of-function, is a hallmark of amyotrophic lateral sclerosis (ALS). Lots of studies about TDP43 mislocalization employ animal models. The understanding of how TDP43 mislocalization contributes to the progression of ALS in vitro human neuromuscular is not sufficient. To examine the role of RBP dysfunctions contributing to neuromuscular junction disruption and neuron death, we developed a co-culture setup for iPSC-derived motor neuron spheres and myotubes to generate fully human models of healthy and ALS in vitro neuromuscular junctions. We differentiated MN spheres from human-derived iPSCs to co-culture with C2C12 myotubes or human primary skeletal muscles. Neurofilament-positive axons elongated and innervated myosin heavy chain-positive muscle fibres, with acetylcholine receptor clusters formed at connections with neurons. Neuromuscular junction formations are verified by postsynaptic marker AChR and presynaptic marker synaptophysin co-localization. Muscle contractions were observed following glutamic acid activation of motor neurons and were effectively halted by neuromuscular junction antagonists. This validated the physiological relevance of the model and established a functional assay for studying neuromuscular pathophysiology.

Currently, we are investigating how TDP-43 mislocalization contributes to neuromuscular junction (NMJ) disruption. To induce TDP-43 cytoplasmic aggregation after NMJ maturation, we employ nanobodies tagged with a nuclear export signal (NES). We then assess NMJ integrity by the number and size of junctions, neuronal loss, muscle contractility, and synaptic function following TDP-43 mislocalization. Future work will focus on examining how TDP-43 mislocalization disrupts protein-RNA networks using covalent crosslinking and RNA-seq. Through this project, we aim to uncover the molecular mechanisms by which TDP-43 mislocalization compromises neuromuscular junction integrity in ALS.

#### **#4: Éanna Ryan. Exploiting Oligogenic Models of ALS to Identify Pathogenic Mechanisms**

##### **Background**

Amyotrophic Lateral Sclerosis (ALS) is a fatal disease resulting in muscle weakness, paralysis and ultimately death, often within 2-3 years of symptom onset. These symptoms arise due to the loss of motor neurons from the brain and spinal cord. Treatment options are limited and have little impact on halting progression of the disease. Mutations in ~20 genes have been linked to monogenic forms of ALS, with the C9orf72 hexanucleotide repeat expansion mutation being the most common known cause of ALS. Oligogenic cases have also been identified where individuals harbour more than one known ALS mutation, including in genes with truncating and putative loss-of-function variants.

##### **Aims**

The aims of this study are (1) to establish and characterise monogenic and novel oligogenic induced pluripotent stem cell-motor neuron (iPSC-MN) models of ALS, (2) phenotypic characterisation of the models and (3) transcriptomic profiling of the iPSC-MNs and bioinformatic analysis to identify dysregulated molecular pathways.

##### **Methods**

C9orf72-mutant and isogenic control iPSC-MNs were differentiated from ALS patient-derived iPSCs using a cocktail of small molecules to induce dual-SMAD inhibition and ventral spinal cord patterning. Motor neuron marker expression was validated via immunocytochemistry and western blotting. Target gene expression was knocked down using Accell siRNA and assessed via qRT-PCR and western blotting. Paired-end mRNA sequencing was performed using a NovaSeq X Plus system and bioinformatic analysis was carried out using the limma package in the R computing environment.

##### **Results & Discussion**

The iPSC-MN model system has been successfully established and expression of spinal motor neuron markers confirmed. Putative loss-of-function ALS genes NEK1 and UNC13A were successfully knocked down. Differentially expressed genes have been identified among numerous comparison groups and pathway enrichment analysis is ongoing. This analysis will provide insight into dysregulated pathways relevant to neurodegeneration in ALS that can be targeted in the future development of diagnostic and therapeutic drug treatment options.

---

**#5:** Laura R. Rodríguez. Human 5xFAD iPSC models with inducible and tunable pathology

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder causing significant suffering and growing healthcare burden worldwide. With an aging population, AD prevalence is expected to rise sharply in the coming decades. Despite extensive research, the etiology and pathophysiology of AD remain elusive, and while some antibody-based therapies have shown disease-modifying potential, they are insufficient as standalone treatments, highlighting the need for deeper investigation. A critical barrier to progress is the limited translational relevance of current models. Mouse models fail to fully replicate human AD pathology, and existing human induced pluripotent stem cell (hiPSC) based models often show mild phenotypes and limited neuronal dysfunction. This gap underscores the urgent need for more advanced human models to better understand disease mechanisms and identify effective therapeutic targets.

To address these limitations, we developed a novel hiPSC model harboring five familial AD mutations present in the widely used 5xFAD mouse model. Using CRISPR/Cas9 and homology-directed repair, we introduced Cre- and Flp-inducible expression cassettes for mutated APP (Swe/Flo/Lon) and PSEN1 (M146L/L286V) into the AAVS1 safe harbor loci. This design allows precise temporal and cell type specific control over the induction of AD pathology. Importantly, we validated that these mutations can be activated through two distinct approaches: (1) by inducing recombination in the edited cells before differentiation or (2) by first differentiating the edited iPSCs into neurons and subsequently triggering recombination. This dual strategy demonstrates the model's high versatility for studying disease onset and progression. Neurons derived from recombined lines exhibited elevated A $\beta$ 42/A $\beta$ 40 ratios, particularly in the 5-mutation line, aligning with key AD pathological features. Combined with forward programming or traditional differentiation to neuronal and glial lineages, this platform offers broad applications for tunable disease modeling, including drug screening, and 3D culture systems, providing a robust tool for advancing Alzheimer's disease research.

**#6:** Melissa Barber. RELN-DAB1: a novel pathway that interacts with APOE to modulate microglial activation and Alzheimer's disease-risk

Recent genetic studies have shown RELN-DAB1 to be a novel pathway that significantly modifies disease-risk in both familial and late-onset cases of Alzheimer's disease (AD). While preclinical studies have shown that RELN counteracts amyloid and Tau pathology in mice, how protection is conferred in the human brain remains unclear. A recent GWAS from the lab predicts that RELN-DAB1 interacts with APOE4 to modify disease-risk. Our analyses of publicly available transcriptomic databases suggest that RELN-DAB1 pathways may alter neuroinflammation and the vascular system in AD, and that RELN-responsive microglial subtypes may be associated with their response to amyloid and tau in vulnerable brain regions. Here, we use a germline N-Reln knock-down mouse model and in-vitro models of human iPSC-differentiated microglia to study how RELN-DAB1 alters microglial activity. We show that a Reln-deficient environment alters microglial function within the adolescent murine brain. We next use siRNA knock-down in human iPSC-microglial models in vitro to understand how RELN-DAB1 genetic status may alter microglial responses to stressors in AD. In conclusion, we propose that RELN-DAB1 is a novel immune-modulatory pathway that interacts with APOE to alter AD-risk through its effects on microglial function in the human brain. This work may allow the development of new candidates for biomarkers and drug discovery for AD.

---

**#7:** Ben Clarke. Multi-omic analysis reveals ERAP2-dependent dysregulation in VCP-ALS astrocytes

Deleterious astrocyte reactivity is increasingly recognised in contributing to neurodegenerative diseases including in amyotrophic lateral sclerosis (ALS). However, the molecular determinants of cell autonomous astrocyte reactivity are incompletely understood. Previous meta-analysis of human induced pluripotent stem cell (hiPSC)-derived ALS astrocytes identified endoplasmic reticulum aminopeptidase 2 (ERAP2) as commonly upregulated across various genetic forms of ALS including C9orf72, FUS, SOD1 and VCP. Here, we report that ERAP2 expression is increased in ALS postmortem tissue and is associated with poor prognosis. We therefore investigated the effects of siRNA mediated knockdown of ERAP2 in hiPSC derived VCP mutant ALS and control astrocytes. This revealed partial amelioration of multiomic signatures in VCP mutant astrocytes, suggesting a role for ERAP2 in cell autonomous astrocyte reactivity in ALS.

**#8:** Hilary Carswell. A 'stroke-on-a-chip' microfluidic model using mouse primary neuronal cultures-a pathway for the future development of a human iPS stroke model.

Stroke is an unmet clinical need and new therapies are desperately needed. Thousands of agents are neuroprotective in rodents but none, other than revascularisation, have translated to the clinic, in part due to species divergence. Therefore human stem cell models of stroke are needed for improving our understanding and development of treatments for stroke.

We have developed a 'stroke-on-a-chip' microfluidic model using mouse primary neuronal cultures that has two main features:

- 1) Our model replicates the spatial and temporal gradients of oxygen glucose deprivation (OGD) spreading from 'core' to 'penumbra' found *in vivo* which cannot be achieved using existing *in vitro* systems. This provide crucially important information on critical thresholds of OGD that induce loss of neuronal viability, connectivity, electrical function and protein synthesis in core and penumbra, all of which could be salvaged by interventional therapeutics.
- 2) Our multi-chamber microfluidics system allows quantitative, real-time measurements of cell-cell responses in remote and proximal regions to the ischaemic insult. This crucially important knowledge on spatial definition of cellular reactivity and their modulation by therapeutics cannot be acquired in conventional systems.

Future work, funded by NC3Rs, will aim to develop, validate, and apply this model using human induced pluripotent stem cell (iPS)-derived neuronal cells instead of rodent neuronal cultures to transform it into a human-based stroke model.

In summary, we hope to make our enhanced, "humanised" stem cell microfluidics model the "go-to" model for some areas of stroke research that currently require the use of animals to help address the 3Rs principles and to help overcome the hurdles of species divergence. Given that hypoxia is an independent risk factor for vascular dementia and Alzheimer's disease, our model could also advance our understanding of other neurodegenerative diseases.

---

**#9: Rana Fetit.** Can ES-derived human oligodendrocytes recapitulate known regional and functional transcriptional heterogeneity?

Oligodendrocytes are cells that wrap nerves with a protective layer called myelin. Myelin provides nerves with energy, allows signals to travel quickly along them and its loss contributes to neurodegenerative diseases. Using snRNA-sequencing of adult post-mortem white matter, our lab found that human oligodendrocytes are heterogeneous and can be grouped into 3 different subtypes. Some of these subtypes are exclusive to humans and are found in varying amounts depending on region (brain or spinal cord) and whether a person is healthy or not. It remains unknown whether these transcriptional subtypes have different propensities to myelinate axons and provide metabolic support.

To assess biological function we need to, first, grow the different subtypes in-vitro. We used GFP+ human embryonic stem cells(hESCs) to generate forebrain and spinal cord oligodendrocytes. Oligodendrocytes were maintained for two weeks in culture, then the expression of the different subtype markers was assessed using flowcytometry. Oligodendrocytes from both differentiation routes were "confused", expressing all genetic markers of the three subtypes rather than becoming one specific type. We transplanted oligodendrocyte progenitors from both differentiation routes into immunocompromised Shiverer (Shi/Shi;Rag2<sup>-/-</sup>) mice to assess their differentiation in-vivo. Human-mouse chimeras could not efficiently drive oligodendrocyte subtype differentiation. To overcome this, we assembled an expression vector to generate a stable hESC line expressing inducible CRISPRa/i machinery as a Landing Pad in the AAVS1 site. The introduction of guideRNAs to this cell line will switch on genes chosen from available transcriptomic and epigenomic datasets to drive our in-vitro cultures to the specific oligodendrocyte subtypes to answer the questions regarding their function.

This is important, because once we understand how these subtypes differ, we can identify targets to improve human oligodendrocyte function in ageing and neurodegenerative diseases, such as multiple sclerosis and dementias, where oligodendrocyte function is disturbed, and where improving this will aid neuroprotection.

**#10: James Evans.** \*\*\*FLASH TALK SPEAKER\*\*\*

## Modelling oligodendrocyte dysfunction in Parkinson's disease

Neuronal aggregation of alpha-synuclein ( $\alpha$ Syn) drives cellular dysfunction in Parkinson's, yet genetic and single-cell data highlight a significant oligodendroglial component to the disease. First, the genetic risk for PD is enriched within genes specifically expressed in oligodendrocytes, and second, that oligodendrocyte-related genes are altered early in the disease process, prior to mDA neuronal genes. Despite this, the underlying pathways by which oligodendrocytes contribute to the disease process, and ultimately neuronal degeneration, are unknown. We optimised an experimental paradigm to generate highly enriched populations of oligodendrocytes from hiPSCs and characterised their molecular and functional identity. In order to investigate the mechanisms by which oligodendrocytes contribute to synucleinopathy, we generated hiPSC-derived oligodendrocytes from controls and donors with SNCA mutations, and investigated the effect of SNCA mutation on oligodendrocyte protein homeostasis, lipid metabolism, and oligodendrocyte function. We further investigated how oligodendrocytes handle misfolded protein, and characterised the process and consequences of protein oligomerisation in oligodendrocytes. We demonstrate that  $\alpha$ Syn aggregation in oligodendrocytes triggers an inflammatory cascade mediated by pattern recognition receptors. This inflammatory response disrupts physiological oligodendrocyte functions and promotes activation of other glia. Together, our data reveal  $\alpha$ Syn-induced mechanisms by which oligodendrocytes contribute to cellular dysfunction and a broader glial inflammatory response in Parkinson's.

---

**#11: Lizzie Glennon. Complex modelling of neurodegeneration using iPSC derived cells and applications to drug discovery.**

### Introduction

The role of non-neuronal cells in neurodegeneration is poorly understood. Therefore, more complex human models including non-neuronal cells could better help drug discovery and development. We have developed methodologies for co-culturing two of the main central nervous system (CNS) cell types involved in amyotrophic lateral sclerosis (ALS): microglia and motor neurons.

### Methods

RBi001-A iPSCs purchased from EBiSC were differentiated into microglia-like cells using a modified version of Haenseler et al (2017) protocol. Motor neurons were purchased from FujiFilm Cellular Dynamics (01279). To confirm generation of specific cell types cells were stained with cell type specific primary antibodies. For live cell imaging motor neurons were transduced with NeuroLight lentivirus (4808, Sartorius), or stained with NeuO (01801, Stem Cell Technologies) and cells were imaged using the Opera Phenix™ or Incucyte SX5™. Secreted proteins were quantified using MSD V-plex assay or ELISA.

