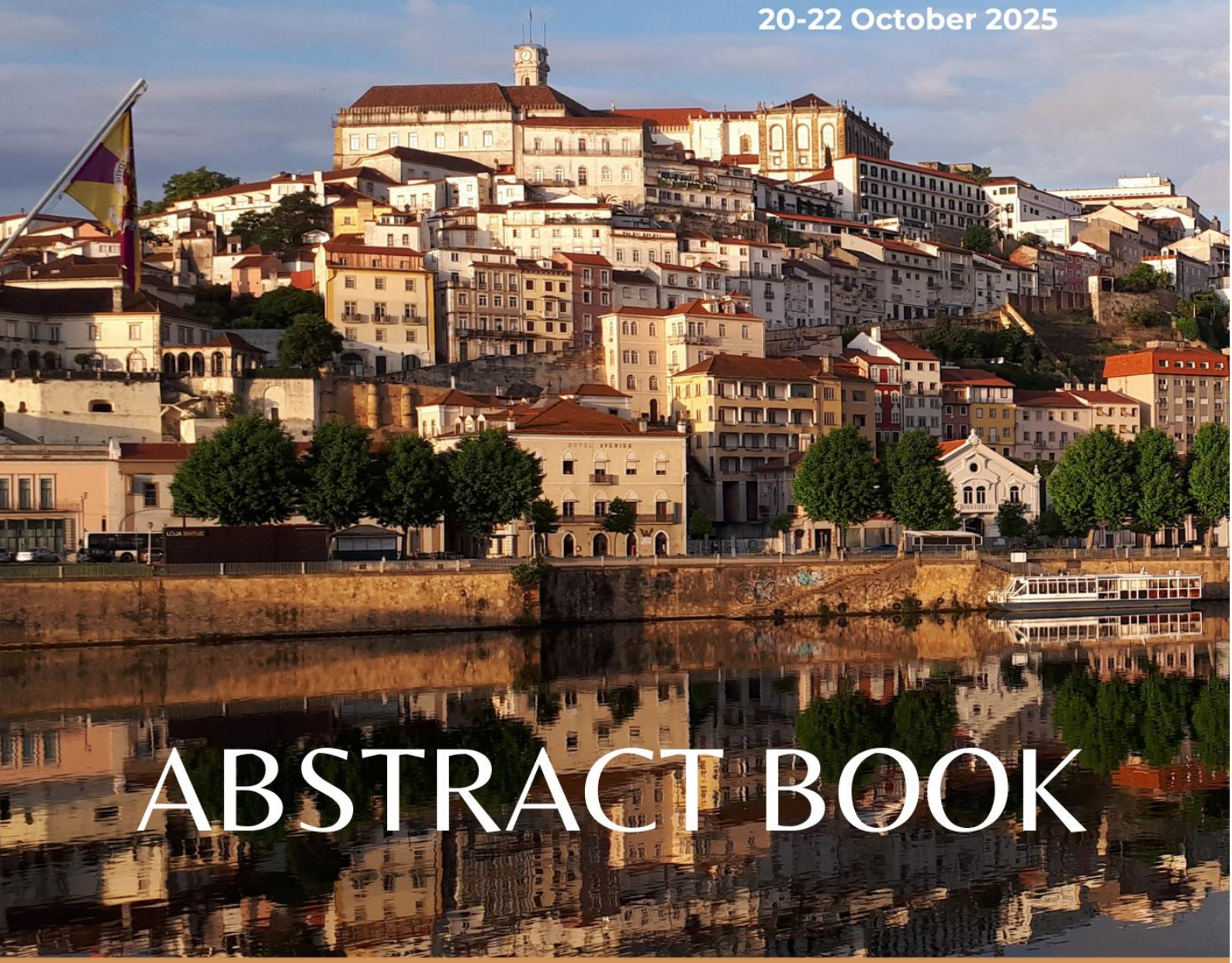
Coimbra
Portugal

NECTAR 2025

35th ANNUAL SYMPOSIUM OF THE NETWORK FOR EUROPEAN CNS TRANSPLANTATION AND RESTORATION

Colégio da Trindade | University of Coimbra | Portugal

20-22 October 2025



ABSTRACT BOOK

ORGANIZATION

LOCAL SUPPORT

GOLD SPONSORS

SILVER SPONSORS

BRONZE SPONSORS

INDEX

KNOW NECTAR2025 ABSTRACT BOOK

INDEX

SPONSORS 5

ORGANISATION & SUPPORT 14

SHORT PROGRAM 22

DETAILED PROGRAM 25

INVITED SPEAKERS 36

ABSTRACTS 40

E-POSTERS 110

SPONSORS

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Alzheimer's Research Charity

The Alzheimer's Research Charity (previously Campaign for Alzheimer's Research in Europe) was registered with the Charity Commission England approximately 25 years ago for the purpose of raising funds for scientific research into Alzheimer's disease (registration no. 1077279).

To date funds have been raised in the UK predominantly through i) bequests and ii) sponsorship from charitable trusts registered to support medical research. Smaller sums have been raised through fund raising events, but this is not a significant activity of the charity.

Over the course of its history the charity has funded laboratory-based research projects, travel grants and academic meetings always with a view to increasing knowledge of the degenerative processes underlying Alzheimer's disease and with an eye to therapeutic interventions.

This year, the charity has opened an International branch based in France to promote UK-European research collaborations. The Alzheimer's Research Charity (France) is registered as an Association for the purpose of supporting medical research into Alzheimer's disease and conforming to the Répertoire National des Associations (RNA) in compliance with French law according to 1 July 1901, RNA no. W242005127.

The Alzheimer's Research Charity is supported by Chairman, Mrs. Jaqueline Perry (King's Counsel) and a Board of Trustees including:

- Professor Konrad Beyreuther (beta-amyloid biochemstry, Heidelberg)
- Professor Stephen Dunnett (animal models, Cardiff)
- Professor Philippe Hantraye (design, testing and validation of drug, cell and gene therapy, MIRCen., Fontenay-aux-Roses, Paris)
- Professor Colin Masters (Alzheimer's pathology and biochemistry, Melbourne)
- Mrs. Patricia Naughton (extended family suffering from inherited Alzheimer's disease)
- Dr. Sarah-Jane Richards (previously molecular biology, Cambridge, retired HM Coroner)

CONTACTS

UK Office

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International Office

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SILVER SPONSORS

Cure Parkinson’s

EVERYTHING WE DO IS TO MOVE US CLOSER TO OUR GOAL, OF FINDING NEW TREATMENTS TO SLOW, STOP OR REVERSE THE PROGRESSION OF PARKINSON'S. YOUR DONATIONS AND YOUR INVOLVEMENT IN RESEARCH WILL LEAD TO THE BREAKTHROUGH WE ALL WANT TO SEE.

Curing Parkinson's needs world-class collaborative science involving researchers, clinicians, the pharmaceutical industry and, most importantly, people who are living with Parkinson's. This collaboration is at the heart of our research programme.

Our leadership and funding enables the world's leading neuroscientists and neurologists to prioritise, together, the next generation of drugs for clinical trial. We're acting with urgency, for people currently living with Parkinson's, with a focus on research which has potential to translate into the clinic within five years.

We've made significant progress towards our goal. As well as reshaping the approach to Parkinson's research, we've directly funded, or secured funding, for over £100 million of clinical trials searching for a cure for Parkinson's. But there's so much more that we need to do.

Cure Parkinson’s is the only organisation in the UK solely dedicated to finding a cure for Parkinson’s, one of the fastest growing neurological conditions in the world.

Cure Parkinson’s was set up in 2005 by four people living with Parkinson’s. Frustrated by the lack of progress in research or curative treatments, Tom Isaacs, Sir Richard Nichols, Air Vice Marshal Michael Dicken and Sir David Jones set up Cure Parkinson’s to focus on research projects with the potential to slow, stop or reverse the progression of Parkinson’s – to find a cure.

Since then, the charity has made significant progress in the quest for a cure. Cure Parkinson’s has directly funded millions of pounds of research, made scientific discoveries and opened new avenues of research.

As of March 2024, the charity has awarded funding for research in 38 institutions across 11 countries to 56 individual Principal Investigators.

CONTACTS

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 https://cureparkinsons.org.uk/

Sartorius

Sartorius is a leading international partner of life sciences research and biopharmaceutical manufacturing, known for its innovative technologies and consistent growth. Headquartered in Göttingen, Germany, the company operates through two main divisions: Bioprocess Solutions and Lab Products & Services. The Bioprocess Solutions division provides equipment and consumables for the production of biopharmaceuticals, including filtration systems, bioreactors, and purification technologies. The Lab Products & Services division supports laboratories with precision instruments and lab essentials. Sartorius has built a strong reputation for enabling efficient and scalable production of advanced therapies, including biologics and cell-based treatments.

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BlueRock

BlueRock Therapeutics is a clinical-stage biotechnology company pioneering regenerative medicine through cell therapy. Founded in 2016 and now a wholly owned subsidiary of Bayer AG, BlueRock focuses on developing therapies based on induced pluripotent stem cells (iPSCs) to restore function in patients with degenerative diseases. The company's lead program, bemdaneprocel, targets Parkinson's disease by replacing lost dopaminergic neurons. In 2025, BlueRock advanced to a pivotal Phase III trial for bemdaneprocel, marking a major milestone in its mission to transform treatment paradigms for neurological disorders

CONTACTS

CORPORATE HEADQUARTERS

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BRONZE SPONSORS

Cytiva

Cytiva is a global leader in life sciences, providing technologies and services that advance the development and manufacture of therapeutics. Cytiva supports researchers and biopharmaceutical companies with tools for drug discovery, development, and production. Its portfolio includes process chromatography systems, cell culture media, single-use technologies, and bioprocessing equipment. With over 15,000 employees worldwide, Cytiva plays a critical role in enabling the production of biologics, vaccines, and cell and gene therapies. The company is known for brands like ÄKTA, Amersham, HyClone, and Whatman, which are widely used in labs and manufacturing facilities across the globe.

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SciComPt

SciComPt Network is an association that was born out of an initiative by science communicators to serve the community working in the field of science communication. With members across the country, from the islands to the north and south, who are either fully dedicated to science communication or do it as a side activity, and including communicators from communication offices, science museums, journalism, etc., SciComPt represents a diverse, multidisciplinary and dynamic community.

Functioning as a true network, SciComPt aims to map and highlight the work of science communicators in Portugal, promoting partnerships, knowledge sharing and new opportunities that enable symbiotic growth among all its actors. We also aim to help shape the identity of science communicators in Portugal — while maintaining the uniqueness of each one — in order to enable the community, the professional field and science itself to flourish.

CONTACTS

Portuguese Network of Science and Technology Communication | SciComPt Network

Email : info@scicom.pt **Website**: https://www.scicom.pt/

ORGANISATION & SUPPORT

MEET NECTAR2025 ORGANIZERS & SUPPORTERS

ORGANIZERS

GeneT

GeneT is a flagship initiative by the University of Coimbra aimed at establishing a Center of Excellence (CoE) in gene therapy in Portugal's Centro region. Funded under the European Union's Horizon Europe program, the project seeks to create a robust research and innovation hub focused on gene therapy, particularly for rare and severe diseases. By leveraging the University of Coimbra's existing strengths in red biotechnology and translational research, GeneT aspires to become a national and international reference point for scientific and industrial excellence in the field.

The project integrates frontier research, advanced manufacturing, and clinical trial capabilities to accelerate the development of gene-based therapies. It will support investigator-initiated clinical studies, foster public-private partnerships, and provide infrastructure for the development and manufacturing of advanced therapeutics. The initiative is designed to enhance Portugal's competitiveness in biotechnology while addressing unmet medical needs through cutting-edge science. By uniting academic expertise, industrial collaboration, and regulatory engagement, GeneT aims to transform the landscape of therapeutic innovation and position the University of Coimbra as a leader in the global gene therapy ecosystem.

CONTACTS

Center for Neurociences and Cell Biology (CNC-UC)
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NECTAR

NECTAR (Network for European CNS Transplantation and Restoration) is a multidisciplinary scientific community dedicated to advancing research in central nervous system (CNS) repair and regeneration. Established in the early 1990s, NECTAR brings together neuroscientists, clinicians, and industry experts focused on developing and translating innovative therapies for neurological disorders. The network emphasizes collaborative research in areas such as cell transplantation, gene therapy, neuroprotection, and neuroinflammation, with the goal of restoring function in patients affected by conditions like Parkinson's disease, spinal cord injury, and stroke.

NECTAR hosts an annual symposium that serves as a key platform for sharing cutting-edge discoveries and fostering international collaboration. These meetings feature presentations from leading researchers, early-career scientists, and patient advocacy groups, creating a dynamic environment for scientific exchange and translational dialogue. By integrating basic science with clinical perspectives, NECTAR plays a pivotal role in shaping the future of neurorestorative medicine in Europe and beyond. Its commitment to innovation, education, and patient-centered research continues to drive progress in the field of CNS therapeutics.

CONTACTS

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CiBB

The Centre for Innovative Biomedicine and Biotechnology (CIBB) is a leading research unit of excellence at the University of Coimbra, dedicated to advancing biomedicine and biotechnology. Formed through the integration of the Center for Neuroscience and Cell Biology (CNC-UC) and the Coimbra Institute for Clinical and Biomedical Research (iCBR-FMUC), CIBB brings together over 700 members, including more than 320 PhD researchers. It serves as a multidisciplinary hub that connects the Faculties of Medicine, Pharmacy, Sciences and Technology, and Economics, as well as the Coimbra University Hospital and the Institute of Interdisciplinary Research. CIBB's mission is to foster cutting-edge research, promote advanced training, and translate scientific discoveries into clinical and industrial applications.

CIBB plays a central role in national and international biomedical innovation, hosting major initiatives like the GeneT project and MIA-Portugal. With state-of-the-art facilities and a strong focus on translational research, the center supports a wide range of programs in neuroscience, aging, gene therapy, and regenerative medicine. Its strategic partnerships with biotech and pharmaceutical industries, along with participation in European training networks, position CIBB as a key player in shaping the future of healthcare. Through its commitment to excellence, collaboration, and societal impact, CIBB continues to attract talent and funding, reinforcing its status as a flagship institution in Portugal's scientific landscape.

CONTACTS

Main Location (Polo I)

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University of Coimbra

The University of Coimbra is one of the oldest and most prestigious universities in Europe, founded in 1290 and located in the historic city of Coimbra, Portugal. Renowned for its rich academic tradition and cultural heritage, the university has played a pivotal role in shaping Portuguese education and intellectual life for centuries. Its iconic campus, a UNESCO World Heritage Site, features architectural landmarks such as the Joanina Library and the Royal Palace. The university offers a wide range of programs across disciplines including medicine, law, engineering, humanities, and sciences, attracting students and researchers from around the world.

Today, the University of Coimbra is a dynamic center for innovation, research, and international collaboration. It hosts several cutting-edge research units, such as the Centre for Innovative Biomedicine and Biotechnology (CIBB) and the Institute for Interdisciplinary Research, and leads major initiatives like the GeneT project in gene therapy. With a strong commitment to sustainability, inclusion, and academic excellence, the university continues to evolve while honoring its centuries-old legacy. Its vibrant student community and global partnerships make it a key contributor to scientific advancement and cultural exchange in Europe and beyond.

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LOCAL SUPPORTERS

Faculty of Law, University of Coimbra

The Faculty of Law at the University of Coimbra (Faculdade de Direito da Universidade de Coimbra – FDUC) is one of the oldest and most prestigious law schools in Europe, with a legacy dating back to the 13th century. Deeply rooted in the university's founding, FDUC has played a pivotal role in shaping legal education and jurisprudence in Portugal and across the Lusophone world. Its historic building, located in the heart of the university's UNESCO World Heritage campus, reflects centuries of academic tradition. The faculty offers undergraduate, master's, and doctoral programs in law, combining rigorous legal theory with a strong emphasis on civic responsibility and critical thinking.

FDUC is renowned for its influential alumni, including prominent jurists, politicians, and scholars who have shaped Portuguese legal and political systems. The faculty maintains a vibrant academic environment, hosting international conferences, legal clinics, and research centers focused on constitutional law, human rights, and comparative legal studies. With a commitment to both tradition and innovation, the Faculty of Law continues to be a cornerstone of legal scholarship and public service, fostering a new generation of legal professionals equipped to address contemporary global challenges.

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APAHE

APAHE (Associação Portuguesa de Ataxias Hereditárias) is a national non-profit organization founded in 2006 to support individuals affected by hereditary ataxias—rare, incurable, and degenerative genetic disorders. Recognized as a Private Institution of Social Solidarity (IPSS) since 2007, APAHE was created to fill a critical gap in Portuguese civil society by advocating for the rights and needs of patients and their caregivers. The association works to raise public awareness about the physical and psychological impact of these conditions, promote social inclusion, and foster community among affected families. Through educational campaigns, events, and partnerships, APAHE aims to improve quality of life and access to care for those living with hereditary ataxias.

In addition to advocacy, APAHE organizes member gatherings, fundraising initiatives, and collaborative research efforts to advance understanding and treatment of ataxias. The organization maintains strong ties with national and European networks, including the European Federation of Neurological Associations (EFNA), to amplify its impact and share best practices. APAHE's commitment to visibility and support ensures that patients are not isolated and that their voices are heard in healthcare and policy discussions. By building a compassionate and informed community, APAHE continues to make a meaningful difference in the lives of those affected by these challenging neurological conditions.

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Coimbra Municipality

Coimbra Municipality is a dynamic administrative and cultural hub in central Portugal, known for its historical significance, academic excellence, and strategic development initiatives. As the seat of the University of Coimbra—one of the oldest universities in Europe—the municipality plays a vital role in shaping the region's intellectual and civic life. Its governance focuses on sustainable urban planning, cultural preservation, and inclusive public services. In recent years, Coimbra has invested heavily in housing, healthcare, and education, with the 2025 municipal budget allocating €249.1 million to key sectors including social housing and accessible rental programs.