### Results

Functional microglial positive for well-defined markers (e.g. PU.1, CD11B) were generated. Microglia numbers doubled over a 72-hour time period, and consistently phagocytosed zymosan particles over 24 hours. Transduction of iCell Motor Neurons with NeuroLight lentivirus was optimised for long-term live cell imaging. A reduced growth factor co-culture media was identified for future studies assessing neurodegeneration. We demonstrated that co-culture with microglia alters the dendritic arborisation of motor neurons, leading to longer and more branched neurite trees.

### Conclusions

We developed a methodology for long-term labelling of motor neurons for quantitative longitudinal cellular profiling in co-culture. This allows on-going assessment of neurodegeneration *in vitro* without perturbing the cells in culture. This will be a valuable tool for drug discovery, to investigate the effect of non-cell autonomous neurodegeneration and microglia dysfunction.

**#12: Poulomi Banerjee. \*\*\*FLASH TALK SPEAKER\*\*\***

**Hyperphosphorylated tau aggregates drives microglial activation and synapse loss in microglia-containing cortical organoids**

Hyperphosphorylated aggregates of tau are a pathological hallmark of several neurodegenerative diseases. Exaggerated microglial inflammation and synapse loss are well documented in tauopathy preclinical models and human post-mortem studies of diseases containing tau aggregates, such as Alzheimer's disease (AD) and progressive supranuclear palsy (PSP). Here, we investigated whether tau aggregates trigger human microglial activation, leading to synapse loss. To address this, we first investigated the impact of *in vitro* generated hyperphosphorylated tau (ON4R) on the immune state of microglia. Human Induced pluripotent stem cell-derived microglia (iPSC-MG) exposed to hyperphosphorylated tau for 24 hours exhibited significant immune activation, as indicated by increased CD68 reactivity and upregulation of immune-related genes. Notably, treatment with the Toll-like receptor 4 antagonist TAK242 attenuated the tau-induced proinflammatory responses. Using microglia-containing cortical organoids treated with hyperphosphorylated tau for 10 days, we further assessed the non-cell-autonomous effects of tau-driven microglial activation on neurons, astrocytes, and synapses. Our data indicate that tau activates iPSC-MG, increases GFAP levels, and reduces synapse number without significant changes in neurite area. We further corroborated these observations in post-mortem samples of PSP patients. Future studies will focus on uncovering the molecular mechanisms underlying microglial activation, increased astrocyte reactivity, synapse loss through single cell proteomics

---

**#13:** Tom Cremer. Understanding mitochondrial control of tau hyperphosphorylation

Changes in brain energy metabolism and accumulation of hyperphosphorylated Tau (pTau) are two major hallmarks of Alzheimer's disease. We recently discovered that neuronal mitochondrial metabolism, downstream of the ApoE4 genotype, is a direct regulator of pTau in iPSC-derived NGN2 neurons. Our data indicates that neuronal mitochondria regulate Tau proteastasis via mTOR-mediated signaling. In addition, our data point out that the mitochondrial inhibitor Berberine can safely reverse the effects of mitochondria on pTau in ApoE4 neurons. To validate these results in a more relevant model for Tau in AD, we will develop an ApoE4 x 3R/4R Tau iPSC-model. Herein, I will also test whether other metabolism-targeting candidate interventions can prevent ApoE4-induced accumulation of pTau. These data have generated novel insights into the pathways that connect early ApoE4-mediated metabolic changes to pTau, which pave the way for discovering novel candidate interventions that might reverse these pathways *in vitro* and *in vivo*.

**#14:** Miguel Minaya. \*\*\*SCMND travel award\*\*\*

4R-tau disrupts molecular networks, including SALL1, which promotes tau aggregation

Tauopathies are a group of neurodegenerative disorders characterized by the deposition of abnormal filamentous accumulations of the tau protein in the brain. Splicing defects in MAPT exon 10 lead to an imbalance between 3R- and 4R-tau isoforms, a key factor in the development of tauopathies such as frontotemporal dementia (FTD-tau) and progressive supranuclear palsy (PSP). However, it is still unclear whether increased 4R-tau expression is sufficient to drive the core pathologic processes across 4R tauopathies. To determine whether increasing 4R-tau levels is sufficient to drive pathologic changes in human neurons, induced pluripotent stem cells (iPSC) were differentiated into neurons and treated for 14 days with an isoform switching antisense oligonucleotide that increases 4R-tau (4R-ASO). We identified 532 significantly dysregulated protein-coding genes ( $FDR-BH \leq 0.05$ ) in iPSC neurons treated with 4R-ASO compared to controls treated with a non-targeting scrambled ASO (Scr-ASO). A subset of these genes were differentially expressed in iPSC neurons from MAPT mutation carriers and in human brains with FTLD-tau and AD, pointing to their possible involvement in pathological processes. These genes are enriched in key dementia-related pathways, including glutamatergic synapses and protein secretion. We also found that SALL1, a zinc finger transcriptional repressor and part of the histone deacetylase (HDAC) complex, is significantly elevated in 4R-ASO-treated neurons, MAPT mutant neurons, FTLD-tau brains, and AD brains. Using a FRET-based tau seeding biosensor assay, we assessed the impact of SALL1 expression on tau aggregation. Overexpression of SALL1 significantly increased integrated FRET density, while silencing SALL1 led to a significant decrease compared to controls. Thus, increased 4R-tau triggers dysregulation of key tauopathy pathways, including cellular signaling, synapse, and lysosome functions, ultimately leading to tau aggregation. These findings point to a number of new therapeutic targets for tauopathies.

---

**#15: Lois Keavey** Investigating non-cell autonomous effects of MAPT mutations through mixed genotype neuron-astrocyte co-cultures

In frontotemporal dementias (FTD), alternative splicing of the MAPT gene, and post-translational modifications results in pathological accumulation of hyperphosphorylated tau, which leads to synaptic loss and neuronal death. Specific astrocytic pathologies occur in FTD, however their in disease pathogenesis remains understudied. Previously, tau knockout astrocytes have been shown to promote the upregulation of synaptoprotective genes in neurons, and implantation of control mouse astrocytes into tau mutant mice rescues cortical neuron death. Similarly, we have found there are cell autonomous effects of MAPT splicing mutations on astrocyte function. We are now investigating the non-cell autonomous effects of MAPT mutations in astrocytes on neuronal health and activity.

We have generated neuron-astrocyte co-cultures derived from isogenic pairs of iPSCs containing heterozygous and homozygous MAPT S305N mutations, using NGN2 viral induction and directed differentiation to produce neurons and astrocytes from neural progenitor cells (NPCs) respectively, under homogenous and heterogeneous co-culture conditions. Co-cultures have been processed for single cell sequencing, cellular survival and phenotype analysis through immunofluorescence. Through single cell analysis of gene expression patterns of cells in heterogeneous co-cultures in comparison with homogenous co-cultures, processing data using Seurat and UMAP reduction, we aim to identify gene transcripts altered by non-cell autonomous effects in neurons and astrocytes due to interactions between cell types and genotypes.

Co-cultures containing mixed genotype combinations of neurons and astrocytes produced differential gene expression profiles to those in homogenous co-cultures, indicating of an important role of astrocytes on neuronal function in tauopathies. We are now investigating whether heterogeneous co-culture of neurons with corrected or mutant astrocytes will rescue or confer hyperexcitability neuronal phenotypes, respectively. By investigating astrocyte-neuron interactions, and non-cell autonomous effects of changes to astrocytes in FTD, we are beginning to gain a better understanding of the role of astrocyte dysfunction in pathology, and their potential as a therapeutic target.

**#16: Francesco Paonessa**. Targeting the acetyltransferase NAT10 corrects pathologies in human frontotemporal neurons and extends lifespan in an *in vivo* Drosophila tauopathy model

Disruption of the neuronal nuclear membrane and nucleocytoplasmic transport are emerging as hallmarks of neurodegenerative diseases, including Alzheimer's disease and frontotemporal dementia (FTD). Using iPSC-derived neurons, we previously reported that missense and splicing mutations in the MAPT gene, which encodes the microtubule-associated protein tau, result in nuclear envelope deformation and impaired nucleocytoplasmic transport. These defects are likely driven by microtubule mechanical stress, resembling the phenotype observed in laminopathies like the Hutchinson-Gilford Progeria Syndrome (HGPS). Remodelin, a small molecule inhibitor of the acetyltransferase NAT10 has been shown to correct nuclear membrane defects in HGPS by modulating microtubule dynamics.

In this study, we used human iPSC-derived neurons from FTD-MAPT patients carrying the MAPT IVS10+16 mutation to investigate the potential of NAT10 inhibition in tauopathies. Treatment of FTD neurons with Remodelin reduced microtubule elongation rates, restored nuclear lamina shape and corrected defects in nucleocytoplasmic transport, all cellular phenotypes associated with tau-related neurodegeneration. Similarly, knockdown of NAT10 also corrects nuclear lamina defects in human MAPT IVS10+16 neurons. Finally, we demonstrate that NAT10 inhibition and haploinsufficiency restore neuronal nuclear shape and extend lifespan *in vivo*, in a Drosophila model of tauopathy.

These results highlight the potential of iPSC-derived neuronal models for studying the cellular mechanisms of neurodegenerative diseases and their use for preclinical drug discovery. Our findings identify NAT10 as a key mediator of neuronal pathologies in tauopathies and suggest that targeting NAT10 may represent a promising therapeutic approach for tau-related neurodegenerative diseases.

---

**#17: Olivia Soper. \*\*\*FLASH TALK SPEAKER\*\*\*****Generation of Human Hippocampal Organoids**

The human hippocampus is a highly plastic brain region critical for learning and memory. Unlike most brain areas, where neurogenesis ceases after development, the hippocampus uniquely sustains neurogenesis into adulthood. Dysregulation of this process has been implicated in various neurological and psychiatric disorders including Alzheimer's disease and depression, highlighting its potential as a therapeutic target. However, progress in understanding hippocampal neurogenesis has been limited by the absence of robust *in vitro* models that accurately reflect the complexity of this process in humans.

Here, we present an optimised protocol for generating human induced pluripotent stem cell derived hippocampal organoids. These hippocampal organoids exhibit 3D, self-organizing structures that mature over time and contain neural stem cells, immature neurons, dentate granule cells, hippocampal neurons, and astrocytes. Importantly, these organoids exhibit ongoing neurogenesis, maintain their neural stem cell population, and display neuronal activity making them a suitable model to study human hippocampal neurogenesis *in vitro*.

We further demonstrate the utility of these hippocampal organoids by exposing them to dexamethasone, a corticosteroid commonly prescribed in elderly patients and associated with cognitive impairment. Dexamethasone treatment disrupted neural stem cell activity and altered neurogenesis. Transcriptomic analysis via RNA sequencing revealed activation of the senescence-associated secretory phenotype, along with pathways related to cellular stress responses and DNA damage repair.

Our findings establish a reproducible 3D human hippocampal organoid model as a powerful platform to investigate hippocampal function and neurogenesis in both physiological and pathological contexts. This advancement holds significant potential for exploring novel therapeutic strategies targeting hippocampal-related disorders.

**#18: Áine Heffernan. Understanding the consequence of LRRK2 dysregulation in human stem cell-derived astrocytes'**

Mutations in leucine-rich repeat kinase 2 (LRRK2), which increase kinase activity, are a common genetic cause of Parkinson's disease (PD). The non-cell autonomous role of glial cells in neuronal dysfunction is increasingly recognised in PD, particularly impairment of astrocytic homeostatic functions. However, the role of LRRK2 in this context is unclear. To investigate this, human stem cell-derived LRRK2 mutant astrocytes were generated harbouring pathogenic mutations, LRRK2R1441C/+ and LRRK2G2019S/+, and loss of function mutations, LRRK2-/ and LRRK2D2017A/D2017A pseudokinase.

Firstly, unbiased mass spectrometry analysis investigating the cell autonomous effect of LRRK2 dysregulation in astrocytes highlighted impairments in the innate immune response across LRRK2 mutants which was validated by immunofluorescence. A similar phenotype was noted in LRRK2-/ astrocytes and when cells were subject to a LRRK2 kinase inhibitor (GNE-0877), suggestive of a novel loss-of-function role for the pathogenic mutants.

Functional bioenergetic analysis revealed reduced glycolytic activity and mitochondrial respiration in LRRK2R1441C/+ astrocytes. However, increased oxygen consumption and glycolytic activity was noted in LRRK2-/ and LRRK2D2017A/D2017A astrocytes.

Astrocytes are important non-cell autonomous contributors to disease pathogenesis, but the effect of LRRK2 mutant astrocytes on neuronal physiology has not yet been investigated. In this study, LRRK2R1441C/+ astrocytes were noted to affect synaptic number and induce an immature neuronal electrophysiological phenotype in control neurons in co-culture, supporting non-cell autonomous mechanisms in LRRK2-mediated PD. Future work aims to understand the mechanism underlying this phenotype utilising single-cell transcriptomics.

In summary, astrocytes with LRRK2 mutations exhibit alterations across bioenergetic and inflammatory profiles and cause neuronal dysregulation. Ongoing work intends to mechanistically explore the involvement of LRRK2 in modulating these pathways via orthogonal approaches (pharmacological and overexpression manipulation).

---

**#19: Orjona Stella Taso, Novel TDP-43 aptamers identify early aggregation events in C9orf72 mutant human motor neurons**

**Background:** TDP-43 mislocalisation and aggregation, may contribute to pathogenesis through both gain- and loss-of-function mechanisms, and is a key hallmark of ALS. To understand the precise molecular events underlying TDP-43 proteinopathy, model systems, including human iPSC-derived motor neurons (hiPSC MNs), are crucial. Whilst TDP-43 pathology has been observed in hiPSC MNs, this has primarily been observed under specific cellular stress conditions (1). Furthermore, the degree of pathology, from mislocalisation to bona fide aggregate formation, varies depending on the mutation present, culture conditions, and the nature of the stressor(s) applied. Additionally, loss-of-function mechanisms are rarely evaluated. Despite TDP-43 proteinopathy being the fundamental hallmark in ALS pathology, current methods lack robustness in detecting early aggregation events.

**Objectives:** To address this, here we present a robust protocol, optimised for hiPSC MNs (2), that measures concomitant (i) gain-of-function (GoF), with our novel TDP-43 RNA aptamer (TDP-43APT) used to detect early aggregation events; and (ii) loss-of-function (LoF), using *in situ* hybridisation (ISH) to detect STMN-2 mRNA – the loss of which is a sensitive correlate of loss of TDP-43 splicing function.