The municipality also champions innovation and international collaboration through projects like Coimbra CityLab and the European Campus of City-Universities (EC2U). These initiatives aim to position Coimbra as a smart, resilient city that integrates digital transformation with community engagement. The local government actively supports participatory budgeting, youth programs, and environmental sustainability, reflecting its commitment to transparent governance and citizen empowerment. With a blend of tradition and forward-thinking policies, Coimbra Municipality continues to evolve as a model for regional development and urban excellence.

CONTACTS

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SHORT PROGRAM

FOLLOW NECTAR2025 SESSIONS

Day 1 | 20 October 2025

12h00 | Registration

13h30 |Welcome

13h45 | Session 1. Clinical 1

15h15 | Session 2. Patients Advocacy

15h45 | Coffee Break

16h15 | Session 3. Disease Modelling 1

17h45 | Data Blitz 1

18h30 | Cocktail and Meet the speakers

Day 2 | 21 October 2025

8h30 Registration

8h45 Session 4. Clinical 2

10h30 Coffee Break

11h00 Data Blitz 2

11h45 Session 5. Disease Modelling 2

13h30 Lunch

14h30 Data Blitz 3

15h15 Session 6. Mechanistic

17h00 Special Session

17h15 Coffee Break

17h45 Data Blitz 4

18h30 NECTAR General Assembly

20h30 Social Dinner

Day 3 | 22 October 2025

8h30	Registration
8h45	Session 8. Aging
10h30	Coffee Break
11h00	Session 9. Pre-Clinical
12h45	Wrap up & Conference closure

DETAILED PROGRAM

EXPLORE NECTAR2025 SESSIONS

Day 1 | Mon, 20 Oct 2025

12:00 - 13:29 | **Registration** | *Atrium*

13:30 - 13:44 | Welcome | *Church*

Luís Pereira de Almeida | Coordinator of GeneT, CiBB, University of Coimbra

Romina Aron Badin | President of NECTAR

13:45 - 15:14 | Session 1 | Clinical 1 | *Church*

Chair of the session | Romina Aron Badin | MIRCen CEA, France

Clinical assessment for innovative therapies in Huntington's disease; new perspectives
Anne-Catherine Bachoud Levi

New avenues in the treatment of Frontotemporal Dementia
Isabel Santana

Bridging patient expectations and therapeutic advancements in Rare Neurological Diseases
João Durães

15:15 - 15:45 | Session 2 | Patients Advocacy | *Church*

Chair of the session | Luis Pereira de Almeida, GeneT, CNC, CIBB, Faculty of Pharmacy, University of Coimbra

Interactions of Patients & Researchers: APAHE & GeneT example
Magda Santana Célia Costa

15:45 - 16:14 | **Coffee Break** | *Cloisters*

16:15 - 17:44 | Session 3 | Disease Modelling 1 | *Church*

Chair of the session | Tilo Kunath, University of Edinburgh, UK

Exploring brain development and genomic instability at the single-cell Level in Huntington's Disease
Elena Cattaneo

Modeling and Treating Polyglutamine Diseases Using Viral Vectors
Rui Nobre

OC#1 | Single-cell analysis of the developing HD+ human fetal striatum reveals early molecular disruptions already present at 12 weeks
Oliver Bartley Sophie Precious Anne-Marie Mcgorrian Rachael Hills Mariah Lelos Anne Rosser

OC#2 | Immune-evasive strategies for allogeneic neural grafts in non-human primate models of Huntington's disease
Quentin FUCHS Apirahmee JEYAKUMARAN Donya El Akrouti Noëlle DUFOUR Audrey FAYARD Sophie LECOURTOIS Romina ARON BADIN Anselme PERRIER

17:45 - 18:30 | Data Blitz 1 | Church

Chair of the session | Alessandro Fiorenzano, University of Naples Federico II, Italy & Lund University, Sweden

DB#1 | Directly Reprogrammed Human Neurons and Astrocytes Reveal Age-Related Difference
Roland Zsoldos Anna A. Abbas Kinga Vörös Ali F. Dadawalla Chandramouli Muralidharan Julie Bouquety Idris Jimoh Ágnes Varga Balázs Kis Melinda Gazdik Zsófia Koltai Vivien Pillár Ármin Sőth Anikó Göblös Jenny J. Johansson Lajos Kemény Roger A. Barker Johan Jakobsson Attila Szűcs Karri Lamsa Mária Judit Molnár Janelle Drouin-Ouellet Karolina Pircs

DB#2 | Anti-inflammatory potential of helminth-derived peptides in vitro: a review
Sienna Stucke Prof. Eilis Dowd Aonghus Feeney

DB#3 | CD200-based cell sorting enables a safer, more reproducible cell therapy for HD
Cinta Gomis Marc Estarellas Francisco J Molina-Ruiz Georgina Bombau Josep M Canals

DB#4 | Choreic-like Movements in a Transgenic Rat Model of Huntington's Disease
Olivia Edwards Patricia Garcia Jareño Mariah Lelos Anne Rosser

DB#5 | In vitro microglial model of early neuroinflammation after CNS injury
Eszter Perhacs James B. Phillips Aminul Ahmed

DB#6 | Striatal differentiation of hypoimmunogenic non-human primate iPSCs for allogeneic cell therapy of Huntington's disease

Apirahmee Jeyakumaran Quentin Fuchs Donya El Akrouti Noëlle Dufour Romina Aron-Badin Anselme L Perrier

DB#7 | Mesenchymal stromal cells tackle mitochondrial and autophagy deficits in SCA3, supporting neuronal recovery

Inês Barros António Silva Dina Pereira Sónia P Duarte Sara M Lopes Rita Perfeito Diogo Tomé Ramiro Almeida Rui J Nobre Luís Pereira de Almeida Catarina O Miranda

18:30 - 21:00 | **Get together and meet the speakers | Cocktail** | *Cloisters*

Day 2 | Tue, 21 Oct 2025

08:30 - 08:44 | **Registration** | *Atrium*

08:45 - 10:29 | Session 4 | Clinical 2 | *Church*

Chair of the session | Nicole Déglon, Lausanne Univ. Hospital, Switzerland

Are we ready for gene therapy trials in spinocerebellar ataxia type 3 (SCA3)?
Thomas Klockgether

Future Cell Replacement - Safer Faster Delivery and Tuning Graft Function
Harry Bulstrode

Novel brain machine interfaces: Prostheses, optogenetic and sonogenetic therapies for visual restoration
Serge Picaud

OC#3 | Ascl1 and Ngn2 as Key Factors in Direct Glial Reprogramming in Spinocerebellar Ataxia 3
Margarida Pereira Inês Barros Sara Lopes Sónia Duarte Dina Pereira Rita Perfeito Rui Nobre Luís Pereira de Almeida Catarina Oliveira Miranda

10:30 - 10:59 | **Coffee Break** | *Cloisters*

11:00 - 11:44 | Data Blitz 2 | *Church*

Chair of the session | Catarina Miranda, GeneT, CiBB, University of Coimbra

DB#8 | Deciphering the Role of VLMCs in DA Graft Function Using Lineage-Restricted hPSC
María García Garrote Edoardo Sozzi Malin Åkerblom Petter Storm Malin Parmar

DB#9 | Functional integration of human striatal progenitor-grafts in a HD animal model
Marta Ribodino Linda Scaramuzza Christian Cassarino Marta Morrocchi Gabriela Berenice Gomez Gonzalez Roberta Parolisi Edoardo Sozzi Giacomo Turrini Valentina Cerrato Simone Diana Erika Gallo Paola Conforti Riccardo Tognato Greta Galeotti Chiara Cordiglieri Maria Cristina Crosti Martina Lorenzati Linda Ottoboni Malin Parmar Simone Maestri Dario Besusso Elena Cattaneo Annalisa Buffo

DB#10 | Molecular lineage tracing of stem cell derived dopamine grafts
Petter Storm Malin Åkerblom Yu Zhang Tomas Björklund Malin Parmar

DB#11 | Enhancing cell therapy for PD – Assessing clonal expansion and graft diversity
Jana Bonsberger

DB#12 | Chemogenetic Modulation of Pluripotent Stem Cell Therapy for Parkinson's disease
Athena Stamper Sahil Patel Elise Garcon Yujun Wang Dimitri Kullmann Roger Barker Harry Bulstrode

DB#13 | Extrinsic modulation of co-grafted neural progenitors enhances dopamine neuron specification and functional maturation
Edoardo Sozzi María García Garrote Janitha Mudannayake German Ramos Passarello Andreas Bruzelius Sara Corsi Greta Galeotti Mette Habekost Linda Scaramuzza Dario Besusso Anders Björklund Elena Cattaneo Alessandro Fiorenzano Petter Storm Malin Parmar

DB#14 | Unravelling the central breathing circuitry to study CNS-related breathing disorders
Kevin Law Walid Bahbah Rebecca Bricker Lachlan Thompson

11:45 - 13:29 | Session 5 | Disease Modelling 2 | *Church*

Chair of the session | Rui Nobre, GeneT, CiBB, University of Coimbra

The Gut–Immune- Brain Axis in Parkinson's: A Window for Disease Modification
Sandra Morais Cardoso

From Scar to Repair: Defining the Roadmap for Mammalian Spinal Cord Regeneration
Monica Sousa

Single-Cell Perspective of Neuroinflammation in Parkinson's Disease
Vania Broccoli

OC#4 | Forecasting fate and function: gene signatures of hPSC-derived dopamine cells
Haley Clark Kevin Michael Mark Ebel Lucas Harvey Corey Anderson Jim Xu Edmund Tam Alyssa Petko Elizabeth Pereira Dan Wilkinson Maya Srinivas Ryan Smith Carlos Paladini Stefan Irion

13:30 - 14:30 | **Lunch** | *Cloisters*

14:30 - 15:14 | Data Blitz 3 | *Church*

Chair of the session | Sónia Duarte, GeneT, CiBB, University of Coimbra

DB#15 | In vitro Parkinson's disease modelling with NPC-derived dopaminergic neurons
Yawei Liang

DB#16 | Assessment of cryogel microcarriers for improving the engraftment of iPSC-DAPs
Saoirse Ryan Giulia Comini Tommy Patton Sarah Crudden Niamh Moriarty Clare Parish Ben Newland Declan McKernan Eilís Dowd

DB#17 | A 3D model to characterize the maturation of an iPSC-derived mDA progenitor cell therapy
Sonia Bernal Haley Clark Melissa Jones Dan Wilkinson Mark Ebel Kevin Michael Nathanial Adams Lilia Carpenter Lucas Harvey Luendreo Barboza Deven LoSchiavo Jim Xu Maya Srinivas Ryan Smith Carlos Paladini

DB#18 | Strategies to enhance stem cell migration and differentiation upon cerebellar transplantation
Daniel Henriques Ricardo Moreira Frederico Pena Pedro Perdigão Agnete Kirkeby Clévio Nóbrega Luís Pereira de Almeida Liliana Mendonça

DB#20 | Generation of multi-regional neural spheroids to model brain circuitry in vitro and for multi-circuit cell replacement in vivo
Laura Ancellotti Serena Viventi Yu Kwan Mong Tien Jonathan D Teo Kevin Law Lachlan H. Thompson

DB#21 | Calcium dysreguation potentially drives vulnerability in PARK2 and PARK7-linked Parkinson's disease dopaminergic neurons
Francesco Gubinelli Andreas Jonas Huber Ferdinand Francesco Bela Graziotto Danish Anwer Arek Kendirli Franz Bauernschmitt Martin Kerschensteiner Lena F. Burbulla

15:15 - 16:59 | Session 6 | Mechanistic | *Church*

Chair of the session | TBD

Reshaping Neural Connectivity with Tunneling Nanotubes: Role in Development and Neurodegenerative Diseases
Chiara Zurzolo

Brain organoids uncover age-related neuronal vulnerability in Parkinson's disease
Michela Deleidi

The yin-and-yang of astrocytes in Parkinson's disease
REBECCA MATSAS

OC#5 | Development of a delivery strategy for neural microtissues in PD cell therapy
Nicolas Prudon Jérôme Hardouïn Lucia Cordero-Espinoza Caleb Anderson Marlène Martins William Tilmont Ines Januario-Neves Guillaume Dabée Blanche Tamarit Andrea Sovera Anaïs

Machado-Hitau Marie Lacaze Jens Schroeder Kevin Alessandri Erwan Bezard Emilie Faggiani Maxime Feyeux

17:00 - 17:14 | Session 7 | Ethics & Biomedical Law | *Church*

Chair of the session | Luís Pereira de Almeida, GeneT, CNC, CiBB, Faculty of Pharmacy, University of Coimbra

Gene editing - ethical challenges?
André Dias Pereira

17:15 - 17:45 | **Coffee Break** | *Atrium*

17:45 - 18:29 | Data Blitz 4 | *Church*

Chair of the session | Liliana Mendonça, GeneT, CiBB, University of Coimbra

DB#22 | Exploring glucocorticoid receptor function in dopaminergic neuron protection
Maria Roberta Iazzetta Andreas Bruzelius Edoardo Sozzi Daniela Di Girolamo Rosaria Di Martino Gennaro Andolfi Annalisa Fico Petter Storm Lucia Altucci Gabriella Minchiotti Malin Parmar Fiorenzano Alessandro

DB#23 | Exploring pathology in directly reprogrammed iDANs from PD patient cells
Kerstin Laurin Mette Habekost Janko Kajtez Tilo Kunath Roger A. Barker Malin Parmar

DB#24 | Neurorestoration by AAV-GDF5 ± GDNF in a rat model of Parkinson's
Fionnuala Wilson Martina Mazzocchi Louise Collins Gerard O'Keeffe Aideen Sullivan

DB#25 | The effect of a loaded collagen hydrogel on engraftment of RM3.5 iPSC-DAPs
Sarah Crudden Giulia Comini Tommy Patton Saoirse Ryan Niamh Moriarty Clare Parish Eilís Dowd

DB#26 | Evaluation of Novel Anti-Aggregation Compounds Against α-Synuclein
Tommy Patton Eilis Dowd Declan McKernan Andrew G. Cairns Jörgen Ådén Fredrik Almqvist

DB#27 | Oligodendrocyte maturation & myelination defects in PD due to oxidative stress
Jose Maria Salazar Campos Lisa Evangelista Sarah Jaekel Lena F. Burbulla

DB#28 | Strain-specific α-Synuclein effects on brain function and pathology in non-human primates
Fayard Audrey Fenyi Alexis Lavisse Sonia Barret Olivier Brammoulle Yann Lecourtois Sophie Jouy

Christophe Guillermier Martine Jan Caroline Gipchtein Pauline Petit-Fontyn Fanny Nardin Basha Dehay Benjamin Bezard Erwan Melki Ronald Hantraye Philippe Aron Badin Romina

18:30 - 19:30 | General Assembly | ***Church***

20:15 - 00:30 | **Social Dinner** | *Convento de São Francisco | Church*

Day 3 | Wed, 22 Oct 2025

08:30 - 08:44 | **Registration** | *Atrium*

08:45 - 10:29 | Session 8 | Aging | *Church*

Chair of the session | Tilo Kunath, University of Edinburgh, UK

The role of focused ultrasound in the treatment of neurodegenerative diseases: A double hit approach in Parkinson´s disease
Jose A. Obeso

Neuroendocrine based therapies in ageing and brain disorders
Cláudia Cavadas

Circadian dysfunction as a biomarker in cognitive aging
Luisa V. Lopes

OC#7 | First BPAN Microglia Model Shows Pro-Inflammatory Phenotype
Gamze Özata Dr. Rachel Wise Dr. Aida Cardona-Alberich Naiyareen Mayeen Prof. Dr. Lena Burbulla

10:30 - 11:00 | **Coffee Break** | *Cloisters*

11:00 - 12:44 | Session 9 | Pre-Clinical | *Church*

Chair of the session | Romina Aron Badin | MIRCen CEA, France

Novel approaches for treating Parkinson's disease
Merja Voutilainen

Harnessing genetic scissors to rewrite polyglutamine disorders
Nicole Déglon

Machado-Joseph disease: of mechanism and therapy
Luís Pereira de Almeida

OC#8 | Brain-targeted and pH-sensitive liposomes encapsulating small-molecule drugs to enhance differentiation of human iPSC-derived neuroepithelial stem cells into neurons

R Moreira D Henriques K Leandro J Panão-Costa JN Moreira C Nóbrega L Pereira de Almeida L Mendonça

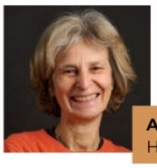
12:45 - 13:00 | **Closing** | *Church*

INVITED SPEAKERS

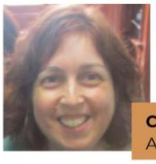
MEET NECTAR2025 SPEAKERS

INVITED SPEAKERS

André Dias Pereira
Center Biomed. Law, Univ. Coimbra | Portugal



Anne-Catherine Bachoud Levi
Henri Mondor hospital | France

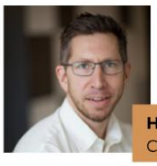


Célia Costa
APAHE | Portugal

Chiara Zurzolo
Institut Pasteur | France

Cláudia Cavadas
CIBB, Univ. Coimbra | Portugal

Elena Cattaneo
INGM, University of Milan | Italy



Harry Bulstrode
Cambridge Stem Cell Institute | UK

Isabel Santana
ULS Coimbra & Univ. of Coimbra | Portugal

João Durães
ULS Coimbra & Univ. Coimbra | Portugal

José Obeso
CINAC | Spain

Luís Pereira de Almeida
GeneT, CiBB, Univ. Coimbra | Portugal

Luísa V. Lopes
GIMM, Univ. Lisboa | Portugal

Magda Santana
GeneT, CNC, CiBB, Univ. of Coimbra | Portugal

Merja H. Voutilainen
University of Helsinki | Finland

Michela Deleidi
DZNE, Univ. of Tübingen | Germany

Mónica Sousa
i3s, Univ. of Porto | Portugal

Nicole Deglon
Lausanne Univ.Hospital | Switzerland

Rebecca Matsas
Hellenic Pasteur Institute | Greece

INVITED SPEAKERS

Rui Nobre
GeneT, CiBB, University of Coimbra | Portugal

Sandra Cardoso
CiBB, Univ. Coimbra| Portugal

Serge Picaud
Institut de la Vision, INSERM, CNRS, Paris | France

Thomas Klockgether
DZNE, University of Bonn

Vania Broccoli
CNR & IRCCSO San Raffaele | Italy

ABSTRACTS

READ NECTAR2025 ABSTRACTS

Day 1 | October 20th 2025

SESSION 1 | CLINICAL 1

[Invited Speaker]

Clinical assessment for innovative therapies in Huntington's disease; new perspectives

Anne-Catherine Bachoud Levi[1,2,3]

[1]Université Paris Est/ Assistance Publique-Hôpitaux de Paris, France
[2]Ecole Normale Supérieure-Paris Sciences et Lettres, France
[3]Institut national de la santé et de la recherche médicale, France

Accurate and sensitive clinical assessment is essential to evaluate the safety and efficacy of innovative therapies in Huntington's disease. Existing tools, originally designed for transplantation studies, rely on lengthy evaluations with limited sensitivity, heterogeneous impacts across domains, and a lack of standardisation, which hinders direct comparison between cognitive and motor functions. There is a pressing need to redefine core assessment protocols to meet modern therapeutic and methodological demands.