**Methods:** hiPSCs-derived MNs from a C9orf72 mutation background ( $n=3$  lines), their isogenic controls ( $n=3$ ), and control ( $n=3$  lines) were differentiated into motor neurons (3). The presence of TDP-43 pathology was examined via our dual GoF and LoF TDP-43 detection assay with (i) TDP-43APT and STMN-2 ISH. Inverse correlation was measured using digital image analysis.

**Results:** TDP-43 pathology was identified in C9orf72 iPSC-derived motor neurons at day 7 of terminal differentiation, but not in isogenic controls, without stressor application.

**Discussion:** Here we present a robust dual staining method to assess, by inverse correlation, LoF and GoF of TDP-43 including the identification of early disease events that were not previously observed in hiPSC-derived models. The results underscore the critical importance of understanding the primacy of pathogenic events, emphasizing how this knowledge could significantly impact the development of therapies tailored to different stages of the disease.

**#20: Mark Gurney, Transcriptomic profiling and comparative analysis of human iPSC-derived reactive astrocytes**

Astrocytes, a predominant glial population in the human central nervous system, normally play critical roles in neuroprotection, immunity, and homeostasis. However, upon exposure to specific neuroinflammatory cues, astrocytes assume a pro-inflammatory, neurotoxic phenotype. Neurotoxic astrocytes promote chronic neuroinflammation, a common driver of many neurodegenerative diseases. Thus, identifying and targeting the signaling pathways that lead to the formation of neurotoxic astrocytes can provide novel therapeutic approaches to curb neurodegeneration. The preclinical development of new drugs targeting neurotoxic astrocytes requires scalable, robust and reproducible assays that harness accurate, translational and accessible *in vitro* models of the neurotoxic phenotype. Here, we aimed to assess the validity of commercially available human iPSC-derived astrocytes exposed to neuroinflammatory stimuli as a representative model of reactive neurotoxic astrocytes.

To achieve this, we performed bulk RNA-sequencing (RNA-seq) on human iPSC-derived astrocytes (2 different lots of cells, in technical triplicate) stimulated with the proinflammatory "TIC" cytokine cocktail (30 ng/mL TNF $\alpha$ , 3 ng/mL IL-1 $\alpha$ , and 400 ng/mL C1q) for 24 hours, and compared them to resting astrocytes. RNA-seq libraries were generated using the Roche KAPPA mRNA HyperPrep. Gene-level counts were quantified by Salmon and normalised by TMM. Differential expression was performed using Limma, followed by Reactome pathway and GO term GSEA. Results were validated against publicly available human iPSC-derived astrocyte transcriptomic data.

Results confirmed expression of astrocytic markers and demonstrated a distinct shift in the transcriptomic signature of astrocytes upon stimulation with the TIC cocktail. Transcriptional changes were consistent with the acquisition of a pro-inflammatory phenotype and demonstrated low lot-to-lot variability.

Our data indicate that pro-inflammatory human astrocytes can be reproducibly modelled through *in vitro* polarisation of commercially available iPSC-derived astrocytes, a cell type that can be readily accessed at scale for drug screening and other applications.

---

**#21: Sheikh Shahzabe Mukhtar.** Investigating the Effect of PD-Linked GBA1 Mutations on Neuronal Activity and the Autophagy Lysosomal Pathway Using iPSC-Derived Neurons

Heterozygous mutations in the lysosomal hydrolase  $\beta$ -Glucocerebrosidase (GBA1) are one of the biggest genetic risk factors for developing Parkinson's disease (PD). Phenotypes in induced pluripotent stem cell (iPSC) models derived from PD patients with GBA1 mutations result in defects in the autophagy lysosomal pathway (ALP) and electrophysiological function.

Recent evidence has linked synaptic activity to the ALP in multiple models, with synaptic activity upregulating autophosome biogenesis, and impaired autophagy affecting neurotransmitter release. This link supports the possibility of a mechanistic relationship between the ALP and synaptic function in neurons, which provides the possibility of druggable targets that can modulate both dysregulated processes.

In this ongoing project, iPSC-derived GBA1 cortical neurons (CNs) were differentiated with age-and sex-matched controls. Temporal maps of neuronal activity, and the emergence and function of the ALP were created up to 100 days *in vitro* (DIV). Our findings thus far have identified perturbations in electrophysiological maturation and differential ALP activity in GBA1 mutant iPSC-derived CNs, compared to controls.

Further experiments will be conducted at 70 DIV to elucidate the mechanistic link present between both processes, a time point at which they are both active. This will facilitate the identification of therapeutically relevant targets, with the ultimate goal of creating disease modifying treatments that simultaneously address pathogenic changes in synaptic and ALP activity.

**#22: Rebeka Popovic.** PINK1 mDA human neurons display mitochondrial dysfunction not due to mitophagy impairments.

Parkinson's disease (PD) is a progressive neurodegenerative disease characterised by the loss of dopaminergic neurons in substantia nigra, causing deficits in motor function. Mutations in PTEN-induced putative kinase 1 (PINK1) cause neurodegeneration in some autosomal recessive forms of PD. PINK1 is a kinase known to regulate mitophagy, the process of selective removal of faulty mitochondria important for maintenance of cell homeostasis. In this study we generated midbrain dopaminergic (mDA) neurons from patient fibroblasts carrying the W90L and I368N mutations in the PINK1 gene. Our results show that mutant PINK1 mutant mDAs display early mitochondrial dysfunction, as observed by the decrease in mitochondrial membrane potential, complex 1 dysfunction and increase in reactive oxygen species (ROS). Furthermore, we show PINK1 mutant neurons are more susceptible to mitochondrial permeability transition pore opening (mPTP) compared to control neurons. Surprisingly, despite reduced levels of PINK1, one of the key regulators of mitophagy, we observe this process can still occur in mutant PINK1 mDA neurons, suggesting involvement of PINK1-independent mitophagy mechanisms. Together, our results suggest that in dopaminergic neurons mutations in PINK1 lead to mitochondrial dysfunction separate to impairments in mitophagy, as mitophagy can occur in these cells via alternative mitochondrial clearance pathways independent of this kinase.

---

**#23: Alice Sartini. Electrophysiological and Morphological Characterization of human-derived neurons carrying ACTG1 actin mutation**

Human actinopathies are a group of rare autosomal dominant disorders caused by heterozygous missense mutations in genes encoding actin. In particular, mutations in the ACTG1 gene, encoding the  $\gamma$ -cytoskeletal actin, a major isoform in neuronal cells, lead to diseases known as Non-Muscle Actinopathies (NMAs), which may result in severe neurological symptoms.

In developing neurons, the actin cytoskeleton has a crucial involvement in neurite formation, elongation, branching, signal transduction, formation of synaptic structures and in neuronal migration process. In mature neurons, actin is the predominant cytoskeletal component of synapses, both at the pre- and postsynaptic levels. Mutations in actin genes can alter the stability or polymerization of actin filaments, impairing the protein's functionality and causing significant morphological and functional changes in neuronal cells.

As of now, isoform-specific functions of actin in neurons remain poorly understood and the molecular mechanisms driving these disorders remain largely unknown.

In this study, patient-derived induced Pluripotent Stem Cells (iPSCs), wild-type or harboring mutations in the ACTG1 gene, were differentiated in neuronal cells. Electrophysiology and confocal microscopy were used to analyze the relationship between actin mutation and cellular functionality and morphology.

Our results show significant differences between pathogenic and wild-type neurons in amplitude of voltage-dependent currents, as well as a significant increase in neuronal branching throughout the maturation period, highlighting a specific relationship between actin and neuronal features.

Future work will involve a deeper understanding of the mechanisms underlying NMAs and the connection between actin mutations and neuronal abnormalities, ultimately aiding in the development of novel therapeutic approaches.

**#24: Shreya Das Sharma. Loss of TDP-43 causes AMPAR current dysfunction in iPSC derived motor neurons**

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder, characterized by loss of motor neurons (MNs) in the motor cortex, brainstem and spinal cord. A key mechanism underlying MN vulnerability in ALS is glutamate-mediated excitotoxicity mediated by dysfunctional AMPA receptors (AMPA-R), which are ligand-gated ion channels. Here, we used iPSC-derived MNs from sporadic ALS patients – comprises 90% of ALS cases – to further understand AMPAR-mediated excitotoxicity and its implications in sporadic ALS. Using electrophysiology, we found that sALS MNs displayed increased calcium-permeable AMPA-Rs. A key pathology observed in ALS- both genetic and sporadic forms, is the presence of cytoplasmic TDP43 aggregates, leading to TDP43 nuclear loss-of-function. Our pilot data shows that the aberrant calcium-permeable AMPA-R expression was due to TDP43 loss-of-function in the MNs, and this phenotype was reversed after TDP43 re-expression, suggestive of a reversible mechanism. Additionally, unbiased proteo-transcriptomic assays on TDP43 KO MNs showed downregulation of DPP6 gene - auxiliary subunit of the A-type potassium channels -due to mis-splicing of DPP6. Interestingly, in sALS MNs showing AMPA-R dysfunction, DPP6 expression was downregulated. Crucially, overexpressing DPP6 in TDP43 KO MNs reversed the AMPA-R dysfunction. In conclusion, we show DPP6 can be a potential therapeutic target for reversing excitotoxicity in ALS.

---

**#25:** Nazli Eskici. Dual role of DLK1 in GNRH neuron ontogeny

Mutations in Delta Like Non-Canonical Notch Ligand 1 (DLK1), a paternally expressed imprinted gene, underlie central precocious puberty (CPP), yet the mechanism remains unclear. To test the hypothesis that DLK1 plays a role in GnRH neuron ontogeny, we first cut out 75 base pairs in both Dlk1 alleles in a H9 human pluripotent stem cell line (hPSC) with CRISPR-Cas9 and differentiated this clone to GnRH neurons by using previously described protocol comprised of dual SMAD inhibition, FGF8b and Notch inhibition. Ablation of Dlk1 did not, however, accelerate GNRH1 expression or the appearance of TdT-positive cells in a GnRH reporter cell line. Subsequently, we specifically activated Dlk1 expression with a CRISPRa system in the different phases of the GnRH neuron differentiation protocol. Interestingly, induced Dlk1 expression during dSMAD inhibition suppressed the formation of anterior neuronal precursors, almost completely suppressed GnRH neuron fate ( $P < .0001$ ), and induced the expression of Wnt signaling pathway components, markers of proliferating and non-proliferating progenitors, together with dorsal spinal cord interneurons. Conversely, activation of Dlk1 expression during the FGF8b phase of the protocol augmented GNRH1 expression ( $P < .0001$ ).

In conclusion, loss of Dlk1 did not lead to an accelerated pace of GnRH neuron ontogeny, or augmentation of GNRH1 expression [ $n=3$ ] suggesting that CPP in Dlk1 loss-of-function mutation carriers is not attributable directly to the development GnRH neurons. Conversely, our data show that Dlk1 overexpression is a major regulator of GnRH neuron fate, and thereafter further augments GNRH1 expression representing a potential rescue mechanism for human fertility.

**#26:** Ana Carreras Mascaro. Functional assays for ALS drug screening using predictive human cell models

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing neurodegenerative disorder affecting more than 500,000 people worldwide, with a life expectancy of about 2-5 years from the time of diagnosis. ALS is characterized by the presence of ectopic cytoplasmic TAR DNA-binding 43 (TDP43)-positive proteinaceous inclusions, which ultimately results in severe loss of motoneurons in the brain, brainstem, and spinal cord. Despite years of research, there is a clear lack of understanding of ALS biology and predictive preclinical models, which partly explains why existing disease-modifying drugs are only able to mildly prolong patient survival. iPSC-derived motoneurons carrying patient-specific mutations and phenotypes offer a unique opportunity to study ALS biology and identify potential novel targets for drug development.

We have optimized ALS disease models consisting of iPSC-derived human motoneuron cultures, including patient-derived iPSCs and gene-edited lines with patient mutations, in co-culture with primary rat glia or human iPSC-derived skeletal muscle cells, for high content analysis of functional and biochemical parameters. We integrate immunofluorescence analysis of TDP-43 pathology, optical assays of motoneuron and muscle activity, and organelle transport assays to quantify over 50 parameters of neuronal function. Furthermore, we have designed a potent, human-specific, ASO-mediated TDP43 knock-down and we have shown TDP43 miss-localization induced by stressors. Using these validated ALS disease models and assays, we have successfully tested novel TDP43 pathology-modifying drugs in collaboration with industry partners and assessed their effects on disease progression and neuronal health. Our goal at Neurospector is to accelerate preclinical drug development for ALS, by means of predictive, robust, and scalable functional and biochemical assays.

---

**#27: Eilish Mackinnon. \*\*\*FLASH TALK SPEAKER\*\*\***

A standardised framework for evaluating human iPSC-derived microglial cultures

### Introduction

In vitro models of human microglia are invaluable for our understanding of microglia's role in neurodegenerative diseases, like Alzheimer's Disease. Human iPSCs are widely used for the generation of microglia-like cells, with existing protocols aiming to recapitulate the ontogeny and gene expression of primary human microglia. Reproducibility and validity of iPSC-microglia between and within differentiations is a concern within the field, however there is no standardised criteria for validating iPSC-microglia identity. This project aims to establish a standardised framework for the evaluation of human iPSC-derived microglia cultures.

### Methods

An existing microglia differentiation protocol was adapted to standardise the generation of microglia monocultures from iPSCs. Single cell/nuclei technologies were employed to profile transcriptomic and epigenomic signatures of both primary human and iPSC-microglia. Furthermore, single cell transcriptomics were performed on a set of differentially 'aged' iPSC-microglial cultures, using the SMART-seq platform to identify differentially expressed genes alongside novel isoforms and splice variants.

### Results

Preliminary results indicate our iPSC-microglia monocultures exhibit cellular heterogeneity, with key functional and transcriptional similarities to primary human microglia. We have further developed a cold digestion protocol to isolate primary human microglia from fresh cortical tissue, attaining a high yield, purity and viability, with minimal transcriptional artifacts. From this, we intend to establish a primary human microglia single cell sequencing reference database for which differentially aged iPSC-microglial cultures can be compared and functionally evaluated.

### Conclusion

Insights from multi-omic profiling will help define the criteria through which iPSC-derived microglial cultures can be validated for similarity to human microglia and address variability across iPSC-microglial differentiations, to improve reproducibility of downstream translational studies.

---

**#28: Mario Yanakiev. APOE-Dependent Mechanisms of the Response to Amyloid- $\beta$  in iPSC-derived Microglia**

Chronic, dysregulated neuroinflammation is a pathological process largely mediated by abnormal microglial activation. Mounting evidence suggests it may be a core mechanistic factor in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD). In these conditions microglia undergo a phenotypic shift from a homeostatic to a dysfunctional state, contributing to advanced neuronal loss and the spread of toxic protein aggregates. This shift has particularly been linked to the E4 polymorphism of the APOE gene, the strongest genetic risk factor for AD. However, the transcriptomic signatures and biological processes that underlie this functional switch remain unclear, especially during the initial response of microglia to pathological stimuli.

To address this gap, we generated induced microglia-like cells (iMGLs) derived from isogenic induced pluripotent stem cell lines, harbouring either of the three APOE alleles: E3, E4, and E2, the latter being associated with a reduced risk of AD. We first validated the transcriptomic profile of our iMGLs against that of microglia isolated from foetal brain samples and confirmed their cellular and functional identity. We then treated APOE2/3/4 iMGLs with either LPS or amyloid- $\beta$  peptides and performed RNA sequencing to investigate the gene expression patterns and molecular pathways governed by the APOE variants.

Interestingly, we found that APOE2 and APOE4 iMGLs shared a similar transcriptomic signature, distinct from that of APOE3 iMGLs. Upon stimulation, APOE2 iMGLs exhibited gene expression patterns consistent with an immediate immune reaction, whereas APOE4 iMGLs failed to elicit a significant response. Inter-genotype comparisons also revealed that APOE2 transcripts were enriched for genes involved in cholesterol and lipid metabolism, while APOE4 ones showed downregulated expression of genes related to cell cycle progression. Together, these findings highlight APOE-dependent differences in microglial function that could provide further insight into AD risk modulation.

---

**#29:** Tom Campbell. A phenotypic screen for novel small molecules that suppress tau-mediated pathologies in human frontotemporal dementia neurons

Disruption of the neuronal nuclear membrane is a common pathology in neurodegenerative diseases, including Alzheimer's disease (AD) and frontotemporal dementia (FTD). Missense and splicing mutations in the MAPT gene that are causal for FTD (MAPT IVS10+16) lead to mislocalisation of tau protein to the neuronal cell body, changing microtubule dynamics to deform the nuclear membrane, disrupting nucleocytoplasmic transport. Acute depolymerisation of microtubules with nocodazole corrected nuclear membrane shape and ameliorated nucleocytoplasmic transport, demonstrating that these defects are potentially reversible. In addition, we recently found that a small molecule inhibitor of N-acetyltransferase 10 (NAT10) corrected nuclear membrane shape and nucleocytoplasmic transport defects in MAPT mutant neurons. Those studies established that FTD phenotypes are captured by *in vitro* models and can be corrected by small molecule modulation of targets other than the tau protein itself.

We report here a high content imaging-based phenotypic screen to identify novel small molecules that correct nuclear membrane defects in human MAPT IVS10+16 neurons. Screening a 19,786 compound diversity library, we identified compounds that corrected or worsened nuclear membrane defects in MAPT IVS10+16 neurons. Focusing on the positive hits, over 100 compounds were confirmed as correcting the phenotype, with 23 demonstrating robust dose-dependent rescue of nuclear membrane deformation. A common feature of hit compounds was disruption of neuronal microtubules, with a subset also altering neuronal tau protein levels and/or phosphorylation. Identification of small molecules that rescue this tau-dependent phenotype demonstrates the utility of human disease models of tauopathy for the development of novel dementia therapeutics. Finally, in addition to the specific uses of the compounds identified from this screen as potential starting points for development of novel therapeutics for MAPT IVS10+16 and other tauopathies, this screen illustrates the value of human iPSC models of neurodegenerative disease for phenotypic screens to identify potentially disease-modifying targets.

**#30:** Niamh O'Brien. Generation of a *de novo* isogenic ALS iPSC cell bank to investigate converging pathomechanisms in ALS and FTD

This project developed a novel tool for dementia research to investigate genetic forms of ALS and FTD, modelling both aggressive and familial forms of diseases. We have generated a genetically engineered isogenic induced pluripotent stem cell (iPSC) bank with mutations in the FUS and C9orf72 genes. Using CRISPR/Cas9, we have introduced KO, P525L, Delta 14, R495X and Q519fs mutations in the FUS gene on the KOLF2.1J wildtype background. Quality control at each stage confirmed that all iPSCs retained genomic integrity and differentiation ability.

Additionally, we utilised a 2-step scarless CRISPR/Cas9 method to insert a hexanucleotide repeat expansion into the C9orf72 locus in the KOLF2.1J, creating a heterozygous cell line with 600 G4C2 repeats. Characterization of these lines showed similar motor neuron differentiation capabilities between C9orf72 and wildtype iPSCs. Motor neurons derived from the C9orf72 iPSCs exhibited ALS-related pathological features, including increased sense and antisense RNA foci, reduced C9orf72 protein and RNA, and elevated cryptic exon expression in genes affected by TDP-43 nuclear loss such as STMN2.

This tool provides a valuable resource to study and model ALS and FTD. It provides a resource to complement patient iPSC line banks for uncovering commonalities in genetic forms of neurodegeneration and their pathogenesis.

---

**#31: Paolo Marchi.** A multimodal screening platform for endogenous dipeptide repeat proteins in C9orf72 patient iPSC-neurons

Repeat expansions contribute to over 50 neurological and neuromuscular disorders, including the G4C2 expansion in C9orf72, the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). A key driver of C9orf72-mediated ALS/FTD pathology is the unconventional repeat-associated non-AUG (RAN) translation of G4C2 repeats into five neurotoxic dipeptide repeat proteins (DPRs). In order to rapidly and sensitively detect endogenous DPRs in iPSC-neurons, we used CRISPR/Cas9 to integrate a HiBiT nanoluciferase reporter downstream of the G4C2 repeat in C9orf72 patient-derived iPSCs, generating "DPReporter" iPSC lines. We further used an inducible NGN2 expression system (i3Neurons) coupled with miniaturisation and extensive validation to create a scalable model for high-throughput screening. In a small molecule screen using DPReporter i3Neurons, we identified periplocin, an ERK1/2 pathway activator that increased DPR levels in a dose-dependent manner by inhibiting their degradation. Consistent with this, ERK1/2 inhibition using the drug trametinib significantly reduced DPR levels and extended lifespan in a C9orf72 Drosophila model. To further explore DPR regulation, we conducted a CRISPR-based genetic screen targeting all human helicases. This identified telomere-associated helicases as novel regulators of DPR expression—suggesting a mechanistic link between telomeric and C9orf72 repeats. Overall, our DPReporter platform provides a physiologically relevant system for studying RAN translation and endogenous DPR regulation. This approach offers a powerful tool for advancing research into C9orf72-related diseases and provides a template for investigating RAN translation, at scale, in other repeat expansion disorders.

**#32: Georgia Boothe.** Optogenetic modelling of the C9orf72 dipeptide-repeat protein (DPR) pathology in ALS/FTD

Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD) are neurodegenerative disorders that share common pathological hallmarks. A hexanucleotide expansion in the C9orf72 gene is the most common genetic cause of ALS/FTD, responsible for 50% familial cases. This expansion results in the accumulation of five dipeptide repeat proteins (DPRs), poly-GA, GR, PR, PA and GP which form inclusions in neurons and glia of the affected CNS areas. In the multitude of cellular models reported to date, including neuronal models, only poly-GA aggregation could be easily reproduced, whereas the remaining four DPR species typically displayed diffuse distribution. DPR aggregation may contribute to cellular dysfunction and disease mechanisms, however, due to the difficulty of modelling, it could not be efficiently investigated in the cellular setting.

Through the use of the Cry2Olig optogenetic protein module, we have achieved poly-PR and poly-GR condensation/aggregation with spatial and temporal control in cultured cells, including human iPSC-derived motor neurons. Blue-light stimulation induced nuclear poly-PR condensation, enhanced by RNA depletion. Following prolonged, 24-hour light stimulation, cytoplasmic redistribution and aggregation of poly-PR was achieved, and these aggregates were found to sequester TDP-43 protein. This data suggest that poly-PR condensation may constitute an early pathological event, upstream of TDP-43 pathology, in C9-ALS/FTD. We conclude that OptoDPR platform is a useful tool for modelling cytopathologies elicited by DPR aggregation for mechanistic research and drug discovery.

---

**#33:** [Sara Tacconelli](#). Identifying novel synaptic interactors of FUS in different experimental models.

Fused in Sarcoma (FUS) is an RNA/DNA binding protein which has been found to be mutated in some familial cases of Amyotrophic Lateral Sclerosis (ALS). Recent studies have proven that FUS is located at the synapse, where its role remains unknown. The main aim of this project was therefore to identify and validate FUS interactors at the synapse to further our knowledge of the protein's synaptic functions. Mass spectrometry analysis of FUS immunoprecipitations in synaptoneuroosomes was used to generate a dataset of potential synaptic interactors. Immunostaining and proximity ligation assay (PLA) were then used to validate a set of selected interactors in two different models, primary rat cortical neurons and non-transgenic mouse tissue, as well as to investigate the impact of FUS-P525L on these interactions in iPSCs-derived cortical neurons. A total of 622 proteins were identified as potential synaptic interactors of FUS. Gene Ontology, KEGG pathways and functional clustering analysis revealed that many of these proteins could be divided into four groups: RNA-binding proteins, ribosomal proteins, mitochondrial proteins and synaptic vesicle-related/synaptic maintenance proteins. Validation of the interactions with the synaptic proteins revealed that FUS associates with each of these proteins, although with clear protein-specific and neuronal-type-specific differences. The results obtained throughout this study reveal a novel possible role of FUS at the synapse and elucidates how ALS-relevant mutations alter the association between FUS and its synaptic interactors.

**#34:** [Sascha Koppes-den Hertog](#). Cholesterol as a regulator of astrocyte reactivity impaired by ApoE4

Lipid changes in the brain have been implicated in many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Induced-pluripotent stem cell (iPSC)-derived brain cells allow modelling of these diseases, but whether lipid phenotypes are recapitulated remains unknown. Using our lipidomics pipeline, we show that iPSC-derived neurons, microglia and astrocytes display distinct lipid profiles that recapitulate *in vivo* lipotypes. In addition, we show that iPSC-derived astrocytes carrying the Alzheimer's disease risk gene ApoE4 are burdened with cholesterol ester accumulation. This is in line with the cholesterol ester accumulation we see in the human brain. Multi-omic interrogation of iPSC-derived astrocytes revealed that ApoE4/4 and reactive astrocytes show an opposite phenotype of respectively higher and lower storage lipids and lower and higher immunoproteasome and major histocompatibility complex (MHC) class I presentation. Furthermore, we show that free cholesterol can regulate astrocyte reactivity, specifically MHC class I presentation, and that through enhanced cholesterol esterification ApoE4 suppresses MHC class I presentation.

---

**#35: Xu Zhang.** A novel chimeric model investigating human neurological features in Alzheimer's disease

Alzheimer's disease is a complex dementia disease pathologically manifesting with amyloid aggregation, gliosis, tau pathology and neuronal loss. Among the above, misfolding and transmission of abnormal tau is most closely associated with cognitive decline. It is however unclear how Tau pathology becomes induced and what neurons are most sensitive to the initial Tau induction at single cell level, which hampers specific and effective drug development. We have previously shown that human neurons are more sensitive to amyloid induced Tau pathology induction than mouse neurons in NPCs transplantation model. The problem with the model is that they are hard for single neuron isolation. Therefore we have generated a cortical organoid implantation animal model based on the work of Fred Gage. Initial characterization by spatial transcriptomics showed effective neuronal maturation and generation of the different layers of the cortex. In the context of amyloid plaques, the organoid reveals shrinkage, A $\beta$  aggregation and microgliosis at the borders of the implants. Single cells FACS studies confirm the dramatic decrease of neuronal numbers and further revealed initial phospho-tau bearing neurons. We aim for single cell profiling of the tau bearing neurons. In order to do that, we plan to isolate single neurons at optimal time point, label them with novel epitope indexing technique and construct neuronal transcriptomic network. The optimal time point will be determined by non-invasive imaging and biochemistry analysis and pTau neurons will be labelled with oligo ssDNA barcoded antibody and sequenced with tag compatible platform. Finally, we will integrate single nuclei database from primary tissue and indicate neurons transition from healthy to pTau states from which signal cascade of neuronal susceptibility and most significant vulnerability target will be revealed together. By combining a human cortical organoid with an amyloid bearing mouse we have been able to generate a model that embodies the main neuropathological features of AD and further work will help explore the disease pathways leading to AD.

**#36: Timothy Birkle.** Isogenic MAPT S305N/10+3 iPSCs to study 4R tauopathy using i3Neurons

Human induced pluripotent stem cells (iPSCs) are becoming a crucial tool in translational neurodegenerative research. However, they have proven difficult to use to study tauopathies because their epigenetic reprogramming results in production only of the shortest 3R-tau isoform; meanwhile, 3R-tau and 4R-tau are roughly equal in the adult human brain. This is a particular limitation for modelling tauopathies thought to arise from the accumulation of excess 4R-tau, such as corticobasal degeneration and progressive supranuclear palsy.

Here, gene-editing approaches were used to generate novel iPSC-derived cell models of 4R-tauopathy by introducing the S305N and IVS 10+3 mutations into the MAPT gene. Inducible i3-Neuron (i3N) differentiation was enabled by stable NGN2 cassette integration using either PiggyBac or AAVS1 safe-harbour-targeted approaches. S305N/10+3 i3Ns exhibit a near complete shift of 3R-tau to 4R-tau expression, alongside altered tau localisation and disease-relevant tau phenotypes. Moreover, these phenotypes are largely consistent regardless of NGN2 integration method, despite some interesting differences in neuronal differentiation between the resulting cell lines. This work is contributing to the development of screens for genetic or small-molecule modifiers of 4R-tau pathology using i3Ns, and these screens will focus on the pathology-relevant features that are uniquely recapitulated by this model.