Future batteries should be short, simple, repeatable, reliable, and comprehensive, allowing long-term follow-up while limiting retest effects. They must provide multi-domain assessments with standardised procedures to enable cross-domain comparisons and disentangle motor from cognitive performance. Higher sensitivity is required to detect subtle changes over time in small cohorts.

Digitised tools, including SelfCog, DAT, speech analysis, and selfDiag, address many of these unmet needs. Through constrained randomisation, SelfCog limits retest effects by design, allows fair comparison between cognitive domains, and separates cognitive from motor performance. Digital tools offer objective, high-granularity assessments delivered rapidly and remotely, minimising examiner bias and improving data harmonisation across multicentre trials.

A future core assessment protocol for Huntington's disease should leverage these innovations while ensuring rigorous validation. The digital era provides unprecedented opportunities to reshape clinical assessment, supporting the development and evaluation of transformative therapies.

[Invited Speaker]

New avenues in the treatment of Frontotemporal Dementia

Isabel Santana[1]

[1]Center for Innovative Biomedicine and Biotechnology (CIBB-UC)

Frontotemporal Dementia (FTD) is a common cause of early-onset dementia accounting for 20% of cases, second only to Alzheimer's disease.

Pathologically, FTD is characterized by a rapidly progressive neurodegeneration, in particular affecting frontal and temporal cerebral cortex. From a genetic perspective, approximately 30-40% of FTD cases demonstrate familial clustering, with 10-20% showing definitive autosomal dominant inheritance. The genetic architecture is dominated by three major genes that collectively account for approximately 80% of familial cases: C9orf72 (hexanucleotide repeat expansions), the most common genetic cause worldwide, GRN (progranulin mutations) account for 15-25% and MAPT (microtubule-associated protein tau mutations) represent 10-15% of familial cases While no disease-modifying treatments is yet approved for FTD, innovative therapies for genetic subtypes are already targets for clinical trials.

Prioritizing GRN mutations which are dominant in Portugal, we have been involved in clinical trials with humanized monoclonal antibody targeting sortilin to block lysosomal progranulin degradation and thereby elevate both plasma and cerebrospinal fluid progranulin levels and gene-therapy progranulin deliveries with acceptable safety/tolerability.

These advances are expected to translate into effective targeted interventions to alter the course of this devastating illness.

[Invited Speaker]

Bridging patient expectations and therapeutic advancements in Rare Neurological Diseases

João Durães[1,2]

[1]ULS Coimbra, Centro Hospitalar e Universitário de Coimbra, Neurology
[2]Faculty of Medicine, University of Coimbra

Day 1 | October 20th 2025

SESSION 2 | PATIENTS ADVOCACY

[Invited Speaker]

Interactions of Patients & Researchers: APAHE & GeneT example

Magda Santana[1], Célia Costa[2]

[1]GeneT, CNC, CIBB, University of Coimbra
[2]APAHE

Day 1 | October 20th 2025

SESSION 3 | DISEASE MODELLING 1

[Invited Speaker]

Exploring brain development and genomic instability at the single-cell Level in Huntington's Disease

Elena Cattaneo[1]

[1]Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases, University of Milan Fondazione Istituto Nazionale Genetica Molecolare – INGM

[Invited Speaker]

Modeling and Treating Polyglutamine Diseases Using Viral Vectors

Rui Nobre[1]

[1]GeneT, CNC, CIBB, University of Coimbra

[Oral Presentation]

OC#1 | Single-cell analysis of the developing HD+ human fetal striatum reveals early molecular disruptions already present at 12 weeks

Oliver Bartley[1], Sophie Precious[1], Anne-Marie Mcgorrian[1], Rachael Hills[1], Mariah Lelos[1], Anne Rosser[1]

[1]Brain Repair Group, School of Biosciences, Cardiff University, UK

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by CAG expansion in the *HTT* gene (mutant HTT; mHTT). Pathological degeneration first occurs in the striatum with the progressive loss of medium spiny neurons (MSNs), preceded by dysfunctional genetic expression linked to the misfolding mHTT protein. While clinical symptoms typically emerge in adulthood, converging evidence collected from HD models suggests that the disease process begins much earlier, likely during early brain development. However, it is currently unknown at what point this mutation first results in MSN expression differences, particularly in the human context.

To address this, we performed single-cell RNA sequencing on developing striatal tissue (whole ganglionic eminence; WGE) collected from human HD-positive and control fetuses at 12 weeks post conception. This allowed us to generate a comprehensive atlas of early striatal development in a HD context and directly compare the developing striatum of HD-positive and control tissues.

Analysis revealed widespread transcriptional alterations across the developing HD striatum, with significant deviations in pathways related to protein ubiquitination, DNA repair, and telomere maintenance. These findings indicate early disruption of fundamental cellular processes essential for genome stability and proteostasis, linked to the expanded mutant HTT protein. Importantly, many differentially expressed genes overlapped with those previously identified in postnatal and adult HD brain, demonstrating that commonly disturbed molecular programs are already misregulated during the earliest stages of striatal development.

This study provides the first single-cell resolution profile of the HD-positive human fetal striatum and establishes that pathogenic mechanisms eventually associated with MSN degeneration are initiated prenatally. These insights open avenues for exploring early interventions that could modify disease trajectory.

[Oral Presentation]

OC#2 | Immune-evasive strategies for allogeneic neural grafts in non-human primate models of Huntington's disease

Quentin FUCHS[1,2], Apirahmee JEYAKUMARAN[1,2], Donya El Akrouti[1,2], Noëlle DUFOUR[3,2], Audrey FAYARD[1,2], Sophie LECOURTOIS[1,2], Romina ARON BADIN[1,2], Anselme PERRIER[2,1]

[1]Université Paris-Saclay, CEA, Molecular Imaging Research Center, 92265, Fontenay-aux-Roses, France
[2]Université Paris-Saclay, CEA, CNRS, Laboratoire des Maladies Neurodégénératives : mécanismes, thérapies, imagerie, 92265, Fontenay-aux-Roses, France
[3]Université Paris-Saclay, CEA, Molecular Imaging Research Center, 92265, Fontenay-aux-Roses, France.

Neural grafts derived from human pluripotent stem cells hold significant promise for treating neurodegenerative diseases. While patient-specific autologous immune pluripotent stem cells (iPSC) grafts avoid immune rejection, their clinical translation is limited by complex manufacturing and high costs. Although off-the-shelf allogeneic iPSC products are a scalable alternative, immune rejection remains a critical barrier as the prolonged use of immunosuppressive drugs poses significant risks. Our goal is to explore immune-evasive strategies for allogeneic neural grafts in non-human primate models of Huntington's disease. Because previously published data refute the immune-evasion capacity of the MHC-matched allogeneic striatal graft, we prioritized an "MHC cloak" approach.

First, we generated Macaca fascicularis-iPSCs, and inactivated *B2M* and *CIITA* genes using CRISPR-Cas9 ribonucleoprotein. These MHC-cloaked iPSCs retained their pluripotency and ability to differentiate into synaptically active neurons. Ex vivo mixed lymphocyte reactions with naïve macaque PBMCs revealed that double knockout iPSC-derived neuronal grafts lower T-cell mediated allogeneic responses.

Although MHC cloaking can disable the T-cell-mediated immune response, it is likely insufficient by itself because allogeneic grafts lacking self-MHC-I trigger a "missing-self" cytotoxic response by natural killer (NK) cells. To target this innate response, we are testing membrane-bound inhibitors of NK cells, as well as secreted immunoregulatory cytokines and chimeric proteins, to condition the local microenvironment of the graft to inhibit the missing-self response.

Our long-term goal is to validate in non-human primates an immune-evasive strategy relevant to allogeneic stem cell replacement therapies for Huntington's disease and potentially other neurodegenerative disorders.

Day 1 | October 20th 2025

DATA BLITZ 1

[Data Blitz & E-poster]

DB#1 | Directly Reprogrammed Human Neurons and Astrocytes Reveal Age-Related Difference

Roland Zsoldos[1,2], Anna A. Abbas[1], Kinga Vörös[1], Ali F. Dadawalla[1], Chandramouli Muralidharan[3], Julie Bouquety[4], Idris Jimoh[5], Ágnes Varga[1,2], Balázs Kis[1], Melinda Gazdik[1,2], Zsófia Koltai[1], Vivien Pillár[1,2], Ármin Sőth[1], Anikó Göblös[6], Jenny J. Johansson[3], Lajos Kemény[6], Roger A. Barker[7], Johan Jakobsson[3], Attila Szűcs[1], Karri Lamsa[8], Mária Judit Molnár[5], Janelle Drouin-Ouellet[4], Karolina Pircs[1,2,3]

[1]HCEMM-SU Neurobiology and Neurodegenerative Diseases Research Group, Institute of Translational Medicine, Semmelweis University, Budapest, HU
[2]HUN-REN-SZTAKI-SU Rejuvenation Research Group, Budapest, HU
[3]Laboratory of Molecular Neurogenetics, Lund University, Lund, SE
[4]Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada, CA
[5]Institute of Genomic Medicine and Rare Disorders, Faculty of Medicine, Semmelweis University, HU
[6]HCEMM-Szeged University, Szeged, HU
[7]Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
[8]HCEMM-USZ, Human Neuron Physiology and Therapy Core Group, Szeged, Hungary

Age is the greatest risk factor for neurodegenerative diseases (NDDs), yet the molecular links between physiological aging and NDD pathogenesis remain poorly understood. Autophagy—a lysosomal degradation pathway essential for maintaining cytoplasmic homeostasis—declines with age and contributes to both neuronal and glial dysfunction. To investigate how aging alters autophagic flux in the human brain, we use direct reprogramming of human dermal fibroblasts to generate induced astrocytes (iAs) and induced neurons (iNs), which retain donor-specific genetic and epigenetic signatures.

We aim to generate iAs and iNs from a total of 50 healthy donors, ranging in age from 24 to 86 years. Neuronal and astrocytic identity is verified by immunocytochemistry and high-content automated microscopy using cell-type- (TAU or ALDH1L1) and autophagy-specific markers (BECN1, LC3, p62, LAMP1), under both basal and stress-induced autophagy. Our first results using 5 young and 5 old donors indicated cell-type-specific age-related autophagy impairments, but via different mechanisms: while in iNs we found an impairement in autophagosome formation and/or turnover, in iAs autophagy flux was blocked likely at the lysosomal degradation step. These changes may contribute to age-related decline in brain homeostasis and increased vulnerability to neurodegeneration. We are currently validating these initial findings across the full donor cohort. In parallel, we are collecting samples for multi-omic autophagy profiling using a range of molecular assays—including RT-qPCR, genome-wide DNA-methylation arrays, bulk RNA-sequencing, mass-spectrometry, metabolomics, and functional readouts via patch-clamp electrophysiology —to compare young andold cells.

Finally, we are establishing co-culture systems with iNs and iAs to investigate autophagy dynamics in a more physiological neuron–glia environment, increasing the relevance and robustness of our model.

Our long-term goal is to identify key cell type–specific regulators of autophagy and to explore rejuvenation strategies. This approach could inform future therapies for NDDs, where impaired autophagy and accelerated aging are often observed.

[Data Blitz & E-poster]

DB#2 | Anti-inflammatory potential of helminth-derived peptides in vitro: a review

Sienna Stucke[1], Prof. Eilis Dowd[1], Aonghus Feeney[1]

[1]University of Galway

Helminths are parasitic worms that secrete a plethora of immune regulatory molecules which allow them to dampen inflammatory responses by their host's immune system to ensure their survival within the host. Their ability to have a compatible existence with their host has led to research into the potential therapeutic effects of helminth-derived molecules for suppression of overactive immune and inflammatory responses in a wide variety of diseases.

This systematic review aims to synthesize the published data on helminth-derived peptides/polypeptides (HDPs) with a focus on determining the extent to which they modulate the inflammatory response in in vitro cellular models of inflammation. In accordance with PRISMA 2020 guidelines, a predefined systematic search of the PubMed, Web of Science and Medline databases identified relevant studies published up to August 2025, and 79 articles were included after screening.

We found that most published studies used LPS or Concanavalin A stimulated macrophages, peripheral blood mononuclear cells or dendritic cells as the cellular model of inflammation. Twenty helminth species from which >60 isolated HDPs were derived were tested in these models, with the nematodes, Haemonchus contortus and Acanthocheilonema viteae, and the trematode, Fasciola hepatica, the most explored species. A common property of these molecules was to ability to significantly reduce the expression or production of pro-inflammatory cytokines such as IL-12, IL-1β, IL-6 and TNFα, and significantly increase the expression or production of anti-inflammatory cytokines such as IL-10, TGFβ and IL-4. The effects on other cytokines, including IFNγ which is known to have both pro- and anti-inflammatory effects, were less consistent, with HDPs either decreasing or increasing the levels of this cytokine.

This systematic review synthesizes the existing literature in this field and shows that the HDPs secreted by several helminth species have consistently demonstrated effects though modification of cytokine levels and, as such, have therapeutic potential in conditions in which overactive immune and inflammatory responses play a pathogenic role.

[Data Blitz & E-poster]

DB#3 | CD200-based cell sorting enables a safer, more reproducible cell therapy for HD

Cinta Gomis[1,2], Marc Estarellas[1,2], Francisco J Molina-Ruiz[1,2], Georgina Bombau[1,2], Josep M Canals[1,2]

[1]Universitat de Barcelona, Barcelona, Spain
[2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

Huntington's disease (HD) is a neurodegenerative disorder marked by the progressive loss of striatal projection neurons (SPNs), making it a compelling candidate for cell replacement therapy (CRT). Human pluripotent stem cell (hPSC)-derived neural progenitor cells (hNPCs) show strong therapeutic potential. Yet, clinical translation is hindered by the heterogeneity of cell populations generated through current hPSC differentiation protocols, compromising reproducibility and safety.

To address these challenges, we optimized Good Manufacturing Practice (GMP)-compliant cryopreservation protocols for hPSCs and their neural derivatives, including early neuroepithelial progenitors (hNEPs) and striatal-committed hNPCs. These protocols preserve cell viability and identity, enabling biobanking, quality control, and distribution. We also defined optimal seeding densities and attachment conditions, providing a reproducible framework to transition from research-grade to clinically applicable protocols.

Striving to increase graft reproducibility and safety, we implemented an immunomagnetic sorting strategy to enrich for post-mitotic neuroblasts (hNBs) within hNPC cultures. Using CD200 as a surface marker, we consistently isolated CD200high hNBs exhibiting SPN lineage commitment. Upon transplantation into the adult mouse striatum, CD200high hNBs retained striatal marker expression and continued to differentiate for at least one month. Notably, CD200-based selection reduced the number of proliferating cells and eliminated neural rosette-like structures within the grafts, thereby minimizing the risk of overgrowth and tumor formation.

Our work establishes a robust pipeline for translating hPSC-based CRT for HD, combining GMP-compliant cryopreservation with CD200-based cell sorting to meet key safety and regulatory benchmarks.

[Data Blitz & E-poster]

DB#4 | Choreic-like Movements in a Transgenic Rat Model of Huntington's Disease

Olivia Edwards[1], Patricia Garcia Jareño, Mariah Lelos, Anne Rosser

[1]Cardiff University

A range of genetic mouse models are available to study Huntington's disease (HD), although most have CAG repeats well in excess of that seen in adult HD and a relatively aggressive disease progression. The transgenic F344tgHD rat expresses a 51 CAG repeat fragment and exhibits a slow progressive phenotype that may be more typical of human adult-onset HD.

We sought to characterise motor and cognitive changes in the F344tgHD transgenic rat model, with a focus on understanding the biological basis of the choreic-like movements.

Wild type (WT), low mHTT expressing (mHTT ^{low}) and high mHTT expressing (mHTT ^{high}) rats were assessed for motor and cognitive deficits. Choreic-like movements were quantified longitudinally, with pharmacological modulation at 5 and 18 months of age. The 5-choice serial reaction time task (5-CSRTT), probing cognitive function, was undertaken at 12 months of age.

Choreic-like movements were observed in mHTT ^{high} rats as early as 2 months of age and persisted throughout the animal's lifetime. Pharmacological challenge indicated these to be dopamine-mediated-behaviours. In the 5-CSRTT, mHTT ^{high} rats performed significantly fewer total trials, were significantly less impulsive and omitted more responses compared to WT rats.