---

**#37: Ropafado Mzezewa. Inducing pathological insults in hIPSC-derived neural cultures to model Alzheimer disease in vitro**

Alzheimer's disease (AD) is the leading cause of age-related neurodegeneration clinically characterized by the progression of cognitive decline and dementia. Neuropathologically, the disease is hallmark by accumulation of extracellular  $\beta$ -amyloid ( $A\beta$ ) plaques, intracellular neurofibrillary tangles containing tau and granulovacuolar degeneration (GVD). Accumulation of aggregates proteins results in massive loss of synapses and ultimately neuronal degeneration. Among the most vulnerable neuronal populations are basal forebrain cholinergic neurons (BFCNs), which innervate the cortex and hippocampus, and which undergo severe degeneration in AD. As such, the main symptomatic treatments target inhibition of enzymatic acetylcholine (ACh) degradation. However, despite continued efforts of intense drug development, no effective disease modifying therapies exist, partly due to the lack of physiologically relevant human models for drug development.

This project aims to establish a disease relevant human AD model to enable the development of a high-throughput phenotypic drug screen platform. Disease-modifying effects are measured through ACh release detected by sniffer cells. In this model we utilize hPSC-derived BFCNs with APOE4/APPmut genetic background co-cultured with glial cell types. We show that mutant and wild type differentiated neuronal cultures express LHX8+/CHAT + markers, confirming BFCN identity. Furthermore, we observe endogenous population of GFAP+ astrocytes within the neuronal cultures. We apply key pathological insults to the model that induce tau fibril aggregation/phosphorylation, cellular ageing, and disrupted kinase and lysosomal machinery. Upon exposure of tau fibrils to the culture we re-capitulate AD-like pathology by detecting increased phosphorylation of tau and dysregulated synapse. Inducing cellular ageing in the cultures by inhibition of the neddylation pathway resulted in an increase in DNA damage, cell death, senescence marker expression and aberrant neuronal activity detected through microelectrode arrays.

These findings support that our model recapitulates AD hallmarks and thus stands as a physiologically competent platform for high-throughput drug screening.

**#38: Rebecca Gabriele & Marieta Vassileva. From Bench to High-Throughput: Optimising iPSC-based assays for Drug Discovery**

**Introduction:**

The Alzheimer's Research UK UCL Drug Discovery Institute, part of the Drug Discovery Alliance (DDA), expedite the translation of promising new therapeutic targets into small molecule drug discovery projects and the development of next generation treatments for dementia. Human induced pluripotent stem (hiPSC) cells are a key resource in this drug discovery process as they provide a source of reliable cells for in vitro studies. hiPSCs can be used to study cellular phenotypes in different contexts. For drug discovery applications where large numbers of compounds are screened, cellular assays must be highly reliable and reproducible. We applied and refined high-throughput methods to overcome scalability issues for hiPSC enabling their utility in screening and validation of large number of compounds.

**Methods and results:**

We established methodologies using high-throughput automation to develop and validate miniaturised (96- and 384-well) cell-based assays using hiPSC-derived microglia, astrocytes, and i3 neurons. We have established suite of phenotypic readouts in control and compound-treated conditions, including: kinetic phagocytosis by microglia of fluorescently labelled SH-SY5Y and neurite outgrowth (Incucyte), measuring gene expression changes (real-time PCR), recording real-time neuronal firing with (MEA), monitor metabolic changes (Seahorse XF). Workflow validation with tool compounds confirmed reproducibility of assays for a range of applications.

**Conclusion:**

We present a suite of validated hiPSC assays that can be used to evaluate the effects of genetic differences or compounds in miniaturised and highly automated formats amenable to drug screening. In vitro studies using hiPSC models are an informative tool in the drug discovery process allowing interrogation of complex biology in human cells at scale. These human cellular tools bring preclinical studies and clinical outcomes closer, allowing predictions of drug responses in human relevant cell types and may improve the translational hurdle from lab to human in the development of targeted therapies for neurodegenerative diseases.

### **#39: Matthew Reid. A Drug Discovery Pipeline for Inhibitors of Seeded Aggregation of Wildtype Tau**

**Background:** Tauopathies, including Alzheimer's disease (AD), are defined by the pathological aggregation of tau proteins, a process linked to neurodegeneration. Misfolded tau spreads in a prion-like manner, recruiting native tau and propagating across brain regions. Existing models often rely on mutant tau or recombinant seeds, which fail to fully mimic tau conformations seen in AD, limiting therapeutic discovery.

**Methods:** We developed a screening pipeline using HEK cells expressing wildtype 3R/4R tau seeded with AD post-mortem tissue-derived aggregates, ensuring disease relevance. As a complementary approach, primary mouse neurons expressing mutant tau were seeded with recombinant aggregates, offering a comparative model for validating hits. Additionally, an iPSC-derived neuronal model of seeded wildtype tau aggregation is under development to further enhance physiological relevance.

**Results:** The pipeline successfully identified inhibitors of tau aggregation. In HEK cells, these inhibitors blocked AD-derived tau seeding, while in the primary neuron model, they prevented recombinant tau-induced aggregation. This dual approach demonstrates the inhibitors' efficacy across wildtype and mutant tau systems, bridging model relevance and translational potential.

**Conclusion:** This study establishes a robust pipeline for tau aggregation inhibitor discovery, integrating wildtype tau relevance with mutant tau models. The addition of an iPSC-neuronal model will further enhance the physiological fidelity of the pipeline. The mechanisms of the successful compounds are currently being determined to understand their mode of action, and will progress toward lead optimisation and preclinical evaluation, advancing therapeutic development for AD and related tauopathies.

### **#40: Antonio Rocco Fisciardi: Investigating the interaction of APOE4 with TDP43 proteinopathy in driving neurodegeneration**

Alzheimer's disease has several known genetic risk factors, among which APOE4 is the strongest. A post mortem study of severe cases of Alzheimer's disease found that up to 80% of patients possessed with TDP43 inclusions. This suggests TDP43 inclusions may play a role in the progression of Alzheimer's disease. This project aims to investigate if a dual-hit model of both APOE4 expression and TDP43 mislocalisation can result in a synergistic increase in neurodegeneration in iPSC-derived cortical neurones.

We optimised a technique for generating neurones expressing cortical layer 2-3 markers from human iPSCs via over-expression of various transcription factors. This is in addition to incubation with various small molecules to prevent proliferation and push differentiation towards a cortical pathway. Immunostaining and RTQPCR techniques were used to identify these population of neurones. We intend to create our dual hit APOE4-TDP43 mislocalisation model via overexpression of APOE4. Through the use of TDP43-GFP tagged iPSCs, and the expression of GFP-specific nanobodies tagged with a nuclear export signal we will achieve TDP43 mislocalisation. To confirm the generation of an Alzheimer's like phenotype we will perform quantification of Alzheimer's disease biomarkers such as amyloid beta and phospho-tau will be performed. Neurodegeneration will be quantified via a multiplex cell viability assay.

Currently we have successfully improved upon the current methods for cortical neuronal generation, from a 50% population yield to 70%. In addition to this we have had success in generating neurones expressing a biomarker profile reminiscent of a deeper cortical layer.

Overall our results show great progress as we are successfully generating cortical neurones and as several previous studies provided sufficient justification that demonstrates a role for TDP43 mislocalisation in Alzheimer's disease, thus the establishment of a synergistic effect on the progression of neurodegeneration could open new pathways for research and clinical studies.

---

#### #41: Hannah Clarke. Comparison of hiPSC-derived dopaminergic neurons

Mutations in the alpha synuclein gene (SNCA) cause autosomal dominant Parkinson's disease (PD), with loss of dopaminergic neurons in the substantia nigra, and aggregation of alpha synuclein. The generation of midbrain dopaminergic neurons (mDA) aids the modelling of neurodegenerative diseases such as PD *in vitro*. Alongside ongoing efforts to refine differentiation methods, we optimised a protocol in adherently cultured cells that produces a cell population highly enriched (80%) in mDA neurons from patient human induced pluripotent stem cells (hiPSCs).

Adherent cultures enable modelling of aspects of the midbrain environment, which, aside from mDA neurons, also contains other cell types, such as astrocytes. However, the role and function of these non-neuronal cells in synucleinopathies is less well understood. To better model the mDA neurons in their three-dimensional environmental niche, we utilised our small molecule-based differentiation protocol to produce enriched mDA precursor cells (NPCs) and subsequently midbrain organoids.

Employing single cell multiome sequencing, we characterised cell types in our three-dimensional organoid model. The comparison of these organoid cell populations versus the adherently cultured mDA neuronal cell populations highlights differences and commonalities in cellular diversity, cellular maturity, and regulatory landscapes.

In addition, contrasting hiPSCs from patients with and without SNCA mutations not only aids the identification of gene expression changes underlying pathology, it also highlights differences in the regulatory landscapes.

Taken together, this in-depth approach from 2D to 3D models allows us to delineate the role of non-neuronal cells and their interaction with neurons in SNCA associated PD.

#### #42: Johanna-Katharina Maninger. Unravelling the Role of APOE and BIN1 in Shaping Microglial Function in Late-Onset Alzheimer's Disease

Late-onset Alzheimer's disease (LOAD) is the leading cause of dementia, with genetic factors playing a critical role in its pathology. APOE and BIN1 are the two most significant risk genes identified in genome-wide association studies (Bellenguez et al, 2022; Blanchard et al, 2022), yet their combined influence on microglial function remains unexplored. Approximately one-third of individuals with LOAD carry at least one APOE4 allele along with a BIN1 risk SNP. Therefore, understanding the interaction between these genes is key to unlocking potential new therapeutic targets for Alzheimer's disease.

To model this interaction, we have generated CRISPR-mediated BIN1 knockout iPSC lines on both APOE33 and APOE44 backgrounds. Using these lines, we assessed microglial function through calcium signalling assays and live imaging assays, including zymosan and myelin phagocytosis, A $\beta$  and transferrin endosomal uptake, as well as LDL and DQ-BSA uptake. These assays evaluated phagocytosis, endosomal/lysosomal function and lipid accumulation.

Our work has revealed a reduction in early endosome area, alterations in lipid homeostasis and changes in lysosome number. For some phenotypes BIN1 appears to have a potential modulatory effect. Our findings indicate that BIN1 status may modulate APOE phenotypes. Together, with forthcoming transcriptomic data, these results enhance our understanding of the molecular underpinnings of microglial dysfunction in Alzheimer's disease and highlight the potential for BIN1 as a therapeutic target.

#### #43: Luise Schlotterose. Traumatic Brain Injury: Insights into Astrocyte and Neuronal Interactions

Traumatic brain injuries (TBI) are a major cause of death and disability worldwide, particularly in children. TBIs occur when external forces damage the brain, triggering cellular events that can lead to motor impairments, cognitive deficits, and long-term disability. Even mild TBIs can have lasting effects, including an increased risk of neurodegenerative diseases. Current treatments primarily manage symptoms rather than preventing long-term complications, making the development of effective therapies a critical challenge.

A critical aspect of TBI pathology is the disruption of the brain's microenvironment, particularly increased permeability of the blood-brain barrier. This "leakiness" allows inflammatory molecules and immune cells to infiltrate brain tissue, exacerbating injury. Astrocytes and neurons play a key role in this process: astrocytes regulate neurovascular signalling and support neurons, while neurons influence astrocytic responses. However, the precise mechanisms governing astrocyte-neuron interactions post-TBI remain unclear.

We complement the field of TBI research by developing a highly modular, cost-effective *in vitro* model using a 3D-printed tool that induces mechanical injury through cavitation, enabling precise and reproducible trauma application to cultured cells.

Our *in vitro* model utilizes primary human brain cells and induced pluripotent stem cell (iPSC)-derived brain cells, allowing us to replicate key hallmarks of TBI, including reactive oxygen species (ROS) release, proinflammatory cytokine production and cell swelling in injured astrocytes. Our findings demonstrate that injured astrocytes release factors that trigger neuronal cell death, with early evidence implicating lipid droplets as key mediators—visualized using super-resolution microscopy. Identifying these factors and their mechanisms is a central focus of our research.

By uncovering the molecular pathways driving astrocyte-neuron interactions post-TBI, our work highlights potential therapeutic targets to mitigate neuronal death and improve recovery outcomes, providing crucial insights into TBI pathophysiology and drug discovery.

#### #44: Aleksandar Rakovic. Selective vulnerability of dopaminergic neurons in Parkinson's disease links PRKN and differential expression of CHCHD2 and GPNMB

The mechanism(s) causing selective vulnerability of dopaminergic neurons in Parkinson's disease (PD) remain largely elusive. To improve our understanding of mitochondrial involvement and related pathways suggested to play a role in this selective vulnerability, we used tyrosine hydroxylase (TH)-mCherry reporter-induced pluripotent stem cells generated by CRISPR/Cas9. Upon differentiation into a dopaminergic neuron-containing cell culture, we sorted neurons into pure TH-positive and TH-negative neurons. We characterized mitochondrial function in both dopaminergic and non-dopaminergic neurons from PD patients and controls and identified differentially expressed genes between patients and controls in both cell populations. Dopaminergic neurons had a lower mitochondrial membrane potential than non-dopaminergic neurons. Furthermore, ATP levels were lower in PRKN mutation carriers than controls, and mitochondrial mass was reduced in PRKN mutation carriers only in the TH-positive but not in TH-negative neurons. Importantly, in PRKN mutation carriers, we showed elevated levels of dopamine which can present a significant source for toxic, oxidized dopamine. Using unbiased RNA sequencing, we detected increased levels of CHCHD2 and decreased expression of GPNMB in TH-positive neurons from Parkin mutation carriers compared to healthy controls. This suggests a possible interaction of these three PD genes in response to a dopaminergic neuron-specific increase in oxidative stress, which further leads to the selective vulnerability of dopaminergic neurons.

---

**#45: Mosi Li. Investigating Microglia's Role in Alzheimer's Disease Progression with a Microglia-Deficient Mouse Model**

**Background:** Microglia, the resident macrophages of the brain, play crucial roles in both normal brain development and the pathogenesis of neurodegenerative conditions such as Alzheimer's disease (AD). Despite their recognized importance, the precise contributions of microglia to the advancement of AD pathology are not yet fully understood.