These data suggest that alterations of the dopamine system may be instrumental in the manifestation of choreic-like movements in the F344tgHD rats, and that F344tgHD rats also demonstrate motivational deficits. This data will contribute to a larger longitudinal analysis of F344tgHD rat behaviour that aims to provide a more slowly progressive HD model to test cell therapies and other therapeutic approaches.

[Data Blitz & E-poster]

DB#5 | In vitro microglial model of early neuroinflammation after CNS injury

Eszter Perhacs[1,2], James B. Phillips[1], Aminul Ahmed[2]

[1]University College London, UK
[2]King's College London, UK

Microglia are key mediators of acute inflammatory responses in the injured central nervous system (CNS), orchestrating both detrimental pro-inflammatory processes and reparative mechanisms that shape outcomes after injury. Early microglial activation is particularly critical in determining prognosis, as it drives secondary processes within the injured tissue that can either exacerbate damage or aid regeneration. Establishing *in vitro* models of early microglial activation is therefore particularly useful for advancing therapeutic strategies aimed at modulating neuroinflammation and promoting CNS repair. This study aimed to establish a simple and robust assay to investigate microglial activation which is relevant to the early stages of CNS injury.

BV2 microglial cells were cultured in 96-well plates and stimulated with pro-inflammatory factors - lipopolysaccharide (LPS) and/or interferon-gamma (IFN-γ) - for 24 hours. A range of outcome measures were assessed to evaluate microglial characteristics, including metabolic activity (PrestoBlue assay), expression of genes (RT-qPCR) associated with microglial activation, protein expression (immunocytochemistry), and nitric oxide production (Griess assay).

NOS2 gene expression, coding for inducible nitric oxide synthase (iNOS), demonstrated a pronounced ~30-fold increase following 24 hours of IFN-γ stimulation, while other outcome measures showed smaller, non-significant responses, highlighting iNOS as a robust and sensitive indicator of microglial activation in this assay. The optimised *in vitro* model provides a platform for investigating early neuroinflammatory processes in CNS injury and for evaluating candidate therapeutics, including stem-cell-derived factors and bioactive biomaterials.

[Data Blitz & E-poster]

DB#6 | Striatal differentiation of hypoimmunogenic non-human primate iPSCs for allogeneic cell therapy of Huntington's disease

Apirahmee Jeyakumaran[1,2], Quentin Fuchs[3,2], Donya El Akrouti[3,2], Noëlle Dufour[3,2], Romina Aron-Badin[3,2], Anselme L Perrier[2,3]

[1]Université Paris-Saclay, CEA, Molecular Imaging Research Center, 92265, Fontenay-aux-Roses, France
[2]Université Paris-Saclay, CEA, Laboratoire des Maladies Neurodégénératives : mécanismes, thérapies, imagerie, 92265, Fontenay-aux-Roses, France.
[3]Université Paris-Saclay, CEA, Molecular Imaging Research Center, 92265, Fontenay-aux-Roses, France.

Huntington's disease (HD) is a genetic neurodegenerative disorder characterised by progressive loss of medium spiny neurons (MSNs) in the striatum. Off-the-shelf allogeneic human PSC-derived cell therapy products for HD are scalable but are not fully tolerated by the host immune system in the absence of immunosuppression, and prolonged use of immunosuppressive drugs carries significant risks. Immune-evasive strategies for allogeneic grafts (e.g HLA-cloaking) are under investigation. However, such genetic modifications may impair the capacity of the donor line to differentiate into therapeutically relevant grafts. Here, we address this problem in vitro.

Macaca fascicularis iPSCs (Mac-iPSC) were produced and *B2M* and *CIITA* were inactivated using CRISPR–Cas9. These MHC-cloaked iPSCs expressed canonical pluripotency markers. We then assessed their neural differentiation and maturation capacity. Striatal progenitors were derived and cryopreserved from both wild-type (WT) and MHC-cloaked Mac-iPSCs. We compared the ability of each donor line to produce MSN precursors in 2D cultures (MSNp), mature into MSNs or astrocytes in 3D spheroids. We confirmed absence of MHC class I expression in all derivatives from the MHC-cloaked line and lack of MHC class II in purified astrocytes derived from those cloaked cells. To mitigate the “missing-self” NK response against MHC-cloaked derivatives, we evaluated expression of membrane-bound NK-inhibitory ligands. Transgene expression was assessed after lentiviral transduction of MSNp matured into striatal spheroids.

Our next goal is to compare, in vivo in nude rodents, the survival and maturation of single-cell MSN progenitor suspensions versus MSN spheroid suspensions after grafting, and to determine which format yields therapeutically relevant, MSN-containing grafts. We are currently conducting pilot experiments to test the ability of spheroids of different sizes to tolerate injection through needles of different gauges.

[Data Blitz & E-poster]

DB#7 | Mesenchymal stromal cells tackle mitochondrial and autophagy deficits in SCA3, supporting neuronal recovery

Inês Barros[1,2,3,4] , António Silva[1,2] , Dina Pereira[1,2,3,4] , Sónia P Duarte[1,2,3,4] , Sara M Lopes[1,2,3,4] , Rita Perfeito[1,2,3,4] , Diogo Tomé[1,3,5] , Ramiro Almeida[1,3,5] , Rui J Nobre[1,2,3,4,6] , Luís Pereira de Almeida[1,3,4,6,7] , Catarina O Miranda[1,2,3,4]

[1]CNC-UC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal;
[2]iiiUC - Institute for Interdisciplinary Research, University of Coimbra, Portugal;
[3]CiBB - Center for Innovative Biomedicine and Biotechnology, Coimbra, Portugal;
[4]GeneT - Center of Excelence of Gene Therapy, Portugal;
[5]iBiMED- Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal.
[6]ViraVector, Viral Vector for Gene Transfer Core Facility, University of Coimbra, Portugal.
[7]FFUC - Faculty of Pharmacy, University of Coimbra, Portugal.

Spinocerebellar ataxia type 3 (SCA3) is caused by an expanded CAG repeat in the MJD1/ATXN3 gene, producing mutant ataxin-3 that impairs cellular function and leads to neurodegeneration. We previously showed that repeated mesenchymal stem cell (MSC) treatments improved both phenotype and neuropathology in SCA3 mice. Moreover, in MSC-treated SCA3 mice, we observed a reduction in soluble ataxin-3 and a trend toward decreased protein aggregation in the cerebellum [1].

To understand the mechanisms behind this therapeutic effect, we performed *in vitro* co-cultures of MSCs with SCA3 neurons and *in vivo* MSC treatments in transgenic mice, examining pathways known to be impaired in the disease and potentially involved in ataxin-3 accumulation and aggregation.

MSC therapy corrected abnormalities in autophagosome maturation, mitochondrial turnover, and mitochondrial biogenesis in both models. These pathways may play a critical role in re-establishing protein recycling and indirectly contribute to reduced ataxin-3 levels. Furthermore, the MSC secretome prevented the presynaptic loss induced by mutant ataxin-3 in cortical neurons, as assessed by the number of synapsin and bassoon puncta per axonal length.

Finally, MSCs induced the phosphorilation of tyrosine kinase receptors (TRK-Rs) involved in key neuronal functions in an SCA3 cellular model, and normalized the levels of multiple proteins from a defined panel of neurological markers (Olink) in the cerebrospinal fluid (CSF) of SCA3 mice treated via intracerebroventricular MSC injection.

These findings highlight that MSCs exert neuroprotective effects in SCA3 through autophagy, mitophagy, and synaptic recovery. Understanding these mechanisms paves the way for RNA-based therapies to deliver MSC-derived mediators as potential treatments for SCA3.

[1] **Miranda CO*,** *et al*. **Molecular Therapy** 2018 Jul 12. pii: S1525-0016(18)30314-9. doi: 10.1016/j.ymthe.2018.07.007. Epub 2018 Jul 12

Day 2 | October 21st 2025

SESSION 4| CLINICAL 2

[Invited Speaker]

Are we ready for gene therapy trials in spinocerebellar ataxia type 3 (SCA3)?

Thomas Klockgether[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Spinocerebellar ataxia type 3 (SCA3) is the most common autosomal dominantly inherited adult-onset ataxia disease worldwide. SCA3 takes a progressive course and leads to increasing disability and premature death. It is caused by unstable expansions of polyglutamine encoding CAG repeats within the ATXN3 gene, resulting in the formation of abnormally elongated, misfolded ataxin-3 protein (ATXN3). Targeted therapies for SCA3 are being developed, and first safety trials of antisense oligonucleotides (ASOs) have been initiated. In the future, preventive intervention in mutation carriers before clinical onset will be a realistic option. With the advent of disease-modifying treatments for SCA3, there is the need to identify biological markers that are sensitive to disease-related change before and after clinical manifestation.

In the European Spinocerebellar ataxia type 3/Machado-Joseph disease Initiative (ESMI), we prospectively investigated a large cohort of SCA3 mutation carriers before and after onset. We determined the sequence and extent of plasma mutant ATXN3 and neurofilament light chain (NfL), as well as MRI measure changes along the disease course.

Mutant ATXN3 concentrations were constant throughout the entire disease course without major changes over time. NfL levels became abnormal in SCA3 mutation carriers more than 20 years before onset. The earliest MRI abnormality was volume loss of medulla oblongata occurring 4.7 years before onset. The responsiveness of markers depended on the disease stage. Across all stages, pons volume had the highest responsiveness exceeding that of clinical outcome assessments, such as the Scale for the Assessment and Rating of Ataxia (SARA). Lower age and lower medulla oblongata volume were predictors of SARA progression.

Knowledge of the progression of biological markers in these individuals can help researchers to design trials of interventions aimed at slowing clinical progression or delaying the onset of ataxia.

[Invited Speaker]

Future Cell Replacement - Safer Faster Delivery and Tuning Graft Function

Harry Bulstrode[1,2]

[1]Cambridge Stem Cell Institute
[2]Cambridge University Hospitals

Cell replacement has long promised a one-off therapy for motor Parkinson's, but important hurdles remain. Variable delivery, survival and engraftment compromised outcomes in historic trials, and may be contributing to equivocal early PET and clinical outcomes in ongoing commercial trials.

Conventional straight needle delivery systems developed for rodents cannot effectively deliver to human brain volumes. Multiple needle trajectories are required, achieving suboptimal target volume coverage, slow delivery, excessive reflux and high cumulative risks of blood vessel injury and stroke. To address these issues, we have devised and patented Spiramed Technology, enabling rapid local delivery of cells to large brain target volumes via a single needle trajectory. We have completed *in vitro,* human cadaveric, and *in vivo* large mammal testing of two generations of prototype. Compared to conventional delivery devices, Spiramed eliminates the risks associated with multiple needle passes through access cortex, allows delivery of greater therapy volumes more rapidly without reflux, and offer more consistent target volume coverage. Delivery through our system demonstrated no significant difference in cell survival compared to the cells that had only experienced thawed recovery. We anticipate reducing total operative time for bilateral cell delivery to 60-90 mins for bilateral surgery.

Even with the optimal surgery, we anticipate that some patients may not achieve optimal outcomes- either early or after years of disease progression- raising the question of 'rescue' strategies. Many dopaminergic cell lineages are oxygen-sensitive, and specific regulation of Hypoxia Inducible Factor (HIF) signalling is already possible using clinically-available agents with established safety profiles. Therefore we are exploring hypoxia phenotypes through physiological, genetic and pharmacological modulation of HIF signalling- in RC17-derived human mDA progenitors and their neuronal progeny equivalent to commercial cell products (STEMPD - Novo Nordisk). We are further applying the dopaminergic differentiation, calcium imaging and GRABDA dopamine reporter approaches developed for this work, as the basis for exploring Designer Receptor mediated regulation of graft function in CRISPR-engineered RC17 derivatives.

[Abstract submission]

Novel brain machine interfaces: Prostheses, optogenetic and sonogenetic therapies for visual restoration

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Visual restoration is certainly the greatest challenge for brain-machine interfaces with the high pixel number and high refreshing rate. After photoreceptors degeneration, the remaining retinal circuit can be reactivated with a photovoltaic prosthesis resulting in visual acuity close to 1/20.

We demonstrated the prosthesis efficacy in non-human primate prior to the clinical trials. As an alternative, we also demonstrated efficacy of optogenetic therapy with form vision and the ability to grab objects. Our studies on non-human primates allowed the selection of the microbial opsin and AAV prior to the successful clinical trial.

When patients have lost the eye to brain connection, we are developing sonogenetic therapy relying on ultrasound activation of cortical neuronal following a gene therapy to express a mechanosensitive ionic channel. A proof of concept was achieved in rodents prior to the first demonstration of efficacy in non-human primates.

These technologies offer great hopes for restoring vision in blind patients but also for controlling other neuronal circuits in the nervous system.

[Oral Presentation]

OC#3 | Ascl1 and Ngn2 as Key Factors in Direct Glial Reprogramming in Spinocerebellar Ataxia 3

Margarida Pereira[1,2,3,4], Inês Barros[1,2,3,4,5], Sara Lopes[1,2,3,4], Sónia Duarte[1,2,3,4], Dina Pereira[1,2,3,4], Rita Perfeito[1,2,3,4], Rui Nobre[1,2,3,4,6], Luís Pereira de Almeida[1,3,6,7], Catarina Oliveira Miranda[1,2,3,4]

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Spinocerebellar Ataxia 3 (SCA3) is an autosomal dominant neurodegenerative disorder caused by a CAG expansion in the ATXN3 gene, leading to a toxic polyglutamine protein that causes neuronal impairments and currently lacks effective treatments.

Direct reprogramming of glial cells into induced neurons (iNeurons) has shown promise in various neurodegenerative conditions, though its application in ataxias remains largely unexplored. This strategy may help restore neuronal populations and function, offering a potential therapeutic avenue.

We evaluated the reprogramming efficiency of two transcription factors (TFs), Neurogenin 2 (Ngn2) and Achaete-scute homolog 1 (Ascl1), delivered independently or in combination. Both can convert astrocytes into glutamatergic and/or GABAergic neurons. Astrocyte-specific targeting was achieved using lentiviral (LV) and AAV5 vectors with a Cre-Flex system (GFAP::Cre with FLEx-CAG::TF-P2A-mCherry), enabling lineage tracing via mCherry fluorescence.

In wild-type mice, stereotaxic LV injections into the striatum showed robust mCherry expression and increased NeuN immunoreactivity, confirming Cre-Flex system efficiency and neuronal induction. Increased Iba1 expression was observed only in TF-delivered conditions (but not in sham, GFP, or mCherry controls), suggesting a specific microglial response rather than general inflammation.

To assess therapeutic potential in symptomatic SCA3 transgenic mice (69Q, 4–6 weeks old), we injected AAV5 constructs into the cerebellar vermis. AAV presence was confirmed via ITR sequence analysis. Behavioral tests revealed improvements in gait parameters, though no significant changes were seen in rotarod or beam walking, possibly due to advanced disease stage at treatment.

These findings indicate that Ngn2 and Ascl1 effectively mediate glia-to-neuron reprogramming and may guide to therapeutic strategies in MJD. Future studies will explore deliver of RNA sequences and comparison with AAV-mediated delivery of the TFs.

Day 2 | October 21st 2025

DATA BLITZ 2

[Data Blitz & E-poster]

DB#8 | Deciphering the Role of VLMCs in DA Graft Function Using Lineage-Restricted hPSC

María García Garrote[1], Edoardo Sozzi[1], Malin Åkerblom[1], Petter Storm[1], Malin Parmar[1]

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Cell replacement therapy using human pluripotent stem cell (hPSC)-derived ventral midbrain dopaminergic (DA) progenitors is a promising disease-modifying strategy to restore striatal dopamine levels and improve motor function in Parkinson's disease (PD) patients. Although these progenitors are homogeneous at the time of transplantation, they give rise to DA grafts containing a heterogeneous mix of mature cell types, including DA neurons, astrocytes, and vascular leptomeningeal cells (VLMCs). The influence of non-neuronal cells on key parameters for graft functionality - DA neuron survival, maturation, and subtype specification - remains unclear.

Using the MACSima spatial proteomics platform, we identified extracellular matrix (ECM)-rich hPSC-derived VLMCs closely associated with both the host vasculature and therapeutic DA neurons in canonical intrastriatal DA grafts. Accordingly, recent barcode-based lineage tracing from our group revealed that DA neurons, astrocytes, and VLMCs share a common progenitor, with lineage commitment occurring at the final cell division. Thus, mature grafts devoid of VLMCs cannot be generated by simply sorting progenitors prior to transplantation.

To overcome this limitation, we engineered lineage-restricted hPSC lines using CRISPR/Cas9 to knock-in Cre recombinase at a VLMC-specific locus, enabling targeted ablation of VLMCs via Cre-dependent killing systems. We first validated that VLMC-specific Cre knock-in does not impair DA differentiation under our high-yield GMP-compatible protocol. Upon terminal differentiation, VLMC-Cre cultures successfully generated mature tyrosine hydroxylase-positive (TH^+) neurons *in vitro*.

This approach will allow us to determine whether VLMCs contribute to the survival, maturation and specification of the clinically relevant DA neurons. The resulting insights gained will pave the way for rational design of optimized graft composites, enhancing the efficacy of next-generation stem-cell based therapies for PD.

[Data Blitz & E-poster]

DB#9 | Functional integration of human striatal progenitor-grafts in a HD animal model

Marta Ribodino[1,2], Linda Scaramuzza[3,4], Christian Cassarino[3,4], Marta Morrocchi[3,4], Gabriela Berenice Gomez Gonzalez[1,2], Roberta Parolisi[1,2], Edoardo Sozzi[5], Giacomo Turrini[1,2], Valentina Cerrato[1,2], Simone Diana[1,2], Erika Gallo[1,2], Paola Conforti[3,4], Riccardo Tognato[6], Greta Galeotti[3,4], Chiara Cordiglieri[4], Maria Cristina Crosti[4], Martina Lorenzati[1,2], Linda Ottoboni[7], Malin Parmar[5], Simone Maestri[3,4], Dario Besusso[3,4], Elena Cattaneo[3,4], Annalisa Buffo[1,2]

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Human embryonic stem cell (hESC)-derived neuronal progenitors hold strong potential for treating several neurodegenerative pathologies. To be effective, cell products need to properly restore lost neuronal populations and to functionally integrate in the host tissue and reconstruct damaged circuits.

In this perspective, we examined the therapeutic potential of second generation human striatal progenitors (sg-hSP) derived from hESCs via a morphogen-guided protocol (Conforti et al, Cell Reports 2022) after transplantation in the quinolinic acid-induced HD rat model.

Long term single nuclei-RNA sequencing (snRNAseq) analyses revealed, with unprecedented resolution, that hSP-grafts generate a diverse population of striatal-relevant cell types, including medium spiny neurons, CGE- and MGE-derived interneurons, and regionalized astrocytes, recapitulating key aspects of ventral telencephalic development. Immunohistochemistry confirmed stable graft composition over time, supported a neurogenic-to-gliogenic switch post-transplantation, and identified a subset of graft-derived neurons and glia with migratory behavior within host tissue, in line with a remarkable potential of the grafted cells to integrate into the host environment.

Multimodal connectivity analyses, including virus-based tracing, *in vivo* calcium recording and *ex vivo* MEA recordings, demonstrated anatomical and functional integration of the grafts into host circuitries. Moreover, chemogenetic modulation of graft activity influenced locomotion and fine motor behaviors, including grooming, providing direct evidence of functional engagement with the host striatal circuitry.

Altogether, these findings support the ability of hSP-grafts to contribute to the functional reconstruction of host damaged circuits and offer new insights into the therapeutic potential of stem cell-derived cell replacement approaches.

[Data Blitz & E-poster]

DB#10 | Molecular lineage tracing of stem cell derived dopamine grafts

Petter Storm[1], Malin Åkerblom[1], Yu Zhang[1], Tomas Björklund[2], Malin Parmar[1]

[1]Developmental and Regenerative Neurobiology, Lund University
[2]Molecular Neuromodulation, Lund University

Human pluripotent stem cells (PSCs) differentiated into dopamine (DA) neurons provide an unlimited cell source for cell replacement therapy for Parkinson's disease (PD). However, histology and scRNA-seq profiling of resulting grafts in preclinical models have revealed that DA progenitors that are seemingly homogenous at the time of transplantation give rise to heterogenous grafts composed of different mature cell types.

We have used single nuclei RNA-seq combined with molecular barcodes to determine origin and shared lineage of the mature cell types forming in grafts of dopaminergic progenitors currently explored in cell therapy for Parkinson's Disease. Single-cell chromatin accessibility and RNA expression paired maps identified only subtle chromatin accessibility differences among progenitors supporting their homogeneity, and together with the tracing data shows that the PSC-derived floor plate cells used for transplantation are tri-potent, giving rise to dopamine neurons, astrocytes and VLMCs.

In parallel, we have also generated new barcode libraries designs for more efficient capture of the barcode transcript taking advantage of feature barcoding. This will allow for more detailed fate and state mapping in stem cell based regenerative approaches.

[Data Blitz & E-poster]

DB#11 | Enhancing cell therapy for PD – Assessing clonal expansion and graft diversity

Jana Bonsberger[1]

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Human pluripotent stem cell-based therapies are emerging as a promising treatment for Parkinson's disease (PD), with ongoing clinical trials demonstrating the feasibility of targeted regenerative approaches. However, a key challenge in cell replacement therapies for the central nervous system is the limited initial survival of transplanted cells - less than 10% survive and form the graft. This limited initial survival contributes to variability of therapeutic outcomes and complicates dosing strategies in clinical trials. Understanding which progenitor cells survive and mature into functional grafts is critical for advancing translational cell replacement therapies.

To address this, we have developed a molecular barcoding strategy to track the clonal expansion and survival of transplanted mesencephalic dopaminergic (mesDA) progenitor cells. Unique molecular barcodes were introduced via viral transduction into human embryonic stem cell-derived mesDA progenitor cells and subsequently transplanted into 6-hydroxydopamine (6-OHDA)-lesioned nude rats, a preclinical PD model.

Histological and single-nucleus RNA sequencing analyses confirmed efficient barcode integration and recovery. Barcode detection enabled quantification of surviving clones, and using the Chao1 estimator, we calculated that 4,850–9,000 barcoded cells from an initial 300,000 transplanted cells survived and formed the graft.

Our molecular barcoding approach enables a detailed analysis of early graft composition and clonal dynamics. These insights will support the development of strategies to achieve more consistent graft composition and improve initial cell viability. Ultimately, these advances could improve clinical trial outcomes by reducing variability and minimizing the required cell dose, bringing us closer to more reliable and effective cell replacement therapies for PD.

[Data Blitz & E-poster]

DB#12 | Chemogenetic Modulation of Pluripotent Stem Cell Therapy for Parkinson's disease

Athena Stamper[1], Sahil Patel[2], Elise Garcon[1], Yujun Wang[3], Dimitri Kullmann[2], Roger Barker[1], Harry Bulstrode[1]

[1]Cambridge Stem Cell Institute, University of Cambridge
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[3]Cambridge Stem Cell Institute, University of Cambridge.

Parkinsons disease is a chronic neurodegenerative disorder caused, in part, by the loss of dopaminergic neurons within the ventral midbrain (vmDA neurons). Cell replacement using pluripotent stem cell (PSC)-derived vmDA progenitors is already demonstrating promise in early-phase clinical trials. However, the ability to enhance the function of these grafts post-transplant could help further improve clinical outcomes.

The current project aims to explore whether the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) could be used to achieve this goal. DREADDs are engineered G protein–coupled receptors that allow ligand-dependent control of neuronal activity and neurotransmitter release.

Since commonly-studied DREADDs respond to clozapine-N-oxide, a ligand widely used in preclinical research but unsuitable for clinical use, we have instead investigated the application of a novel DREADD that instead responds to a more clinically appropriate ligand. Lentiviral delivery of this novel DREADD in PSC-derived vmDA neurons results in a ligand-dependent increase in neuronal activity, as evidenced by calcium imaging.

We are now exploring whether this, in turn, leads to increases in dopamine release using $GRAB_{DA}$ fluorescent reporters. While these initial and upcoming findings are informative, lentiviral delivery is limited in its suitability for clinical translation. Therefore, using CRISPR targeting, we are now also generating a stable PSC line with this DREADD integrated into the AAVS1 safe harbour locus.

Overall, it is hoped that this work will lay the foundation for future in vivo studies to determine whether changes in neuronal activity and dopamine release induced by the activation of this novel DREADD translate into improvements in motor outcomes. By doing so, such work will ultimately help to advance chemogenetic modulation of PSC-derived vmDA neurons towards clinical translation.

[Data Blitz & E-poster]

DB#13 | Extrinsic modulation of co-grafted neural progenitors enhances dopamine neuron specification and functional maturation

Edoardo Sozzi[1,2], María García Garrote[1], Janitha Mudannayake[1], German Ramos Passarello[1], Andreas Bruzelius[1], Sara Corsi[1], Greta Galeotti[3], Mette Habekost[1], Linda Scaramuzza[3], Dario Besusso[3], Anders Björklund[1], Elena Cattaneo[3], Alessandro Fiorenzano[1], Petter Storm[1], Malin Parmar[1]

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Parkinson's disease (PD), the second most common neurodegenerative disorder, is characterized by the progressive loss of A9 dopaminergic (DA) neurons in the *substantia nigra*, leading to dopamine depletion in the striatum and subsequent motor symptoms. Transplantation of ventral midbrain DA progenitors derived from human pluripotent stem cells, aiming to restore DA neurotransmission in the striatum, is currently being developed and explored in clinical trials. One factor that may influence the maturation and fate determination of DA neurons is the intercellular communication within the graft environment, ultimately affecting the therapeutic outcome.

In this study, we co-transplanted DA progenitors with either glial progenitors, interneuron progenitors derived from the ventral forebrain, or striatal progenitors into the striatum of a preclinical model of PD to assess their role in influencing the development, maturation and function of therapeutic DA neurons.

Our findings show that co-grafts with forebrain interneurons increase the yield of DA neurons and promotes their functional maturation. Furthermore, we demonstrated that co-grafts with striatal neurons promotes functional maturation as well as the acquisition of DA subtype identity. From this data, we identified *EBF3* and *PBX3* as candidate transcription factors that may direct DA neuron specification towards the A9 subtype.

Taken together, our data highlight that the cellular microenvironment, including specific interactions with surrounding cells, influences *in vivo* dopaminergic neuron specification and maturation. These findings can be used to develop more refined and effective cell preparations for replacement therapy in Parkinson's disease.

[Data Blitz & E-poster]

DB#14 | Unravelling the central breathing circuitry to study CNS-related breathing disorders

Kevin Law[1], Walid Bahbah[1], Rebecca Bricker[1], Lachlan Thompson[1,2]

[1]School of Medical Sciences, Faculty of Medicine and Health, University of Sydney
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The signal to breathe originates from the preBotzinger complex (preBotC), the core of the breathing centre in the brainstem. The preBotC have has an intrinsic central pattern generator activity to maintain a rhythm for autonomous breathing. Opioid overdose fatalities arise due to respiratory depression, seen in the US opioid crisis and growing outbreaks in Europe. Despite breathing's fundamental role, research on the central breathing circuitry has mostly relied on rodents and largely unexplored in humans.

To model human central breathing circuitry, we developed a regionally-specified hindbrain differentiation protocol using human pluripotent stem cells (hPSCs) to generate preBotC neurons in vitro, and in vivo via neural transplantation.

After neural induction of hPSCs, we finetuned the rostro-caudal and dorso-ventral patterning by titrating a series of small molecules and using reporter cell lines to specify respiratory progenitors and mature preBotC neurons. Function was assessed on multi-electrode arrays (MEA) with opioid treatment to model opioid overdose. For in vivo analysis, we performed intracerebral injections of our transplantable progenitors.

Differentiated hPSCs acquired HOXB4+HOXB5– rhombomere 7 hindbrain and DBX1+ V0 subplate identity, consistent with the developmental origin of preBotC. Mature MAP2+ neurons expressed markers appropriate for also preBotC, including HOXB4+ and NK1R+. Consistent with the intrinsic central pattern generator function of preBotC, neurons exhibited regular, spontaneous bursting activity on the MEA, which was disrupted by opioids. Transplanted preBotC progenitors produced HOXB4+NK1R+HuD+ neurons that survived and innervated ipsilateral and contralateral host brain structures, showing capacity to form a bilateral network, which is important for coordinating bilateral breathing movements.

We demonstrated hour PSC- derived preBotC neurons can model CNS breathing disorders like opioid overdose *in vitro* and, for the first time, be transplanted for *in vivo* disease modelling.

Day 2 | October 21st 2025

SESSION 5 | DISEASE MODELLING 2

[Invited Speaker]

The Gut–Immune- Brain Axis in Parkinson's: A Window for Disease Modification

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Emerging evidence supports the concept of a *gut-first* subtype of Parkinson's disease (PD), in which pathology may originate in the gastrointestinal tract during the prodromal phase, years before motor symptoms appear. Our group developed a mouse model of sporadic PD based on fecal microbiota transplantation from patients, which recapitulates several early and hallmark features of the disease, including intestinal inflammation, compromised gut barrier integrity, systemic immune activation, enteric and central α-synuclein aggregation, dopaminergic neuron loss, and motor deficits.

With the increasing interest in targeting early pathological events along the gut–immune–brain axis, we explored the therapeutic potential of bacterial extracellular vesicles (BEVs), small bioactive particles naturally secreted by commensal gut microbes. BEVs are known to modulate local and systemic immune responses and may represent promising tools for early-stage intervention in PD.

In this model, BEV supplementation prevented local immune cell infiltration and pro-inflammatory cytokine production in the ileum, preserved epithelial barrier function, reduced α-synuclein accumulation in the enteric nervous system, and dampened peripheral inflammation. These protective effects correlated with preserved nigrostriatal dopaminergic neurons and improved motor performance.

Overall, our results support the notion that targeting immune-mediated events at the gut level may interrupt the pathogenic cascade that leads to neurodegeneration. BEVs emerge as a promising strategy to modulate early disease mechanisms within the gut–immune–brain axis and potentially delay PD onset.

[Invited Speaker]

From Scar to Repair: Defining the Roadmap for Mammalian Spinal Cord Regeneration

Monica Sousa[1]

[1]i3S, University of Porto

It is widely documented that the adult mammalian central nervous system (CNS) lacks regenerative capacity. This is attributed to both the inhibitory environment of the scar and the downregulation of developmental growth programs.

Challenging this paradigm, our group recently demonstrated that the spiny mouse (*Acomys cahirinus*) is capable of spontaneous spinal cord repair following complete transection, achieving remarkable functional recovery. Our initial analyses at a chronic post-injury timepoint (8 weeks post-injury) revealed that *Acomys* forms a pro-regenerative extracellular matrix (ECM) at the lesion site, enriched in keratan sulfate proteoglycans (KSPGs). While this ECM signature is essential for repair, longitudinal bulk transcriptomic profiling of the injury site (from 1 day to 8 weeks post-injury) in *Acomys* and the non-regenerative laboratory mouse (*Mus musculus*) revealed that a critical switch in transcriptional trajectories occurs earlier. In *Acomys*, inflammation, ECM remodeling, and fibrotic pathways are initially activated similarly to *Mus,* peaking between 1 day and 1 week post-injury. However, these programs are subsequently downregulated from 1 to 3 weeks post-injury, coinciding with the upregulation of gene networks involved in axonal growth and synaptogenesis. By contrast, *Mus* maintains sustained activation of inflammatory and fibrotic pathways alongside persistent repression of neuroregenerative programs.

These findings suggest that in *Acomys*, an initial scar-forming response facilitates wound closure but is subsequently resolved to allow regeneration. To elucidate the cellular populations and molecular programs enabling this switch thereby promoting scar resolution and regenerative permissiveness, we generated a single-nucleus RNAseq/ATACseq atlas of the spinal cord injury site in *Acomys* and *Mus* at 3 days, 1 week, and 3 weeks post-injury. Our data identify specific microglial subpopulations as key mediators of spinal cord regeneration in *Acomys*.

These findings will be discussed as well as our ongoing efforts to engineer *Mus* models with *Acomys*-like spinal cord regenerative competence.

[Invited Speaker]

Single-Cell Perspective of Neuroinflammation in Parkinson's Disease

Vania Broccoli[1]

[1]San Raffaele Hospital/CNR-Institute of Neuroscience, Milan, Italy

The role of neuroinflammation in Parkinson's disease (PD) has attracted increasing interest in the last decade, as demonstrated by evidence of spread gliosis in post-mortem PD brains. However, the specific effect of glial cells accumulating α-synuclein (αSyn) in the neurodegenerative process remains unclear. While the role of αSyn aggregates in neurons has been extensively studied, their presence in glial cells as confirmed by PD brain autopsies, is yet to be fully explored.

We exploited an inducible-Cre/loxP viral system to selectively accumulate A53T-αSyn in microglial and astroglial cells and characterized these mouse models through multi-scale analysis. Our investigation included the comparative dynamics of the neurodegeneration and immune-cell activation coupled with anatomical and molecular characterization of brain glial cells.

Notably, A53T-αSyn overexpression in striatal astrocytes resulted in 31% loss of nigral-DA neurons, neuroinflammation and huge immune infiltration. Astrocyte changes were mirrored by marked molecular changes especially in immune-related genes. We mapped infiltrated immune cells and their molecular state during the mounting of neuroinflammation. Depletion of selective immune cell types provided evidence of their complex interplay and their specific effects on the progressive neuronal cell loss.

This research underscores the potential of targeting the immune cell infiltrate to develop therapies aimed at slowing neurodegeneration and preserving dopaminergic neurons in PD. Gene and cell approaches to leverage this new knowledge for establishing new strategies with direct therapeutic implications will be presented.

[Oral Presentation]

OC#4 | Forecasting fate and function: gene signatures of hPSC-derived dopamine cells

Haley Clark[1], Kevin Michael, Mark Ebel[1], Lucas Harvey[1], Corey Anderson, Jim Xu[1], Edmund Tam, Alyssa Petko[1], Elizabeth Pereira, Dan Wilkinson[1], Maya Srinivas[1], Ryan Smith[1], Carlos Paladini[1], Stefan Irion[1]

[1]BlueRock Therapeutics

Parkinson's Disease (PD), characterized by the degeneration of dopamine (DA) neurons in the substantia nigra and ventral tegmental area, is among the most common neurodegenerative diseases across the globe. Transplantation of human pluripotent stem cell (hPSC)-derived DA progenitor cells represents a promising treatment option for those afflicted with PD, with numerous advantages over currently available therapies.

Modern differentiation protocols aim to generate batches of midbrain floor plate progenitors that give rise to DA neurons following maturation, but these batches also include populations of other cells with varying regional specifications. Here, we aim to establish specificity and predictive validity across potential *in vitro* and *in vivo* readouts of cell fate and function by characterizing different research-grade DA progenitor cell products.

One on-target and two off-target cell batches identified by analysis of gene expression upon cell product thaw were further investigated in an extended *in vitro* study, which found that these batches also differ in terms of gene expression profiles, neural activity characteristics, and production of dopamine after maturation. When transplanted into the rodent CNS, the progenitor cell batches containing these off-target populations yield grafts with different compositions and sizes**.**

These results demonstrate that several *in vitro* and *in vivo* assays can discriminate between different batches of DA progenitor cells across time, with implications for potency, efficacy, and analytical strategy.