**Methods:** To study the role of microglia in brain development and AD, we employed a genetically engineered mouse model deficient in microglia (*Csf1rΔFIRE/ΔFIRE*). We performed single-nucleus and single-cell transcriptomic analyses focusing on neurons and astrocytes within the cerebral neocortex of these mice to assess cellular perturbations resulting from the absence of microglia. Subsequently, we examined the impact of microglia on amyloid plaque formation and associated pathologies using a combined microglia-deficient and amyloidosis model (*Csf1rΔFIRE/ΔFIRE x APP-PS1*).

**Results:** Our findings indicate in the early brain development there is no substantial transcriptional disturbances in neurons and astrocytes due to the lack of microglia. In the amyloidosis model, the absence of microglia led to a notable reduction in plaque pathology and inflammatory gene expression, suggesting a detrimental role of microglia in plaque pathogenesis. Despite the reduced plaque pathology, plaque-proximal reactive astrogliosis, synapse loss, and neurite dystrophy persisted in the absence of microglia. Allogenic transplantation of microglia into the microglia-deficient AD model led to a reinstatement of plaque burden.

**Conclusion:** These observations emphasize the complex role of microglia in AD, highlighting their involvement in amyloid plaque formation and the progression of neurodegeneration. Our findings indicate that modulating microglial activity might be beneficial for managing Alzheimer's disease. This study provides insights into the role of microglia in AD and suggests the potential for further investigation into microglia targeted therapeutic strategies.

---

**#46: Björn Vahsen. Microglia-dependent synaptic dysregulation and complement activation in C9orf72-ALS iPSC-derived motor neuron-microglia co-cultures**

Amyotrophic lateral sclerosis, the third most common neurodegenerative disorder, is characterised by progressive motor neuron degeneration, leading to progressive paralysis and rapid death. A growing body of evidence supports a role for neuroinflammation in ALS pathophysiology, with microglia strongly implicated. Particularly in ALS patients with the hexanucleotide repeat expansion (HRE) in C9orf72, the most common ALS-associated mutation, widespread microglial activation has been observed and correlated with disease progression. Here, we aimed to investigate motor neuron-microglia interactions in C9orf72-ALS. We generated iPSC-derived motor neuron (MN)-microglia co-cultures from five C9orf72-ALS patients and five age/sex-matched healthy controls, in matched/mismatched genotype combinations, and performed RNA sequencing. We identified pronounced downregulation of synapse and axon-associated pathways in C9orf72 mutant MN-mutant microglia co-cultures, with significantly reduced SYT4 expression. Compared with healthy co-cultures, synaptic pathways and SYT4 expression were decreased in co-cultures of C9orf72 mutant MNs with control and mutant microglia, indicating the presence of the C9orf72 mutation in MNs as the primary driver. This pronounced dysregulation of synaptic and axonal pathways was not replicated in MN monocultures, suggesting a co-culture specific effect that is driven by mutant MNs but dependent on the presence of microglia. Interestingly C9orf72 mutant microglia CD11b-MAC-sorted from co-cultures with C9orf72 mutant MNs upregulated synapse and axon-associated pathways, indicative of enhanced binding or phagocytic uptake of neuronal material. We additionally identified complement activation as the pathway with strongest upregulation in C9orf72 mutant MN-mutant microglia co-cultures compared with matched healthy co-cultures. Specifically, C1QA and C1QB expression was significantly increased in mutant co-cultures, but virtually absent in MN monocultures, suggesting a co-culture dependent activation of the complement pathway. In summary, our data indicate the interplay of primary MN dysfunction and secondary non-cell-autonomous microglial toxicity may drive synaptic and axonal dysfunction in C9orf72-ALS, potentially mediated via the complement pathway.

**#47: Anastasiia Tourbier.** The importance of high-density microelectrode arrays for recording multi-scale extracellular potential and label-free characterization of network dynamics in iPSC-derived neurons

**Abstract:**

Advances in microelectrode array (MEA) technology for in-vitro electrophysiological recordings have made it possible to study neuronal networks across multiple scales, from subcellular properties to network-level dynamics. These devices are essential for exploring the phenotypes of neurological disorders and accelerating drug discovery, offering unique insights into the behaviour of neuronal networks. Key factors such as electrode density, spacing, and size significantly impact signal quality, noise, and sensitivity. To exhaustively characterize neuronal networks, MEAs must combine single-cell and subcellular resolution with high-throughput capabilities, maintaining sensitivity to small extracellular action potentials to capture the full range of network activity. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were utilized to record activity from induced pluripotent stem cell-derived neurons. These systems, with 26,400 electrodes per well, demonstrated the benefits of increased statistical power in longitudinal data collection. HD-MEA recordings were compared to simulated low-density recordings, where adjacent electrodes on HD-MEAs were clustered to mimic larger, lower-density electrodes. Additionally, the AxonTracking Assay, an automated tool for analysing individual axonal arborescences from multiple neurons simultaneously, was used to evaluate axonal structures and network functionality in the recorded cultures. Results showed that higher electrode density and smaller electrode size enhanced sensitivity, allowing for the detection of smaller spikes and capturing the complete spectrum of network dynamics. The high-resolution analysis of network activity, combined with subcellular insights from the AxonTracking Assay, offers a robust platform for drug screening and disease modelling.

**Funding Source:**

This work is funded by the HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.

**#48: Owen Gwydion James.** Investigating the role of Staufen as a modifier of ALS-associated pathology

Many genetic mutations in RNA binding proteins (RBPs) can cause ALS/FTD but clinical variability among patients suggests that secondary genetic factors play a role in modifying disease pathology. Staufen1 is a ubiquitous double-stranded RBP, Staufen2 is its neuron-specific orthologue; both regulate mRNA localization. Lowering Staufen1/2 levels ameliorates degenerative phenotypes in TDP-43 transgenic mice (Scoles D, et al. 2022), though the mechanism is unclear. To investigate the role of Staufen proteins as disease modifiers, we generated a Staufen1/2 double knock-out iPSC line and established two models of acute stress in cortical iNeurons that lead to ALS-associated phenotypes. Sodium arsenite-induced oxidative stress revealed Staufen knock-out formed stress granules more efficiently at lower concentrations of sodium arsenite. Furthermore, after a period of recovery, knockout cells had fewer granules, suggesting that Staufen impacts the kinetics of both stress granule assembly and disassembly. Secondly, we used proline-arginine (PR) dipeptide repeat treatment, which leads to TDP-43 mislocalisation and aggregation in iNeurons. To determine how Staufen knockout modifies mRNA localisation and transcriptional response to PR, we performed 3' RNA sequencing on fractionated iNeurons following PR treatment. Together, these analyses investigate the role of Staufen as a genetic modifier of ALS/FTD-associated pathology in iPSC-derived neurons.

---

**#49:** Magda Liczmanska. Investigating the role of Ser-Arg Protein Kinases (SRPK) in neurodegeneration using human stem cell-derived neurons.

Neurodegenerative disorders are characterised by loss of neuron function and neuronal death. They affect millions of people around the world with majority focusing on elder population. With increasingly ageing population neurodegenerative disorders are predicted to become even greater concern.

Protein phosphorylation has been strongly linked to neurodegenerative disorders, (Alonso et al, 1996). For example, increased phosphorylation of the microtubule binding protein Tau leads to reduced interaction with microtubules causing destabilization of cytoskeleton in neurons in Alzheimer's disease. (Vega et al, 2013).

Recently, dysregulated Ser-Arg Protein Kinase (SRPK) signalling has been linked to Alzheimer's disease. SRPK2 levels and activity have been reported in brains of AD patients (ref). In this context, SRPK2 reportedly phosphorylates Tau (Hong et al, 2012) and delta-secretase, a lysosomal asparagine endopeptidase (AEP) which results in autocatalytic cleavage, cytoplasmic translocation and increased enzymatic activity (Wang et al, 2017), thereby controlling the onset of Alzheimer's disease. Furthermore, dendric complexity in an Alzheimer's disease mouse model is rescued by SRPK2 depletion, linking SRPK2 activity with structural degeneration in neurons (Hong et al, 2012). Our research will focus on investigating the role of SRPK in neurodegeneration by identifying SRPK substrates in human iPSC-derived neurons. We have established a large-scale cortical neuron differentiation protocol, which combined with the selective SRPK inhibitor SRPKIN-1, will be employed for global phosphoproteomic analysis. Our aim is to identify key factors in human neurons that are regulated by SRPK phosphorylation, and which may be relevant in neurodegeneration. We will then investigate mechanisms of regulation, increasing our understanding of the role of SRPK in neurodegenerative disorders.

**#50:** Stefanie Fruhwürth. Investigation of microglia-amyloid beta interactions using an in vitro Alzheimer's disease model.

One in 10 people above the age of 65 have Alzheimer's disease (AD), the most common form of dementia. Abnormal amyloid beta ( $A\beta$ ) accumulation and deposition in the form of  $A\beta$  plaques is the critical step in the pathogenesis of the disease. The genetic risk for late onset AD, which accounts for 95% of all AD cases, is estimated to be 70% and largely associated with microglia, the innate immune cells of the brain. However, the precise role of microglia in AD pathogenesis remains elusive. Recent genetic and experimental evidence suggests that microglia are actively involved in the formation of  $A\beta$  plaques.

To specifically study AD pathology in a human in vitro system that is easy to manipulate, my team has established an in vitro AD model using hiPSC-derived microglia, cortical neurons, and astrocytes. By adding  $A\beta1-42$  oligomers to the cultures, we can induce and follow formation of  $A\beta$  plaques and closely mimic the chain of neurodegenerative events seen in AD patients. The different stages of  $A\beta$  plaque formation that we have identified are (i) seeding, (ii) compaction, and (iii) barrier formation. During the seeding phase, these plaques stain positive for  $A\beta$  while during the compaction phase they start to stain positive for the fluorescent dye AmyloGlo which is designed to only bind fibrillar (advanced) aggregates of  $A\beta$ . Importantly, in the absence of microglia or  $A\beta$  oligomers, no plaques are formed emphasizing that microglia are indeed required for plaque formation.

Altogether, our approach will uncover novel mechanistic insights of microglia- $A\beta$  plaque interactions.

---

**#51: Nathasia Mudiwa Muwanigwa. Dysregulation of RNA metabolism in an iPSC derived Neuronal Model of Tauopathy**

Tau protein interactions with ribonuclear proteins (RNPs) have emerged as key contributors to the pathogenesis of tauopathies. These interactions can lead to the sequestration or mislocalization of RNPs, disrupting critical processes such as RNA stability, splicing, and translation. Such disruptions compromise normal cellular functions, making neurons more vulnerable to dysfunction, which in turn accelerates neurodegeneration and disease progression.

This project aims to investigate how MAPT mutations, including S305N, P301S, and P301L, as well as different tau isoform ratios (4R vs. 3R tau), influence the mislocalization and aggregation of RNP granules, contributing to the molecular mechanisms underlying tauopathies. A particular focus will be on RNPs involved in stress granules and nuclear speckles, both of which are crucial for cellular stress responses and RNA processing.

Moreover, since RNP granules rely on microtubules for transport, pathological tau aggregation or phosphorylation may disrupt these interactions, further compromising synaptic health. Nuclear speckles, which regulate pre-mRNA splicing, are also likely to be affected by toxic tau, leading to aberrant splicing and increased neuronal stress. By utilizing iPSC-derived neurons and humanized tau mouse models, this project will explore how tau-driven disruptions in RNA processing contribute to tauopathies, potentially uncovering new therapeutic targets.

**#52: Victoria Lievens. Elucidating Lipid Droplets in Neuronal Models of Dementia**

Lipid homeostasis is essential for cellular function. Lipids regulate a wide range of biological processes and represent over 50% of the adult human brain's dry weight. A growing body of evidence places lipid dysregulation as a central theme in dementia and neurodegeneration, from genetic risk factors to altered lipid profiles. However, the mechanisms linking lipid metabolism to neuronal dysfunction remain unclear. We have developed advanced in-vitro neuronal and glial disease models of AD and PD and identified alterations in lipid metabolism.

Lipid Droplets (LDs) are cellular organelles that are critical for lipid homeostasis and recent evidence supports a potential pathological role for LDs in dementia and neurodegenerative disorders. In animal models of PD, accumulation of LDs precedes degeneration and reduction of LDs delays the onset of neurodegeneration. In AD, risk genes can impact LD homeostasis and LD accumulation preceded the formation of pathogenic protein inclusions. These findings suggest a possible new therapeutic opportunity for disease intervention targeting LDs. However, the functional significance of LDs in human neurons remains elusive.

We found that human dopamine and cortical neurons contain LDs that can be induced in response to stressors. We will present novel findings highlighting cell-type differences in LDs and describe our ongoing work characterizing the dynamics of LDs production and usage. Furthermore, we are exploring the functional interplay between LDs with other organelles in these cell models. We will show that mitochondrial dysregulation can regulate LDs and that LDs manipulation disrupts the endolysosomal pathway in neurons.

To explore underlying mechanisms, we have optimized CRISPR lentiviral-based tools for high-throughput genetic-screens in human cortical and dopamine neurons (~90% transduction efficiency) and are building-up CRISPR libraries to elucidate neuronal dependency on lipid homeostasis that could lead to the identification of novel targets for disease intervention.

---

**#53: Josefine Rågård Christiansen.** Development of a neuronal-glia tri-culture-based in vitro model of Alzheimer's disease for phenotypic drug screening

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and cause of dementia. It is characterized by severe memory loss and cognitive decline resulting from a progressive dysfunction and degeneration of specific neuronal populations including basal forebrain cholinergic neurons (BFCNs). This process is associated with pathological changes hallmark by the accumulation of aggregated proteins (amyloid- $\beta$  and hyperphosphorylated tau) and loss of synapses, as well as neuroinflammation involving activation of microglia, the primary immune cell of the brain. The etiology of AD remains unclear, and while treatment with acetylcholinesterase inhibitors can ameliorate symptoms temporarily by elevating the level of acetylcholine (ACh) from surviving neurons, there is a great need for development of disease-modifying therapies.