Day 2 | October 21st 2025

DATA BLITZ 3

[Data Blitz & E-poster]

DB#15 | In vitro Parkinson's disease modelling with NPC-derived dopaminergic neurons

Yawei Liang[1]

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Parkinson's disease (PD) has been widely modelled *in vitro* to enable mechanistic studies and therapeutic screening. Many existing PD models use induced pluripotent stem cells (iPSCs) differentiated into dopaminergic (DA) neurons, a process that often takes upwards of 40 days. While effective, this long differentiation period can limit experimental throughput and scalability.

This project aims to establish a less time-consuming and more efficient *in vitro* PD model using neural progenitor cells (NPCs), which can differentiate into DA neurons in around 2 weeks, followed by 6-hydroxydopamine (6-OHDA)-induced neurodegeneration. In addition to establishing the degeneration model, this project compared the differentiation efficiency of NPCs cultured in monolayer versus collagen gel matrices, quantifying the proportion of DA neurons generated under each condition.

The NPCs were cultured and differentiated into DA neurons under specific growth factor stimulation. The yield and identity of DA neurons were assessed by immunocytochemistry for tyrosine hydroxylase (TH) and other lineage markers. After differentiation, cells were treated with a range of 6-OHDA concentrations to evaluate dose-dependent neurotoxicity. Cell viability was subsequently measured to obtain the extent of neuronal loss, which was then used to establish an optimal degeneration model.

This provides a time-efficient and scalable alternative to traditional iPSC-based PD models. The NPCs-based model supports investigation of neuroprotective strategies and evaluating candidate therapeutics for Parkinson's disease in a controlled and reproducible setting.

[Data Blitz & E-poster]

DB#16 | Assessment of cryogel microcarriers for improving the engraftment of iPSC-DAPs

Saoirse Ryan[1], Giulia Comini[1], Tommy Patton[1], Sarah Crudden[1], Niamh Moriarty[2], Clare Parish[2], Ben Newland[3], Declan McKernan[1], Eilís Dowd[1]

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[2]The Florey Institute, The University of Melbourne
[3]School of Pharmacy and Pharmaceutical Neuroscience, Cardiff University

Cellular based brain repair is a potential therapeutic option to treat Parkinson's disease (PD) but engraftment remains limited with poor survival and differentiation of Induced Pluripotent Stem cells (iPSCs) *in situ* in the brain.

The aim of this research is to determine if PEGDA-SPA cryogel microcarriers loaded with the neurotrophic factors GDNF and BDNF, can aid engraftment of iPSC-derived dopaminergic progenitors (iPSC-DAPs). The microcarriers bind GDNF and BDNF, releasing them in a controlled manner to aid the growth of cells. The cytocompatibility of the microcarriers with iPSC-DAPs must be assessed.

Therefore an *in vivo* study was conducted with the iPSC-DAPs and the microcarriers without neurotrophic factors. Female cyclosporine-immunosuppressed Sprague Dawley rats received bilateral intrastriatal transplants. The treatment groups were Day 16 RM3.5 iPSC-DAPs alone or the iPSC-DAPs with unloaded microcarriers or the iPSC-DAPs with unloaded fluorescently labelled microcarriers. The rats were sacrificed by transcardial perfusion fixation at Day 4, Day 7 and Day 14 post transplantation (n=9 per day) and histological analysis was carried out.

Results from Human Nuclei staining which assesses cell survival show that there are no significant differences between the cells alone group and the microcarrier groups at any of the timepoints. STEM121 staining was carried out to assess cell engraftment and the results were similar to the Human Nuclei staining.

Therefore, the microcarriers are compatible with the iPSC-DAPs. Further long-term studies are needed to evaluate the potential of the neuroptrophin loaded microcarriers and whether they can aid with engraftment and differentiation of iPSC-DAPs.

[Data Blitz & E-poster]

DB#17 | A 3D model to characterize the maturation of an iPSC-derived mDA progenitor cell therapy

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Human pluripotent stem cells (hPSCs) can be leveraged to generate midbrain floorplate progenitor cell therapies to replace the dopaminergic (DA) neurons lost to Parkinson's Disease (PD). The efficacy of these therapies is dependent on the successful differentiation of the floorplate progenitor cells into adult DA neurons that acquire physiological maturity over several months in the *in vivo* environment.

To study this protracted transition from progenitors to neurons, it is important to have *in vitro* culture systems that support long-term neuronal maturation and mimic the *in vivo* environment. Conventional *in vitro* models have relied on monolayer (2D) cell culture systems, which do not accurately recapitulate the architectural complexity of the *in vivo* environment and can be confounded by cell detachment.

Here we describe a 3D cell culture model developed and leveraged to characterize the maturation of an hiPSC-derived midbrain dopaminergic progenitor cell therapy. Ventral midbrain DA (mDA) progenitors were cultured long-term in 2D and 3D *in vitro* environments in parallel and submitted for molecular (qPCR, flow cytometry, single nuclei RNA sequencing) and immunohistochemical assays at various timepoints across maturation to understand cell fate and function. To assess the relevance of the 3D modeling environment, results were compared to the immunohistochemical characterization of iPSC-mDA cells grafted into the 6-OHDA rodent model of PD.

These findings provide insights into the transition of midbrain floorplate progenitors to DA neurons in the context of a cell therapy for PD and demonstrate the potential of a 3D culture system for modeling the dynamics of iPSC-derived cell therapies upon transplantation.

[Data Blitz & E-poster]

DB#18 | Strategies to enhance stem cell migration and differentiation upon cerebellar transplantation

Daniel Henriques[1,2,3,4], Ricardo Moreira[1,2,3,5], Frederico Pena[1,2,3,4], Pedro Perdigão[1,2,3,6], Agnete Kirkeby[7], Clévio Nóbrega[8], Luís Pereira de Almeida[1,2,3,5], Liliana Mendonça[1,2,3,6]

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Stem cell transplantation is a promising approach for neurodegenerative diseases, but limited migration and differentiation hinder success. In this work, we tested small-molecule drugs to enhance hNESC migration and differentiation. Human induced pluripotent stem cells (iPSC)-derived cerebellar neuronal progenitors (CNPs) were also established as a more specialized cell source for cerebellar regeneration.

From the 8 drug candidates tested to enhance cell migration, 2 mM Ligustrazine treatment for 6 days led to enhanced cell migration by 66.89%. Six weeks after hNESC cerebellar transplantation, 75 mg/kg Ligustrazine-treated mice showed higher percentage of DCX-positive cells compared to controls.

From the 8 drug candidates tested to enhance hNESC differentiation into neurons, 50 nM Smoothened agonist (SAG) led to a 2-fold increase in neuronal markers MAP2 and β3-tubulin protein levels. Six weeks after hNESC cerebellar transplantation in NOD/SCID mice, 30 mg/Kg SAG-treated mice presented a 20-fold increase in NeuN-positive graft-derived neurons. Additionally, 10 nM Crenigacestat (CRE) increased MAP2 and β3-tubulin protein by 93.94% and 83.54%, respectively. NeuN-positive graft-derived neurons showed a significant rise from 35.66% in control mice, to 77.28% in 3 mg/kg CRE-treated mice. Thus, both SAG and CRE enhanced hNESC neuronal differentiation.

The patterning protocol to derive CNPs from iPSCs resulted in significant upregulation of CNPs markers including a 10,033.70-fold increase in *ZIC1* mRNA levels, and flow cytometry showed a population of 97.70% ATOH1-positive and 89.00% ZIC1-positive cells. Two months post-transplantation into mice cerebella, CNPs grafts showed 87.73% NeuN-positive neurons and higher ZIC1-positive cells compared with hNESC indicating improved cerebellar commitment.

These findings support Ligustrazine, SAG, and CRE for enhancing hNESC engraftment success, and CNPs as a promising cell source for cerebellar regeneration.

[Data Blitz & E-poster]

DB#20 | Generation of multi-regional neural spheroids to model brain circuitry in vitro and for multi-circuit cell replacement in vivo

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Current induced pluripotent stem cell (iPSC) derived neural cell replacement therapies generally rely on the transplantation of a single regionalised cell type. However, many neurological conditions affect multiple brain regions, so that replacing a single cell type may not address the requirements for functional circuit repair.

Recently, we have explored combining progenitors patterned with specific neural identity in 2D into multi-regional 3D spheroids, which we have termed “combinoids”. Using iPSC lines engineered to constitutively express fluorescent reporters and live cell imaging, we have shown that mixed preparations of striatal, ventral midbrain and cortical progenitor types robustly self-organise into 3D structures with a predictable spatial arrangement of regional cell types. We have also observed that the combination of progenitors drives maturation of the respective cell types.

Preliminary results show that these combinoids can also be transplanted and survive and integrate as multi-regional neural grafts in athymic mice. Spheroids containing cortical and striatal progenitors mature as neural grafts composed of cells that retain cortical and striatal identity, and notably including specific patterns of innervation of the host corresponding to these distinct neuronal populations.

In conclusion, we describe a multi-regionalised neural spheroid model as a promising transplantation avenue to enable the repair of multiple damaged areas with a single injection of a single combinoid into the affected brain region. This approach offers a new strategy for how cell replacement therapies could be used to treat neurodegenerative diseases.

Day 2 | October 21st 2025

SESSION 6| MECHANISTIC

[Abstract submission]

Reshaping Neural Connectivity with Tunneling Nanotubes: Role in Development and Neurodegenerative Diseases

Chiara Zurzolo[1]

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Tunneling nanotubes (TNTs) are actin-based cellular conduits enabling the transfer of vesicles, organelles, and proteins between cells. Initially discovered in cultured cells, TNT-like structures have now been identified in vivo, including in the developing mouse cerebellum, suggesting a physiological role in early brain connectivity and neuronal network formation.

In contrast, in the adult brain TNTs appear to be induced by stress and inflammation, explaining their involvement in neurodegenerative disease (ND) progression. We have shown that α-synuclein (α-Syn) and Tau aggregates—the hallmarks of Parkinson's and Alzheimer's diseases—spread between cells via TNTs. In Parkinson's models, α-Syn aggregates increase TNT formation and preferentially transfer from neurons to microglia, whereas microglia reciprocally deliver healthy mitochondria to stressed neurons, highlighting a neuroprotective crosstalk.

Using advanced live-cell imaging and co-cultures of human and iPSC-derived neurons and microglia, we found that α-Syn aggregates impair lysosomal and autophagy function specifically in neurons, driving directional aggregate transfer to microglia. Moreover, mitochondrial damage and activation of the cGAS–STING–NF-κB pathway promote TNT formation through pro-inflammatory signaling. Cytokine exposure further enhances TNT networks in both mono- and co-cultures of neurons, astrocytes, and microglia.

Altogether, our findings uncover a dual role of TNTs: physiological mediators of neuronal connectivity during development and pathological facilitators of aggregate and organelle transfer under inflammatory and neurodegenerative conditions.

[Invited Speaker]

Brain organoids uncover age-related neuronal vulnerability in Parkinson's diseas

Michela Deleidi[1]

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Neurodegenerative diseases, such as Parkinson's and Alzheimer's, are marked by the progressive loss of specific neuronal populations. Yet, why certain human neurons are particularly vulnerable remains largely unknown. Human brain organoids derived from induced pluripotent stem cells (iPSCs) provide a powerful platform to study these questions, offering access to disease-relevant human neurons and circuits in a controlled, physiologically relevant setting.

In this talk, I will present recent advances in generating midbrain organoids that closely mimic the regional identity and functional properties of dopaminergic neurons, the cells most affected in Parkinson's disease. I will show how novel differentiation strategies and dynamic bioreactor culture enhance neuronal maturation and subtype specification, enabling the study of mechanisms underlying selective neuronal vulnerability. These organoids can model disease-relevant pathologies, including α-synuclein aggregation and dopaminergic neuron degeneration, and allow detailed investigation of cellular responses at the single-cell level.

I will also discuss how these human organoid models can accelerate therapeutic discovery and support the development of cell replacement strategies. By bridging the gap between human biology and experimental modeling, this work offers new insights into why some neurons are more susceptible to disease and provides a scalable platform to explore interventions for neurodegenerative disorders.

[Invited Speaker]

The yin-and-yang of astrocytes in Parkinson's disease

REBECCA MATSAS[1]

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Alpha-Synuclein (αSyn) plays central role in Parkinson's disease (PD) and the p.A53T mutation causes an early-onset familial PD form with severe manifestations. While the effects on neurons are well studied, the consequences on astrocytes and the astrocytic contribution to PD pathology lag behind.

We differentiated patient-derived p.A53T-αSyn induced pluripotent stem cells (iPSC) to ventral midbrain astrocytes, and performed comprehensive molecular, functional, and proteomic analyses against gene-corrected and healthy controls. To assess the effects of p.A53T-αSyn astrocytes on dopamine neurons, we established neuron-astrocyte co-cultures of iPSC-derived control and mutant cells at all combinations.

Our analyses uncovered cell-intrinsic pathologies in p.A53T-αSyn astrocytes, such as calcium dyshomeostasis, and accumulation of protein aggregates. Proteomic and mechanistic studies demonstrated perturbed protein catabolic processes, with associated disturbances in lysosomal function and mTOR signaling. These deficits reduced the endocytic clearance capacity of p.A53T-αSyn astrocytes and their ability to process exogenous αSyn cargo. Fluorescence and electron microscopy showed that p.A53T-αSyn dopamine neurons co-cultured with p.A53T-αSyn astrocytes displayed Lewy-like pathologies, mirroring histopathological hallmarks identified in post-mortem PD brains, and exacerbated neurodegeneration, in anatomical and functional aspects. Control astrocytes mitigated these pathologies, highlighting their neuroprotective role. Additionally, p.A53T-αSyn astrocytes induced PD-relevant pathology in control neurons.

These findings demonstrate a critical impact of p.A53T-αSyn in disrupting astrocytic protein quality control mechanisms, and establish astrocytes as active contributors to PD neuropathology. Our two-dimensional co-culture model reflects key features of PD pathology, offering a robust platform for mechanistic and drug discovery studies.

[Oral Presentation]

OC#5 | Development of a delivery strategy for neural microtissues in PD cell therapy

Nicolas Prudon[1], Jérôme Hardouïn[1], Lucia Cordero-Espinoza[1], Caleb Anderson[1], Marlène Martins[1], William Tilmont[1], Ines Januario-Neves[1], Guillaume Dabée[1], Blanche Tamarit[1], Andrea Sovera[1], Anaïs Machado-Hitau[1], Marie Lacaze[1], Jens Schroeder[1], Kevin Alessandri[1], Erwan Bezard[2], Emilie Faggiani[1], Maxime Feyeux[1]

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With the rapid expansion of the field of 3D cultures and organoids, interest in their therapeutic use is growing. However, these innovative formats pose unique challenges for clinical translation as their physical and biological properties substantially differ from those of conventional single-cell-based products. Their handling imposes new constraints, such as much faster sedimentation, which must be addressed from the fill-and-finish stage to the final delivery procedure in the target region to ensure accurate dosing and precise cell placement.

In this study, we present the development of a strategy to maintain homogeneity throughout the entire downstream process for the delivery of 3D neural microtissues as a cell therapy for Parkinson's disease. This includes the development of a custom-made delivery solution. Various delivery methods were compared using in vitro tests.

The final selected strategy was validated through in-use testing and led to successful engraftment in a non-human primate, with the presence of dopaminergic (DA) neurons observed 1 month after transplantation.

The development approach described here holds potential for broader applications in other diseases and supports using next-generation cell therapies employing 3D formats.

Day 2 | October 21st 2025

SESSION 7 | ETHICS & BIOMEDICAL LAW

[Invited Speaker]

Gene editing - ethical challenges?

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Day 2 | October 21st 2025

DATA BLITZ 4

[Data Blitz & E-poster]

DB#22 | Exploring glucocorticoid receptor function in dopaminergic neuron protection

Maria Roberta Iazzetta[1,2], Andreas Bruzelius[3], Edoardo Sozzi[3], Daniela Di Girolamo[4], Rosaria Di Martino[5], Gennaro Andolfi[2], Annalisa Fico[2], Petter Storm[3], Lucia Altucci[1], Gabriella Minchiotti[2], Malin Parmar[3], Fiorenzano Alessandro[3,6]

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After extensive research efforts, human pluripotent stem cells (hPSCs) are now a well-established resource for generating dopaminergic neurons in both 2D and 3D cultures, with the goal of modeling related pathologies such as Parkinson’s disease (PD). Identifying the molecular mechanisms underlying human dopaminergic (DA) neurons differentiation is essential for advancing in vitro models that more faithfully recapitulate human brain development and neurodegenerative disorders not fully represented in rodent systems.

By using single-cell transcriptomics analysis, we identified NR3C1 as one of the most significantly enriched genes in functionally mature DA neurons. NR3C1 encodes for the glucocorticoid receptor (GR), a nuclear receptor protein that functions as a ligand-dependent transcription factor mediating different actions of glucocorticoids.

After employing hPSCs to generate DA neurons using both monolayer and brain organoid strategies, our findings suggest that GR plays a role in the acquisition of postmitotic molecular features during differentiation, and furthermore, that it could have a protective role during neurodegenerative phenomena. In vivo studies on rodent models of PD and epidemiological studies in PD patients have suggested that glucocorticoids may exert a neuroprotective effect.

However, the underlying mechanisms remain poorly understood, including whether these effects are cell-autonomous or mediated by non-cell-autonomous pathways. Human midbrain organoid-based PD models offer a valuable and more physiologically relevant platform to investigate this question, potentially yielding important insights for future therapeutic strategies.

[Data Blitz & E-poster]

DB#23 | Exploring pathology in directly reprogrammed iDANs from PD patient cells

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Current approaches to cell replacement therapy for Parkinson's disease (PD) focus on stem cell-derived dopamine progenitors for transplantation. However, future therapies might use patient cells that are directly reprogrammed into induced dopaminergic neurons, either *in vitro* for transplantation using dermal fibroblasts as starting cells, or directly *in vivo* reprogramming brain resident cells such as glia progenitor cells (GPCs). It is known that transplanted dopamine neurons from fetal tissue can acquire pathology in a PD patient over time and unpublished data from our lab shows that dopamine neurons derived from patient induced pluripotent stem cells (iPSCs) acquire pathology faster in pre-clinal PD models compared to those differentiated from human embryonic stem cells (hESCs).

In this study we want to investigate whether the same is true for reprogrammed induced dopamine neurons (iDANs). Over the past five years, we have developed both two- and three-dimensional (2D, 3D) cell culture protocols to directly reprogram human adult dermal fibroblasts (hDFs) and human GPCs *in vitro*. Transitioning into 3D cultures has improved post-transplantation survival and allowed for extended maintenance of reprogrammed neurons *in vitro.* In these previous studies we have shown that we can successfully reprogram hDFs from healthy controls and GPCs derived from hESCs. This study includes hDFs and iPSC derived GPCs from healthy controls as well as genetic and sporadic PD patients, enabling us to explore disease-specific pathology development.

By using our previously established protocols we will reprogram hDFs and GPCs to iDANs to assess dopamine identity and investigate whether reprogrammed patient cells are more susceptible to acquire pathology compared to those from healthy controls. We will also compare outcomes between hDFs, which retain environment and age-associated features, and iPSC derived GPCs, where these signatures have been reset. Understanding how patient-specific disease backgrounds influence reprogramming and vulnerability to pathology is critical for advancing patient-specific cell replacement therapies using direct reprogramming.

[Data Blitz & E-poster]

DB#24 | Neurorestoration by AAV-GDF5 ± GDNF in a rat model of Parkinson's

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Parkinson's disease (PD) is characterised by midbrain dopaminergic neuron degeneration associated with a-synuclein (aSyn) accumulation. Adeno-associated viral vector (AAV)-mediated delivery to the brain of neurotrophic factors (NTFs), including GDNF, has disease-modifying potential in PD. However, clinical trials to date have failed to meet their primary endpoints. One hypothesised reason for this is that aSyn downregulates GDNF's receptor, RET. Growth/differentiation factor 5 (GDF5) is an NTF that operates through Bone Morphogenic Protein receptors (BMPRs), and has neuroprotective effects in *in vivo* PD models.

This study investigated effects of delayed administration of GDF5, and of combined GDF5 and GDNF therapy, in the AAV-aSyn rat model of PD. Following baseline behavioural testing, Sprague-Dawley rats received unilateral intranigral injection of AAV-aSyn, followed after 13 weeks by unilateral intranigral injection of either AAV-Null, AAV-GDF5, or AAV-GDF5 + AAV-GDNF. Behavioural testing was carried out at weeks 12, 16, 20 and 24. Treatment with either AAV-GDF5 or combination of AAV-GDF5 + AAV-GDNF partially rescued motor impairments associated with AAV-aSyn. Animals were perfused after 25 weeks for immunohistochemistry, which showed that both AAV-GDF5 and AAV-GDF5 + AAV-GDNF rescued nigral dopaminergic neurons and striatal terminals from AAV-aSyn-induced degeneration. These data show that intranigral administration of AAV-GDF5, or combination of AAV-GDF5 and AAV-GDNF, has neurorestorative effects in the AAV-aSyn PD model.

We also examined the expression of NTF receptors in paraffin-embedded sections of substantia nigra from PD patients and age-matched controls. RET receptor expression was lower in PD than in healthy controls, but BMPR expression was unaltered in PD. This preservation of GDF5 receptors in the PD brain supports its potential therapeutic application.

[Data Blitz & E-poster]

DB#25 | The effect of a loaded collagen hydrogel on engraftment of RM3.5 iPSC-DAPs

Sarah Crudden[1], Giulia Comini[1], Tommy Patton[1], Saoirse Ryan[1], Niamh Moriarty[2], Clare Parish[2], Eilís Dowd[1]

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Cell-based brain repair using induced pluripotent stem cells (iPSCs) is a promising avenue for the treatment of Parkinson's disease, however, engraftment of these cells in the neurotrophin-depleted environment of Parkinsonian brains remains a hurdle. Biomaterials, such as hydrogels can be loaded with neurotrophins (NTFs) which has been shown to improve engraftment and differentiation of iPSC-derived dopaminergic progenitors (iPSC-DAPs) in athymic rats but has not yet been replicated in immunosuppressed rats.

Therefore, the aim of this study was to determine the effect of the neurotrophin-loaded hydrogels on engraftment of RM3.5 iPSC-DAPs in cyclosporine preconditioned rats.

Hemi-parkinsonism was induced by a 6-hydroxydopamine lesion followed by intra-striatal transplants one month later. There were 4 transplant groups: Cells alone, Cells + NTFs (BDNF and GDNF), Cells + Hydrogel and Cells + NTFs + Hydrogel. All animals received daily immunosuppression and motor function was assessed using Apomorphine-induced rotations. All animals were sacrificed 4 weeks post-transplant and Immunohistochemistry was performed to assess graft survival (HuNu+ and STEM121+ staining) and differentiation (TH+ staining).

The results of the rotations show that the lesion successfully induced parkinsonism in all animals, but no functional recovery was noted in this short study. The HuNu+ and STEM121+ staining revealed surviving grafts in the majority of rats, indicating good overall cell survival but with no improvement with the use of the neurotrophin-loaded hydrogel. There was some evidence of cell differentiation to TH+ dopaminergic neurons across all groups, but this was low, as would be expected at the early post-transplantation timepoint of 4 weeks.

Longer studies would be necessary to ascertain the differentiation capacity of these cells in vivo. Further investigation is warranted to see if the results from the athymic rat study can be replicated in immunosuppressed animals, perhaps investigating different immunosuppressants.

[Data Blitz & E-poster]

DB#26 | Evaluation of Novel Anti-Aggregation Compounds Against α-Synuclein

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Parkinson's disease (PD) is a devastating neurological condition, where current treatments remain purely symptomatic. Developing effective neuroprotective drugs have been impeded by the overreliance on replacing a single neurochemical target, dopamine. The misfolding and aggregation of a key protein α-synuclein has been linked to the PD pathogenesis. Targeting pathogenic α-synuclein could potentially slow disease progression.

In this study, we aimed to evaluate five novel compounds directed at preventing the formation of α-synuclein aggregates thereby ameliorating its toxic effect.

We first sought to establish the effect of α-synuclein aggregator FN075 in SH-SY5Y cells, and SH-SY5Y cells overexpressing the protein, to simulate the pathological characteristics of PD. This was conducted by alamarBlue viability assays as well as its effects on aggregation by western blot & immunocytochemistry. We next determine safe dose range for our five compounds, before assessing their ability to prevent FN075 induced toxicity in vitro.

Viability assays demonstrated 50-100µm of FN075 resulted in significant SH-SY5Y cell loss, however overexpressing cells exhibited a resistance to FN075 toxicity. Western blot revealed an increase trend in α-synuclein in response to FN075 in overexpressing cells. None of the five novel compounds had a significant protective effect against FN075 induced toxicity in SH-SY5Y cells.

Although the novel compounds did not display an ability to prevent cell death in response to FN075, further testing is required to verify these compounds can inhibit α-synuclein aggregation in vitro. Preventing the formation of toxic aggregates or breaking down these misfolded proteins might be the answer to developing a disease modifying therapy for PD.

[Data Blitz & E-poster]

DB#27 | Oligodendrocyte maturation & myelination defects in PD due to oxidative stress

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Traditionally, Parkinson's disease (PD) has been considered a purely neuronal disorder due to it being primarily characterized by the degeneration of dopaminergic neurons in the substantia nigra. However, emerging evidence suggests that glial cells, particularly oligodendrocytes (OLs), may also play a critical role in PD pathogenesis. Recent studies have identified PD risk factors linked not only to dopaminergic neurons but also to OLs. However, how the molecular signature of OLs differs between PD and healthy control brains, and which OL functions and cellular pathways may be involved in midbrain neuron vulnerability remains unknown.

To explore the possibility of a causal role for OLs in PD etiology, we utilized PD-patient-derived and CRISPR-engineered OLs generated from induced pluripotent stem cells (iPSCs) with mutations in the PD-linked genes *DJ-1* or *Parkin*. In these, we uncovered a reduction in both early and mature OLs, accompanied by oxidative stress and mitochondrial dysfunction, which was reversible through antioxidant treatment. Analysis of PD patient brain tissue confirmed decreased myelination, and also transplantation of DJ-1-deficient OLs into *ex vivo* mouse brain slices resulted in impaired myelination capacity.

Collectively, our study sheds light on how loss of functional OLs impact their maturation, cellular stress and myelin formation. These findings highlight the critical role of OLs in PD pathology, and suggest that targeting OL function and restoration may offer new therapeutic avenues for PD.

[Data Blitz & E-poster]

DB#28 | Strain-specific α-Synuclein effects on brain function and pathology in non-human primates

Fayard Audrey[1] , Fenyi Alexis[1], Lavisse Sonia[1], Barret Olivier[1], Brammoulle Yann[1], Lecourtois Sophie[1], Jouy Christophe[1], Guillermier Martine[1], Jan Caroline[1], Gipchtein Pauline[1], Petit-Fontyn Fanny[1], Nardin Basha[1], Dehay Benjamin[2], Bezard Erwan[2], Melki Ronald[1], Hantraye Philippe[1], Aron Badin Romina[1]

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Synucleinopathies are defined by the pathological aggregation of alpha-synuclein (α-syn), a normally monomeric presynaptic protein. In Parkinson's disease (PD), these aggregates form Lewy bodies and neurites, driving neurodegeneration. Evidence supports a prion-like propagation of α-syn by recruiting monomers and spreading between cells. Experimental models using viral vectors to overexpress wild-type or mutant α-syn, inoculation of brain homogenates from transgenic mice or synucleinopathy patients, or synthetic α-syn preformed fibrils (PFF), have demonstrated α-syn propagation and dopaminergic degeneration.

Importantly, α-syn can form structurally distinct fibrillar "strains" with unique biochemical and pathological properties. In vivo, these strains induce distinct patterns of α-syn pathology and neurotoxicity, suggesting they may underlie clinical heterogeneity in synucleinopathies.

Here we performed bilateral inoculation of 18 primates both in the substantia nigra (AAV-CBA-aSynA53T or PBS) and putamen (PFF, patient-derived α-syn or PBS). All animals underwent motor and cognitive testing as well as PET imaging at baseline, 6, and 12 months post-injection. Post-mortem analysis included TH immunocytochemistry to detect nigrostriatal alterations. We also assessed the seeding propensity of the various α-syn inocula by quantifying phosphorylated and aggregated α-syn.

Results show motor deficits varying according to the inocula, and suggest that the combination of AAV injection in the SN and PFF or patient α-syn significantly exacerbates the phenotype. This was further confirmed by PET imaging, looking at the activity of the DAT transporter (18F-FE-PE2I). Post-mortem and biomarker studies are currently ongoing.

Our findings highlight the critical role of α-syn strain diversity and dual-hit models in reproducing the complexity of synucleinopathies and offer a valuable platform for therapeutic and biomarker evaluation.

Day 3 | October 22th 2025

SESSION 8 | AGING

[Invited Speaker]

The role of focused ultrasound in the treatment of neurodegenerative diseases: A double hit approach in Parkinson´s disease

Jose A. Obeso[1]

[1]HM CINAC, Madrid, Spain

The role of focused ultrasound in the treatment of neurodegenerative diseases: A double hit approach in Parkinson´s disease.

[Invited Speaker]

Neuroendocrine based therapies in ageing and brain disorders

Cláudia Cavadas[1,2]

[1]GeneT, CNC-UC, CIBB, University of Coimbra
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[Invited Speaker]

Circadian dysfunction as a biomarker in cognitive aging

Luisa V. Lopes[1,2]

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Cognitive decline is one of the most pressing challenges of aging, yet its underlying mechanisms extend beyond neuronal loss. Emerging evidence reveals that subtle but significant synaptic alterations, exacerbated by stress, metabolic factors, and circadian dysfunction, critically shape the trajectory of brain aging.

In this lecture I will explore how hippocampal vulnerability, altered synaptic signaling, and disruption of circadian rhythms converge to impair memory and learning.

Drawing on experimental and translational findings, we will discuss how stress and early-life adversity exacerbate age-related decline, the role of adenosine signaling in regulating synaptic fate, and novel insights into hippocampal-cortical communication under circadian disruption.

Together, these data support circadian dysfunction as both a biomarker and potential therapeutic target in cognitive aging and may shed light on the underlying biological basis of cognitive resilience across the lifespan.

[Oral Presentation]

OC#7 | First BPAN Microglia Model Shows Pro-Inflammatory Phenotype

Gamze Özata[1], Dr. Rachel Wise[1], Dr. Aida Cardona-Alberich[1], Naiyareen Mayeen[1], Prof. Dr. Lena Burbulla

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Beta-propeller protein-associated neurodegeneration (BPAN) is a rare, childhood-onset disorder caused by mutations in the *WDR45* gene, leading to progressive cognitive decline and neurodegeneration [1]. Neuroinflammation is increasingly recognized as a key contributor to disease progression, but the cellular mechanisms remain elusive. Microglia, the brain's resident immune cells, play complex roles in health and disease, balancing neuroprotection and inflammation.

Here, we report the first successful generation of human microglia from BPAN patients using induced pluripotent stem cells (iPSCs). These iPSCs were differentiated into microglia using established protocols [2] and validated by expression of canonical microglial markers. Strikingly, comprehensive profiling via targeted transcriptomics (Nanostring), secretomics, and cytokine assays reveals that BPAN microglia display a pronounced pro-inflammatory phenotype, both at rest and following inflammatory challenge. This is evidenced by elevated secretion of classic pro-inflammatory cytokines and heightened responsiveness to immune stimuli, suggesting that BPAN mutations may prime microglia toward a disease-associated, neurotoxic state. These findings open new avenues for dissecting the immune landscape of BPAN and highlight the potential of patient-derived microglia for understanding and targeting neuroinflammatory mechanisms in rare neurodegenerative diseases [3]. This model also provides a platform for future studies aiming to identify therapeutic strategies to modulate microglial dysfunction in BPAN.

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2- McQuade A, et al. Development and validation of a simplified method to generate human microglia from pluripotent stem cells. Mol Neurodegener. 2018;13:67. doi: 10.1186/s13024-018-0297-x.

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Day 3 | October 22th 2025

SESSION 9 | PRE-CLINICAL

[Invited Speaker]

Novel approaches for treating Parkinson's disease

Merja Voutilainen[1]

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder affecting 10 million people worldwide. It is primarily characterized by the presence of dense intraneuronal inclusions of misfolded proteins known as Lewy bodies, which contain alpha-synuclein. Additionally, extensive data suggest that the dysregulation of proteostasis may play a crucial role in the initiation and progression of PD. The major clinical symptoms of PD stem from the degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). Currently, there are no medications that can slow or halt the progression of the disease, highlighting an urgent need for new and more effective treatments that can significantly alter its course.

Neurotrophic factors (NTFs) are secretory proteins that regulate neuron survival, neurite growth, and branching. While they have been investigated as potential therapies for PD, their efficacy in clinical trials has often been disappointing. CDNF is a protein with NTF properties that has been shown to protect and restore dopamine neurons in animal models of PD. In Phase I/II clinical studies, CDNF demonstrated safety and some therapeutic effects in PD patients. A major limitation of NTFs and CDNF is the requirement for direct delivery into the brain, as NTFs and CDNF cannot cross the blood-brain barrier (BBB).

My research has demonstrated that novel CDNF fragments can protect DA neurons in both in vitro and in vivo models of PD. Furthermore, our data indicate that these fragments can penetrate the BBB and have a neurorestorative effect in an animal model of PD when administered subcutaneously.

The goal of my research is to understand the mechanism of action and therapeutic effects of these novel BBB-penetrating CDNF-derived polypeptides. This study represents an innovative approach to treating neurodegenerative diseases.

[Invited Speaker]

Harnessing genetic scissors to rewrite polyglutamine disorders

Nicole Déglon[1]

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The combined use of gene transfer and editing technologies has pushed the boundaries of precise genome modification and promoted the development of promising strategies to treat genetic diseases, including pathologies affecting the central nervous system.

In this presentation, I will describe gene editing strategies for polyglutamine disorders and summarize in vivo studies of with the KamiCas9 self-activating system.

[Invited Speaker]

Machado-Joseph disease: of mechanism and therapy

Luís Pereira de Almeida[1,2]

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[Oral Presentation]

OC#8 | Brain-targeted and pH-sensitive liposomes encapsulating small-molecule drugs to enhance differentiation of human iPSC-derived neuroepithelial stem cells into neurons

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Stem cell-based brain transplantation can improve neurodegenerative diseases by replacing lost neurons and promoting bystander effects. We recently reported that neurons from human iPSC-derived neuroepithelial stem cells (hNESC) survive up to 6 months post-cerebellar transplantation in mice. However, poor cell differentiation into neurons in the host brain remains challenging.

This study assessed whether modulating neurogenesis with small-molecule drugs could enhance hNESC differentiation into neurons in the cerebellum. We also developed brain-targeted liposomes to improve drug delivery. Smoothened agonist (SAG), selected after screening neurogenesis-related pathways, was encapsulated in novel PEGylated, pH-sensitive brain-targeted liposomes (Lipos-SAG) to enhance SAG delivery to the brain. Three brain-targeting ligands, DAS (rabies virus-derived peptide), IKRG (peptide targeting TrkB receptor), and RVG-r9 (rabies virus-derived peptide), were tested for cell uptake. DAS and IKRG were selected for conjugation to the liposome surface by post-insertion (DAS-Lipos and IKRG-Lipos). SAG was encapsulated using a pH gradient. The liposomes showed high encapsulation efficiency (82.5–95.6%), 126–135 nm of mean size, and pH-sensitivity (46% higher drug release at pH 5.5 vs. 7.4). DAS- and IKRG-liposomes showed active uptake by hNESC; moreover, 74.91% and 61.27% mature neurons ($NeuN^+$) were observed in hNESC cultures treated with DAS-Lipos-SAG and IKRG-Lipos-SAG, respectively, compared to Controls (30.61%).