In this study, we are developing a human pluripotent stem cell (hPSC)-based in vitro model of AD which we will use for phenotypic screening of a library of small molecules aiming to identify compounds that can enhance neuronal functionality and protect against degeneration. To this end, we are generating and co-culturing BFCNs, astrocytes, and microglia from hiPSC lines carrying genetic variants associated with increased AD risk, specifically combining APP mutations (Swedish/London) with an APOE4 genotype. We have developed and optimized differentiation protocols to generate all the relevant cell types and maintain these in tri-cultures, and we have confirmed that APOE4/APPmut neurons show disease relevant pathological changes compared to isogenic APOE2/APPWT controls. Furthermore, we have developed a robust assay for high-throughput imaging-based quantification of ACh release from the in vitro cultures as a measure of neuronal functionality using "sniffer" cells expressing a genetically encoded fluorescent sensor. Characterization and validation of the model is currently ongoing in preparation for the drug screen; specifically, we are evaluating the degree of neuronal functional impairment in the model and investigating how aspects of cellular ageing and neuroinflammation may be incorporated.

**#54: Rachel O'Donoghue..** Exploring the influence of the protective Apolipoprotein E (APOE) variant, R251G, on the function of APOE- $\epsilon$ 4 iPSC-derived microglia

There are 3 common isoforms of Apolipoprotein E (APOE), the strongest genetic risk factor for Late Onset Alzheimer's Disease (LOAD). APOE- $\epsilon$ 4 increases risk of LOAD, APOE- $\epsilon$ 3 is neutral, and APOE- $\epsilon$ 2 confers protection. Due to their strong genetic contribution to LOAD pathogenesis, the mechanisms by which risk is conferred is a large focus in AD research. APOE is expressed in microglia and is increased particularly when they are activated or stressed. Microglia with an APOE- $\epsilon$ 4 genotype have been shown to have affected phagocytosis, lipid metabolism and cytokine profiles compared to APOE- $\epsilon$ 3. Despite increasing knowledge on microglial APOE biology relevant to the common isoforms, therapeutic targets surrounding their mechanisms are sparse.

The discovery of novel rare protective APOE variants, e.g. R251G, allow for the exploration of a new angle in APOE biology. The R251G mutation, co-inherited with APOE- $\epsilon$ 4, recovers APOE- $\epsilon$ 4-conferred risk to neutral/protective levels. How this mutation modifies the APOE- $\epsilon$ 4 isoform of the protein, and ultimately cellular function, to recover the APOE- $\epsilon$ 4-conferred genetic risk is currently unknown. The mechanism of protection of such rare variants must be understood to better inform how we can manipulate APOE for personalized treatments.

In this project, the R251G mutation has been investigated in iPSC-derived microglia. Using a CRISPR-Cas9 approach, R251G was knocked-into an APOE- $\epsilon$ 4 genotyped iPSC line within an APOE isogenic series. Preliminary data has shown increased pHrodo-low density lipoprotein uptake as well as amyloid uptake by APOE- $\epsilon$ 4 microglia, compared to AD-neutral APOE- $\epsilon$ 3. The R251G mutation recovered this increase. Further experimentation is underway to replicate this data and assess other phenotypes. We aim to understand how this may be a protective response in microglia, and the underlying pathways that are responsible for the recovery of detrimental APOE- $\epsilon$ 4 phenotypes. This will enable the identification of more specific therapeutic targets that can reverse the AD-risk conferred by APOE- $\epsilon$ 4.

---

**#55:** [Ahmad Jibai](#). Investigating Tripartite Synapse pathology in ALS utilizing a hiPSC-derived organoid model

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by motor neuron (MN) degeneration in the brain and spinal cord (van Eijk et al., 2019, *Frontiers in Neurology*). Early-stage synaptic changes and glial dysfunction are thought to contribute to MN degeneration. The involvement of tripartite synapses, consisting of presynaptic neurons, postsynaptic neurons, and astrocytes (a major type of glial cell), remains underexplored.

Recent work by our lab (Broadhead et al., 2022, *Acta Neuropathol*) identified selective vulnerability of tripartite synapses in ALS mouse model spinal cords and human post-mortem tissue. It is hypothesized that tripartite synapse pathology may represent a conserved hallmark of the early stages of ALS.

Our goal is to investigate the role of tripartite synapses in ALS using human iPSC-derived organoids. Using immunohistochemistry in cortical organoids we have validated synaptic and astrocytic labelling. Using high-resolution and super-resolution microscopy, and quantitative image analysis, we demonstrate bona fide synapse and tripartite synapse structures in cortical organoids and evidence of synaptic maturation between 60 and 120 days *in vitro*.

From analysis of human iPSC-derived cortical organoid harbouring C9ORF72 mutations (C9) and gene-corrected controls (C9 $\Delta$ ), we find no change in the number of synapses ( $p=0.5895$ ), and no change in the percentage of synapses contacted by the astrocytic marker, Ezrin ( $p=0.8791$ ), indicating no selective loss of tripartite synapses. However, we observed a significant difference in postsynaptic structure between C9 and C9 $\Delta$  lines ( $p=0.01846$ ), irrespective of whether the synapses were tripartite or non-tripartite.

Contrary to our hypothesis, our findings indicate no such selective tripartite synapse pathology, despite structural synaptic changes in ALS neurons. We are currently developing a protocol to grow spinal organoids from human iPSCs to investigate structural and functional changes in synapses and astrocytes in ALS.

**#56:** [Annika Wagener](#). Astrocyte-Neuron Crosstalk in Parkinson's disease

The preferential degeneration of dopamine (DA) neurons in the substantia nigra is a hallmark of Parkinson's disease (PD). However, growing evidence suggests that astrocytes contribute to PD pathology. DJ-1, a protein associated with familial early-onset but also sporadic forms of PD, is known to be a key regulator of mitochondrial function in DA neurons and is strongly up-regulated in astrocytes in PD. It has been hypothesized that neuron-protective factors released from astrocytes are dynamically regulated by DJ-1 expression, but it remains unclear which molecules are involved in this process.

This project aims to understand the role of astrocytic DJ-1 in the non cell-autonomous protection of neurons by mediating proteome and secretome changes, and how its loss may lead to degenerative changes of DA neurons in PD. We analysed secretome profiles of induced pluripotent stem cell (iPSC)-derived astrocytes from familial DJ-1 and sporadic PD patients versus healthy controls, and identified key candidates, which might be responsible for the potential of astrocytes to protect DA neurons PD-linked pathology. Furthermore, we are investigating the impact of altered DJ-1 in astrocyte-neuron co-culture systems.

Additionally, we are studying mechanisms of dopamine uptake and metabolism in astrocytes, which might aid DA neurons in the clearance of potentially toxic dopamine derivatives. Our data suggests these mechanisms to be substantially diminished in DJ-1 mutant astrocytes, indicating an impaired neuroprotective function.

As DJ-1 is not only relevant for the rare familial form of PD, but also the common sporadic form of PD, our project will be of broad significance to a large cohort of patients, as it will advance our understanding of whether restoration of DJ-1 might prevent midbrain DA neurodegeneration.

---

**#57: Mizuki Morisaki.** Understanding the role of Alzheimer's endocytic risks genes in disease pathogenesis

Endocytosis is an essential cellular process that mediates internalising, sorting and recycling the cellular materials. Dysfunction of the endocytic pathway has been reported in numerous neurodegenerative diseases including Alzheimer's disease where the hallmark pathology of early endosomal enlargement is seen even in the preclinical stages of disease. Furthermore, repeated genome-wide association studies have consistently demonstrated a cluster of risk genes that sit in the endocytic pathway including BIN1, PICALM, CD2AP and SORL1 amongst others.

Both genetic and histopathological data strongly indicate the importance of endocytic function in late onset Alzheimer's disease (LOAD). To capture the complex underlying genetics of LOAD and be able to better understand the role of endocytic dysfunction and its molecular mechanisms we have developed an endocytic pathway specific polygenic risk score (PRS). Using the endocytic PRS we have demonstrated stratification of LOAD patients and controls and identified individuals with very high endocytic PRS to develop novel iPSC lines from.

Many endocytic risk genes associated with Alzheimer's disease risk are highly expressed in microglia. We have therefore differentiated a subset of the novel high endocytic PRS iPSC to microglia to gain preliminary findings on endocytic function, phagocytic capacity and lipid storage.

**#58: Sonia Yiakoumi.** 3D Human iPSC Neurosphere Models Reveal Tau-Dependent Microtubule Dysregulation in Tauopathies

Neuronal development critically relies on the microtubule cytoskeleton, which plays essential roles in cell division, migration, and signal transduction (1). The microtubule-associated protein Tau (MAPT) is predominantly expressed in axons, where it stabilizes microtubules and promotes tubulin polymerization (2). Tau plays a central role in neurodegenerative diseases collectively known as tauopathies, including Alzheimer's disease and frontotemporal dementia (3).

Mutations in Tau have been shown to destabilize the microtubule network, impair axonal transport and neuronal migration (3). Adding to the complexity, the MAPT-AS1 and MAPT-IT1 long noncoding RNAs have been shown to be expressed in neurons and associated with Tau expression. However, the precise roles of these non-coding RNAs and mechanisms linking Tau mutations to altered microtubule dynamics and impaired neuronal development remain incompletely understood.

We have reported human iPSC-derived neurosphere models (4) to assay neural progenitor proliferation and active migration. To model Tau-related developmental defects in a human context, we generated MAPT knockout (MAPT-KO), and MAPT IT1 knockout (MAPT-IT1-KO) induced pluripotent stem cell (iPSC) lines. The iPSCs were differentiated into neuronal progenitor cells (NPCs) and matured as both 2D monolayer cortical neurons and 3D neurospheres. While 2D cultures maintained consistent NPC marker expression, both the MAPT-knockout neurospheres demonstrated impaired growth and aberrant morphology, suggesting that the 3D culture more effectively reveals MAPT-associated phenotypic defects. Live imaging of the neurospheres exhibited significantly reduced tyrosinated tubulin levels in MAPT-IT1-KO cells, indicating a reduction in dynamic microtubules. This finding was corroborated by the knock-out cells' increased resistance to Nocodazole, which selectively depolymerizes dynamic microtubules.

These results highlight the significance of using 3D neurosphere models in studying neuronal proliferation and migration. Preliminary data suggest that the absence of functional Tau may lead to microtubule over-stabilization and reduced dynamicity. Further investigations are necessary to validate these findings and explore their implications for understanding Tau-associated neurodegenerative diseases.

---

**#59:** Jamie Toombs. Multiplexed transcriptome profiling of small molecule perturbations in stem cell-derived neuron models.

**Background:** Cost-efficient screening of clinically relevant models is a major bottleneck in translational neurodegeneration research. Advances in iPSC, single cell RNA-seq, and lipid-modified oligonucleotide barcoding technologies offer an exciting opportunity to converge on a solution to this problem.

**Aims:** Here we establish a technical and computational pipeline to enable highly multiplexed screening of iPSC-derived neurons with small molecule perturbations and scRNA-seq profiling.

**Results:** To generate a reference perturbation dataset, iPSC-neurons from two non-neurodegenerative controls were differentiated for 45 days post-terminal differentiation. We describe the process by which these cultures were treated with 92x small molecules from the Tocriscreen 2.0 Micro panel (1x per well, alongside 4x control wells), lifted as whole single cells, labelled with MULTIseq oligonucleotide barcodes, and sequenced as a single sample with 10X Genomics technology. Analysis reveals the heterogeneity of surviving cell identities, which could be subcategorised to filter for cell populations of choice, and distinct gene perturbation profiles from de-multiplexed treatment conditions.

**Conclusion:** This study establishes a proof of concept for highly multiplexed drug screening of post-mitotic iPSC-neuron models. Although more work is required to develop reliable, highly pure cultures of specific neurons, the method demonstrates a cost-efficient, high resolution way to challenge and measure gene expression response in single, whole, human brain cells.

**#60:** Tatiana A. Giovannucci. Neurofilament light protein (NfL) as a dynamic biomarker: insights from stable isotope labelling kinetics

Neurofilament light protein (NfL) is a key biomarker of neurodegeneration, with cerebrospinal fluid (CSF) levels reflecting disease progression and treatment response. However, interpreting NfL dynamics requires understanding its synthesis, release, and clearance. We applied Stable Isotope Labelling Kinetics (SILK) to quantify NfL turnover *in vitro* and *in vivo*.

Using a <sup>13</sup>C6-leucine tracer, we labelled pluripotent stem cell-derived neurons ( $n=3$ ) and human participants with primary tauopathies ( $n=9$ ). Neuronal lysates and conditioned media were collected at three-day intervals during pulse-chase labelling, while CSF samples were obtained at five timepoints up to 120 days post-labelling. Targeted mass spectrometry measured labelled-to-unlabelled NfL ratios, providing estimates of fractional synthesis, clearance rates, and protein half-life.

We found that NfL translation occurs within hours in *ex vivo* human brain, but labelled NfL is first detectable in CSF ~53 days post-labelling, indicating rapid intracellular synthesis but slow extracellular release. This pattern was replicated *in vitro*, where intracellular NfL showed rapid turnover but delayed secretion. *In vivo*, NfL kinetics were slower than tau (Sato et al, 2018), while *in vitro* NfL half-lives ( $5.06 \pm 0.96$  [Ctrl1] and  $6.95 \pm 2.79$  days [Ctrl2]) were comparable to tau ( $6.74 \pm 0.45$  days).

Our findings suggest that intracellular NfL turnover dynamics can be inferred from extracellular compartments. Applying NfL-SILK to neurons from healthy controls and individuals with neurodegenerative mutations may provide critical insights into biomarker kinetics in health and disease, aiding interpretation of biomarker responses to disease-modifying therapies.

---

**#61: Pragati Thakur.** Role of micro-RNA dysregulation to study TDP-43 mislocalisation in Amyotrophic Lateral Sclerosis

**Background:** The dysregulation of gene expression by micro-RNAs(miR) has been an important area of study to understand amyotrophic lateral sclerosis (ALS) disease pathogenesis. The biogenesis of the miRNAs involves the TDP-43 protein which shows a nucleus to cytoplasm mislocalisation in ALS. Hence there is a need to identify specific miRNAs to target relevant pathways and find drug targets for potential therapeutics for ALS.

**Objectives:** We aim to overexpress a set of 24 miRNAs selected from a previous study to compare the effects on healthy versus mis localised neurons while corroborating our results through patient postmortem tissues samples. Further, we aim to characterize the function of these miRs in iPSC-Motor Neurons using transcriptomics.

**Methods:** The lab has devised a novel human iPSC-based model for on-demand endogenous TDP-43 mis-localisation without any overexpression or stressors. The model was then used to differentiate motor neurons to study the phenotypic effects of miR overexpression. MiR targets were validated in the TDP43 model and in patient-derived human postmortem tissue samples.

**Results:** The first round of experiments included overexpression of 12 miRs in control vs mislocalised cell lines which give us an insight into understanding the phenotypic changes such as neurite complexity and apoptosis. Excitingly, we were able to link activation of a cytokine in the form of an interferon response in TDP43 mislocalized neurons to downregulation of a key miR in our study. Further, we were also able to validate IFN targets in ALS tissue samples.

**Summary:** The use of microRNAs to understand the dysregulation of the TDP-43 machinery makes for an important area of research to target sporadic ALS. The need for understanding the immune signature of ALS makes our study an important step to identify clinical targets for drug development in treating TDP-43 proteinopathies.

**#62: Iris Kruijff.** Understanding the effect of LXR-treatments on Amyloid production in iPSC-derived neurons.

Changes in amyloid precursor protein (APP) processing towards an increased A $\beta$ 42/40 ratio is a major hallmark of Alzheimer's disease (AD). For sporadic AD, it is unknown what drives this increase, but cholesterol has been shown to regulate APP maturation via export from the endoplasmic reticulum (ER). Brain cholesterol metabolism is tightly regulated via the initiation or inhibition of cholesterol synthesis, storage, uptake, and export. The latter two processes are regulated by apolipoprotein E (APOE), a major cholesterol carrier whose levels are regulated by the LXR family of transcription factors. Oxysterols are endogenous regulators of ApoE expression and cholesterol export, and several oxysterols are dysregulated in the AD brain. Here we evaluate the effect of endogenous, and synthetic, LXR agonists on APP processing towards A $\beta$ . We differentiated iPSCs to neurons via NGN2 overexpression and treated neurons with oxysterols and other cholesterol targeting interventions (statins, LXR agonists, ABCA1 inhibitor) and determined their effect on of A $\beta$ 40, A $\beta$ 42 and the ratio of A $\beta$ 42/40. Oxysterol treatment led to an increased A $\beta$ 42/40 ratio, through an increase in A $\beta$ 42, and no change in A $\beta$ 40. Similarly, different synthetic LXR agonists increased the A $\beta$ 42/40 ratio. The increased ratio induced by LXR activation was independent of APOE and APOE genotype and regulated by the cholesterol exporter ABCA1. Our results indicate that oxysterols, via LXR activation, are able to alter APP processing, leading to an increase in the A $\beta$ 42/40 ratio. Altered oxysterol levels, as in the AD brain, might thereby directly affect the A $\beta$ 42/40 ratio and contribute to AD pathogenesis.

**#63: Lukas van den Heuvel.** The making of a Lewy Body: An ultrastructural comparison of aSyn pathology in post-mortem human brain and iPSC-derived human dopaminergic neurons seeded with pre-formed fibrils.

**Objectives:** Lewy Bodies (LBs), aggregates rich in the protein alpha-synuclein (aSyn), are a major pathological hallmark of Parkinson's disease (PD). Despite existing studies of LBs in human post-mortem brain, we are only beginning to understand the mechanisms of their formation and their contribution to neurodegeneration. Cellular models are critical in addressing these research gaps. Recently, iPSC-derived human dopaminergic neurons (iDAs) seeded with preformed aSyn fibrils (PFFs) were shown to develop Lewy body-like pathology, but no ultrastructural comparison of this iDA model with post-mortem PD brain tissue exists. Here, we aim to assess the translational relevance of PFF-seeded iDAs as a model for PD by studying the subcellular components in both modelled and bona-fide Lewy pathology.

**Methods:** Using room-temperature correlative light and electron microscopy (CLEM), we characterised the ultrastructure of various stages of LB pathology in midbrain dopaminergic neurons of two PD-diagnosed donors. Second, we used light-microscopy guided transmission and scanning electron microscopy to image the ultrastructure of aSyn-rich inclusions in the PFF-seeded iDA model.

**Results:** Both post-mortem and iDA inclusions demonstrate a large ultrastructural heterogeneity. While some modelled inclusions display ultrastructural features of pale bodies – loosely packed fibrils associated with mitochondria and structures of the lysosomal-autophagosomal pathway – others are rich in membranes and small vesicles. Strikingly, fifty-six days after seeding with PFFs, ring-shaped iDA inclusions consistently contained large multilamellar structures, which potentially result from repeated mitophagy. Last, three-dimensional ultrastructural reconstructions allowed a quantification of mitochondrial damage within and outside pathological inclusions.

**Conclusion:** Our results not only confirm the complex, heterogeneous nature of Lewy pathology in post-mortem brain, but they also demonstrate that a cellular culture of PFF-seeded iDAs offers an effective model for studying pathological heterogeneity in PD. These results contribute towards a subcellular understanding of the prion-like spreading mechanism of misfolded aSyn that is believed to underly progressive neurodegeneration.

**#64: Natalia Garcia Perez.** Live-cell label-free imaging of microtubules to assess neuronal development and degeneration

Microtubules (MTs) are an integral part of the cytoskeleton of neurons. They are highly dynamic, polarized structures involved in many cellular processes, including migration, intracellular transport and neurodevelopment. Furthermore, defects in microtubule dynamics have been associated with multiple neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Here, we employ second harmonic generation (SHG) microscopy to visualize microtubules in live iPSC-derived neuronal cultures in a label-free manner. SHG is a two-photon, non-linear optical process that enables non-invasive, label-free visualization of non-centrosymmetric structures, such as collagen, myosin and microtubules. We imaged the developing neuronal network at different points in time and found that while the neuronal network grows, expanding its neurites over time, the ability to generate SH signals decreases with time. This suggests that there are time-dependent changes in MT structure which we can quantify in a label-free manner with SHG imaging. Moreover, we developed an assay to locally destabilize MTs during SHG imaging by using a high-power laser pulse in a small area of a neurite or neurite bundle. We show that the local destabilization of MTs causes the SHG signal to disappear beyond the perturbation site and that drug-induced MT stabilization prevents the loss of SHG signals. On the other hand, stabilization of the actin cytoskeleton does not preserve the SHG signals after the perturbation suggesting that, despite close interaction between actin filaments and MTs in the neurites, the local destabilization of MTs cannot be prevented by stabilizing other parts of the neuronal cytoskeleton.

---

**#65:** Emma Knowling. The role of SFPQ liquid-liquid phase separation in amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterised by the degeneration of motor neurons in the motor cortex and spinal cord. While the precise mechanisms of ALS remain unknown, genetic analysis of ALS patients has revealed a strong association between mutations in genes encoding RNA-binding proteins and disease onset and progression. Many of these RNA-binding proteins contain Intrinsically Disordered Regions (IDRs) that facilitate an essential biological process called Liquid-Liquid Phase Separation (LLPS). Many ALS-associated mutations occur within these IDRs and drive aberrant LLPS. LLPS refers to the reversible and controlled formation of liquid-like biomolecular condensates via the self-organisation of biomolecules. These biomolecular condensates facilitate the intracellular spatiotemporal organisation of many processes, such as genome stability, DNA-damage response, and transcription. One RNA-binding protein implicated in ALS is Splicing Factor Proline- and Glutamine-rich (SFPQ), where the nuclear loss of SFPQ is reported as a molecular hallmark of ALS. SFPQ functions through LLPS, mediated by its C-terminal IDR. SFPQ facilitates various essential cellular functions, including paraspeckle formation, intron retention, and transcriptional regulation. However, the role of SFPQ phase separation in the context of ALS has yet to be explored. To investigate this, SFPQ mutants with different phase-separating abilities will be transfected into differentiated motor neurons and imaged using fluorescence microscopy. Additional disease-relevant assays will also be implemented. This work is particularly significant as phase separation underpins many biological functions, and aberrant phase separation is implicated in many neurodegenerative diseases.

**#66:** Esther Muñoz Pedrazo. Dissecting the genetic interactions controlling  $\alpha$ -Synuclein accumulation in Parkinson's Disease

Genetic risk constitutes complex interactions among numerous genomic loci to affect disease susceptibility. Given the simultaneous involvement of numerous genetic factors, and the difficulty to identify the relevant genes affected by genome-wide association studies (GWAS), it has been notoriously difficult to define the most critical pathways for further functional testing. In this project, we are using novel technology to define, in a high throughput and unbiased manner, which GWAS-associated genes cause risk in Parkinson's disease (PD) and how their functional and complex genetic interactions are shaped. The most recent GWAS of PD identified 90 risk variants across 78 genomic loci. Using a novel dual CRISPR inhibition/activation tool that we recently developed, we will up- and downregulate combinations of genes associated with these GWAS signals in human dopaminergic neurons, the preferentially affected cell type in PD. We will then use a combination of CROPseq and CITEseq to correlate these gene combinations in a high throughput manner to the levels of  $\alpha$ -Synuclein, that is a major hallmark of PD. I will focus on gene combinations that show a synergic effect in increasing or decreasing alpha-synuclein levels, and conduct molecular work that my host lab is proficient in to define the causative pathways. This project takes a conceptual new approach and brings functional insight into GWAS and complex human genetic interactions in a major neurodegenerative disease.

---

**#67: Ines Ferreira.** Driving experimental reproducibility and lot-to-lot biological consistency at scale in human iPSC-derived cells enabled by opti-ox technology

Due to a lack of standardised, easy-to-use and readily accessible human cell models, scientists often rely on animal models, primary cells, and/or cell lines that considerably differ from human biology and can be difficult to source at scale. Induced pluripotent stem cell (iPSC)-derived cells are an alternative to these, offering a scalable, human model for disease research.

Directed differentiation to generate the desired cell types from iPSCs through signalling with growth factors and small molecules involves lengthy, complex protocols that are challenging to reproduce, difficult to scale, and lead to heterogeneous populations. Moreover, despite the benefits of forward programming, several challenges remain associated with conventional vector-based methods of transgene expression impacting the efficiency, consistency and purity of the resulting cell populations, as the random integration of TFs can result in gene silencing.

The use of these models makes it difficult to generate consistent data from a scalable source of cells, with experimental variability often preventing scientists from being able to reproduce results over time or replicate other scientists' experiments.

Genomic safe harbour (GSH)-mediated optimised inducible overexpression (opti-ox) of cell type-specific TFs enables highly controlled, consistent and scalable manufacturing of human iPSC-derived cells, addressing these challenges. This technology has been used to deterministically cell program iPSCs into different cell types, including ioGlutamatergic Neurons, ioGABAergic Neurons, ioMotor Neurons, ioSensory Neurons, ioMicroglia, ioOligodendrocyte-like cells, and ioAstrocytes. The resulting cell types are highly defined and consist of homogeneous populations, confirmed by ICC and RT-qPCR. Moreover, whole transcriptome analysis reveals consistent expression profiles across manufactured lots, demonstrating consistency of the cells.

The availability of consistent lots, manufactured at scale, of human iPSC-derived cells has the potential to address the lack of experimental reproducibility seen across research, allowing scientists to accelerate their studies and enhance the reliability of their findings.

**#68: Ines Ferreira.** A versatile toolbox of human iPSC-derived microglia for disease modelling, CRISPR screens, and multicellular in vitro models for neurodegeneration drug discovery

Microglia, the resident macrophages of the brain, play critical roles in neural function by regulating neurogenesis, synaptic remodelling, and serving as first responders to infection. They are also highly implicated in the pathology of neurodegenerative diseases, including Alzheimer's disease (AD). To advance drug discovery efforts in complex diseases such as AD, scientists need a diverse toolkit for advanced research applications to model disease, generate gene knockouts and track cells in co-culture. Using opti-ox™, a deterministic cell programming technology, we have successfully generated human-induced pluripotent stem cell (iPSC)-derived microglia from both male and female genetic backgrounds in a consistent and scalable manner. These derived microglia express key markers, including CD45, P2RY12, CD11b, CD14, IBA1, and TREM2. Functionally, both male- and female-derived microglia exhibit robust phagocytic activity and secrete pro-inflammatory cytokines, however, background-specific responses are observed. To provide new models for investigating mechanisms involved in neurodegeneration, we engineered ioMicroglia in the male genetic background with specific AD-relevant mutations. These include point mutations in TREM2 (R47H) and APOE (C112R), the latter of which exhibits a phagocytic phenotype associated with AD. Developing CRISPR-compatible iPSC-derived cells has traditionally been a lengthy and complex process. To address this, we developed CRISPR-Ready ioMicroglia, which constitutively express Cas9, enabling high-throughput CRISPR screening and significantly reducing workflow duration from months to days. Proof-of-concept experiments validate the functionality of this system, demonstrating efficient single-gene knockouts and arrayed CRISPR screens. To support the development of complex multicellular neurobiology models, we created GFP ioMicroglia, which constitutively express GFP throughout the cytosol for live-cell imaging, antibody-free cell sorting, and cell tracking in co-cultures. These microglia were successfully co-cultured with ioGlutamatergic Neurons and evaluated using live-cell imaging assays. In summary, opti-ox-mediated deterministic programming enables the generation of iPSC-derived microglia from diverse genetic backgrounds and serves as a versatile platform for disease modelling and the development of advanced co-culture systems.

# SCMND 2025 Sponsors



The Company of  
Biologists

BRAIN | THE  
GUARANTORS

ALZHEIMER'S  
RESEARCH UK

FOR A  
CURE

maxwell  
BIOSYSTEMS

3 Brain

AXION  
BIOSYSTEMS  
Imagine • Explore • Discover

bioneer

StemTech

Thermo  
SCIENTIFIC

STEMCELL  
TECHNOLOGIES

Miltenyi Biotec

StemCultures

EBiSC

VectorBuilder  
Revolutionize Gene Delivery

SCALE  
biosciences

10X  
GENOMICS®

revvity

iotaSciences

FUJIFILM  
Value from Innovation



CONCEPT LIFE  
SCIENCES

bit.bio  
THE CELL CODING COMPANY

proteintech®

QIAGEN

tcs  
biosciences

Qkine

UK Dementia  
Research Institute

CURE-ND  
Catalysing a United Response in Europe  
to Neurodegenerative Diseases

DZNE lucidity NewBrain Institute UK Dementia Research Institute