Upon intravenous injection of liposomes encapsulating a near-infrared (NIR) dye, fluorescent *in vivo* imaging and *ex vivo* fluorimetry quantification showed that DAS-Lipos and IKRG-Lipos significantly increased the accumulation of the NIR dye in the mouse brain but not in other major organs. Preliminary data indicate that DAS-Lipos-SAG enhances stem cell differentiation into neurons after cerebellar transplantation.

Overall, our findings show that the novel PEGylated, pH-sensitive brain-targeted liposomes encapsulating SAG improve drug delivery to the brain and enhance the differentiation of transplanted stem cells in the cerebellum into neurons.

E-POSTERS

EXPLORE NECTAR2025 DATA BLITZ

DB#1 | Directly Reprogrammed Human Neurons and Astrocytes Reveal Age-Related Difference

Roland Zsoldos[1,2], Anna A. Abbas[1], Kinga Vörös[1], Ali F. Dadawalla[1], Chandramouli Muralidharan[3], Julie Bouquety[4], Idris Jimoh[5], Ágnes Varga[1,2], Balázs Kis[1], Melinda Gazdik[1,2], Zsófia Koltai[1], Vivien Pillár[1,2], Ármin Sőth[1], Anikó Göblös[6], Jenny J. Johansson[3], Lajos Kemény[6], Roger A. Barker[7], Johan Jakobsson[3], Attila Szűcs[1], Karri Lamsa[8], Mária Judit Molnár[5], Janelle Drouin-Ouellet[4], Karolina Pircs[1,2,3]

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[7]Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
[8]HCEMM-USZ, Human Neuron Physiology and Therapy Core Group, Szeged, Hungary

Age is the greatest risk factor for neurodegenerative diseases (NDDs), yet the molecular links between physiological aging and NDD pathogenesis remain poorly understood. Autophagy—a lysosomal degradation pathway essential for maintaining cytoplasmic homeostasis—declines with age and contributes to both neuronal and glial dysfunction. To investigate how aging alters autophagic flux in the human brain, we use direct reprogramming of human dermal fibroblasts to generate induced astrocytes (iAs) and induced neurons (iNs), which retain donor-specific genetic and epigenetic signatures.

We aim to generate iAs and iNs from a total of 50 healthy donors, ranging in age from 24 to 86 years. Neuronal and astrocytic identity is verified by immunocytochemistry and high-content automated microscopy using cell-type- (TAU or ALDH1L1) and autophagy-specific markers (BECN1, LC3, p62, LAMP1), under both basal and stress-induced autophagy. Our first results using 5 young and 5 old donors indicated cell-type-specific age-related autophagy impairments, but via different mechanisms: while in iNs we found an impairement in autophagosome formation and/or turnover, in iAs autophagy flux was blocked likely at the lysosomal degradation step. These changes may contribute to age-related decline in brain homeostasis and increased vulnerability to neurodegeneration. We are currently validating these initial findings across the full donor cohort. In parallel, we are collecting samples for multi-omic autophagy profiling using a range of molecular assays—including RT-qPCR, genome-wide DNA-methylation arrays, bulk RNA-sequencing, mass-spectrometry, metabolomics, and functional readouts via patch-clamp electrophysiology —to compare young andold cells.

Finally, we are establishing co-culture systems with iNs and iAs to investigate autophagy dynamics in a more physiological neuron–glia environment, increasing the relevance and robustness of our model.

Our long-term goal is to identify key cell type–specific regulators of autophagy and to explore rejuvenation strategies. This approach could inform future therapies for NDDs, where impaired autophagy and accelerated aging are often observed.

DB#2 | Anti-inflammatory potential of helminth-derived peptides in vitro: a review

Sienna Stucke[1], Prof. Eilis Dowd[1], Aonghus Feeney[1]

[1]University of Galway

Helminths are parasitic worms that secrete a plethora of immune regulatory molecules which allow them to dampen inflammatory responses by their host's immune system to ensure their survival within the host. Their ability to have a compatible existence with their host has led to research into the potential therapeutic effects of helminth-derived molecules for suppression of overactive immune and inflammatory responses in a wide variety of diseases.

This systematic review aims to synthesize the published data on helminth-derived peptides/polypeptides (HDPs) with a focus on determining the extent to which they modulate the inflammatory response in in vitro cellular models of inflammation. In accordance with PRISMA 2020 guidelines, a predefined systematic search of the PubMed, Web of Science and Medline databases identified relevant studies published up to August 2025, and 79 articles were included after screening.

We found that most published studies used LPS or Concanavalin A stimulated macrophages, peripheral blood mononuclear cells or dendritic cells as the cellular model of inflammation. Twenty helminth species from which >60 isolated HDPs were derived were tested in these models, with the nematodes, Haemonchus contortus and Acanthocheilonema viteae, and the trematode, Fasciola hepatica, the most explored species. A common property of these molecules was to ability to significantly reduce the expression or production of pro-inflammatory cytokines such as IL-12, IL-1β, IL-6 and TNFα, and significantly increase the expression or production of anti-inflammatory cytokines such as IL-10, TGFβ and IL-4. The effects on other cytokines, including IFNγ which is known to have both pro- and anti-inflammatory effects, were less consistent, with HDPs either decreasing or increasing the levels of this cytokine.

This systematic review synthesizes the existing literature in this field and shows that the HDPs secreted by several helminth species have consistently demonstrated effects though modification of cytokine levels and, as such, have therapeutic potential in conditions in which overactive immune and inflammatory responses play a pathogenic role.

DB#3 | CD200-based cell sorting enables a safer, more reproducible cell therapy for HD

Cinta Gomis[1,2], Marc Estarellas[1,2], Francisco J Molina-Ruiz[1,2], Georgina Bombau[1,2], Josep M Canals[1,2]

[1]Universitat de Barcelona, Barcelona, Spain
[2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

Huntington's disease (HD) is a neurodegenerative disorder marked by the progressive loss of striatal projection neurons (SPNs), making it a compelling candidate for cell replacement therapy (CRT). Human pluripotent stem cell (hPSC)-derived neural progenitor cells (hNPCs) show strong therapeutic potential. Yet, clinical translation is hindered by the heterogeneity of cell populations generated through current hPSC differentiation protocols, compromising reproducibility and safety.

To address these challenges, we optimized Good Manufacturing Practice (GMP)-compliant cryopreservation protocols for hPSCs and their neural derivatives, including early neuroepithelial progenitors (hNEPs) and striatal-committed hNPCs. These protocols preserve cell viability and identity, enabling biobanking, quality control, and distribution. We also defined optimal seeding densities and attachment conditions, providing a reproducible framework to transition from research-grade to clinically applicable protocols.

Striving to increase graft reproducibility and safety, we implemented an immunomagnetic sorting strategy to enrich for post-mitotic neuroblasts (hNBs) within hNPC cultures. Using CD200 as a surface marker, we consistently isolated CD200high hNBs exhibiting SPN lineage commitment. Upon transplantation into the adult mouse striatum, CD200high hNBs retained striatal marker expression and continued to differentiate for at least one month. Notably, CD200-based selection reduced the number of proliferating cells and eliminated neural rosette-like structures within the grafts, thereby minimizing the risk of overgrowth and tumor formation.

Our work establishes a robust pipeline for translating hPSC-based CRT for HD, combining GMP-compliant cryopreservation with CD200-based cell sorting to meet key safety and regulatory benchmarks.

DB#4 | Choreic-like Movements in a Transgenic Rat Model of Huntington's Disease

Olivia Edwards[1], Patricia Garcia Jareño, Mariah Lelos, Anne Rosser

[1]Cardiff University

A range of genetic mouse models are available to study Huntington's disease (HD), although most have CAG repeats well in excess of that seen in adult HD and a relatively aggressive disease progression. The transgenic F344tgHD rat expresses a 51 CAG repeat fragment and exhibits a slow progressive phenotype that may be more typical of human adult-onset HD.

We sought to characterise motor and cognitive changes in the F344tgHD transgenic rat model, with a focus on understanding the biological basis of the choreic-like movements.

Wild type (WT), low mHTT expressing (mHTT ^{low}) and high mHTT expressing (mHTT ^{high}) rats were assessed for motor and cognitive deficits. Choreic-like movements were quantified longitudinally, with pharmacological modulation at 5 and 18 months of age. The 5-choice serial reaction time task (5-CSRTT), probing cognitive function, was undertaken at 12 months of age.

Choreic-like movements were observed in mHTT ^{high} rats as early as 2 months of age and persisted throughout the animal's lifetime. Pharmacological challenge indicated these to be dopamine-mediated-behaviours. In the 5-CSRTT, mHTT ^{high} rats performed significantly fewer total trials, were significantly less impulsive and omitted more responses compared to WT rats.

These data suggest that alterations of the dopamine system may be instrumental in the manifestation of choreic-like movements in the F344tgHD rats, and that F344tgHD rats also demonstrate motivational deficits. This data will contribute to a larger longitudinal analysis of F344tgHD rat behaviour that aims to provide a more slowly progressive HD model to test cell therapies and other therapeutic approaches.

DB#5 | In vitro microglial model of early neuroinflammation after CNS injury

Eszter Perhacs[1,2], James B. Phillips[1], Aminul Ahmed[2]

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Microglia are key mediators of acute inflammatory responses in the injured central nervous system (CNS), orchestrating both detrimental pro-inflammatory processes and reparative mechanisms that shape outcomes after injury. Early microglial activation is particularly critical in determining prognosis, as it drives secondary processes within the injured tissue that can either exacerbate damage or aid regeneration. Establishing *in vitro* models of early microglial activation is therefore particularly useful for advancing therapeutic strategies aimed at modulating neuroinflammation and promoting CNS repair. This study aimed to establish a simple and robust assay to investigate microglial activation which is relevant to the early stages of CNS injury.

BV2 microglial cells were cultured in 96-well plates and stimulated with pro-inflammatory factors - lipopolysaccharide (LPS) and/or interferon-gamma (IFN-γ) - for 24 hours. A range of outcome measures were assessed to evaluate microglial characteristics, including metabolic activity (PrestoBlue assay), expression of genes (RT-qPCR) associated with microglial activation, protein expression (immunocytochemistry), and nitric oxide production (Griess assay).

NOS2 gene expression, coding for inducible nitric oxide synthase (iNOS), demonstrated a pronounced ~30-fold increase following 24 hours of IFN-γ stimulation, while other outcome measures showed smaller, non-significant responses, highlighting iNOS as a robust and sensitive indicator of microglial activation in this assay. The optimised *in vitro* model provides a platform for investigating early neuroinflammatory processes in CNS injury and for evaluating candidate therapeutics, including stem-cell-derived factors and bioactive biomaterials.

DB#6 | Striatal differentiation of hypoimmunogenic non-human primate iPSCs for allogeneic cell therapy of Huntington's disease

Apirahmee Jeyakumaran[1,2], Quentin Fuchs[3,2], Donya El Akrouti[3,2], Noëlle Dufour[3,2], Romina Aron-Badin[3,2], Anselme L Perrier[2,3]

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Huntington's disease (HD) is a genetic neurodegenerative disorder characterised by progressive loss of medium spiny neurons (MSNs) in the striatum. Off-the-shelf allogeneic human PSC-derived cell therapy products for HD are scalable but are not fully tolerated by the host immune system in the absence of immunosuppression, and prolonged use of immunosuppressive drugs carries significant risks. Immune-evasive strategies for allogeneic grafts (e.g HLA-cloaking) are under investigation. However, such genetic modifications may impair the capacity of the donor line to differentiate into therapeutically relevant grafts. Here, we address this problem in vitro.

Macaca fascicularis iPSCs (Mac-iPSC) were produced and *B2M* and *CIITA* were inactivated using CRISPR–Cas9. These MHC-cloaked iPSCs expressed canonical pluripotency markers. We then assessed their neural differentiation and maturation capacity. Striatal progenitors were derived and cryopreserved from both wild-type (WT) and MHC-cloaked Mac-iPSCs. We compared the ability of each donor line to produce MSN precursors in 2D cultures (MSNp), mature into MSNs or astrocytes in 3D spheroids. We confirmed absence of MHC class I expression in all derivatives from the MHC-cloaked line and lack of MHC class II in purified astrocytes derived from those cloaked cells. To mitigate the “missing-self” NK response against MHC-cloaked derivatives, we evaluated expression of membrane-bound NK-inhibitory ligands. Transgene expression was assessed after lentiviral transduction of MSNp matured into striatal spheroids.

Our next goal is to compare, in vivo in nude rodents, the survival and maturation of single-cell MSN progenitor suspensions versus MSN spheroid suspensions after grafting, and to determine which format yields therapeutically relevant, MSN-containing grafts. We are currently conducting pilot experiments to test the ability of spheroids of different sizes to tolerate injection through needles of different gauges.

DB#7 | Mesenchymal stromal cells tackle mitochondrial and autophagy deficits in SCA3, supporting neuronal recovery

Inês Barros[1,2,3,4] , António Silva[1,2] , Dina Pereira[1,2,3,4] , Sónia P Duarte[1,2,3,4] , Sara M Lopes[1,2,3,4] , Rita Perfeito[1,2,3,4] , Diogo Tomé[1,3,5] , Ramiro Almeida[1,3,5] , Rui J Nobre[1,2,3,4,6] , Luís Pereira de Almeida[1,3,4,6,7] , Catarina O Miranda[1,2,3,4]

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Spinocerebellar ataxia type 3 (SCA3) is caused by an expanded CAG repeat in the MJD1/ATXN3 gene, producing mutant ataxin-3 that impairs cellular function and leads to neurodegeneration. We previously showed that repeated mesenchymal stem cell (MSC) treatments improved both phenotype and neuropathology in SCA3 mice. Moreover, in MSC-treated SCA3 mice, we observed a reduction in soluble ataxin-3 and a trend toward decreased protein aggregation in the cerebellum [1].

To understand the mechanisms behind this therapeutic effect, we performed *in vitro* co-cultures of MSCs with SCA3 neurons and *in vivo* MSC treatments in transgenic mice, examining pathways known to be impaired in the disease and potentially involved in ataxin-3 accumulation and aggregation.

MSC therapy corrected abnormalities in autophagosome maturation, mitochondrial turnover, and mitochondrial biogenesis in both models. These pathways may play a critical role in re-establishing protein recycling and indirectly contribute to reduced ataxin-3 levels. Furthermore, the MSC secretome prevented the presynaptic loss induced by mutant ataxin-3 in cortical neurons, as assessed by the number of synapsin and bassoon puncta per axonal length.

Finally, MSCs induced the phosphorilation of tyrosine kinase receptors (TRK-Rs) involved in key neuronal functions in an SCA3 cellular model, and normalized the levels of multiple proteins from a defined panel of neurological markers (Olink) in the cerebrospinal fluid (CSF) of SCA3 mice treated via intracerebroventricular MSC injection.

These findings highlight that MSCs exert neuroprotective effects in SCA3 through autophagy, mitophagy, and synaptic recovery. Understanding these mechanisms paves the way for RNA-based therapies to deliver MSC-derived mediators as potential treatments for SCA3.

[1] **Miranda CO*,** *et al*. **Molecular Therapy** 2018 Jul 12. pii: S1525-0016(18)30314-9. doi: 10.1016/j.ymthe.2018.07.007. Epub 2018 Jul 12

DB#8 | Deciphering the Role of VLMCs in DA Graft Function Using Lineage-Restricted hPSC

María García Garrote[1], Edoardo Sozzi[1], Malin Åkerblom[1], Petter Storm[1], Malin Parmar[1]

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Cell replacement therapy using human pluripotent stem cell (hPSC)-derived ventral midbrain dopaminergic (DA) progenitors is a promising disease-modifying strategy to restore striatal dopamine levels and improve motor function in Parkinson's disease (PD) patients. Although these progenitors are homogeneous at the time of transplantation, they give rise to DA grafts containing a heterogeneous mix of mature cell types, including DA neurons, astrocytes, and vascular leptomeningeal cells (VLMCs). The influence of non-neuronal cells on key parameters for graft functionality - DA neuron survival, maturation, and subtype specification - remains unclear.

Using the MACSima spatial proteomics platform, we identified extracellular matrix (ECM)-rich hPSC-derived VLMCs closely associated with both the host vasculature and therapeutic DA neurons in canonical intrastriatal DA grafts. Accordingly, recent barcode-based lineage tracing from our group revealed that DA neurons, astrocytes, and VLMCs share a common progenitor, with lineage commitment occurring at the final cell division. Thus, mature grafts devoid of VLMCs cannot be generated by simply sorting progenitors prior to transplantation.

To overcome this limitation, we engineered lineage-restricted hPSC lines using CRISPR/Cas9 to knock-in Cre recombinase at a VLMC-specific locus, enabling targeted ablation of VLMCs via Cre-dependent killing systems. We first validated that VLMC-specific Cre knock-in does not impair DA differentiation under our high-yield GMP-compatible protocol. Upon terminal differentiation, VLMC-Cre cultures successfully generated mature tyrosine hydroxylase-positive (TH^+) neurons *in vitro*.

This approach will allow us to determine whether VLMCs contribute to the survival, maturation and specification of the clinically relevant DA neurons. The resulting insights gained will pave the way for rational design of optimized graft composites, enhancing the efficacy of next-generation stem-cell based therapies for PD.

DB#9 | Functional integration of human striatal progenitor-grafts in a HD animal model

Marta Ribodino[1,2], Linda Scaramuzza[3,4], Christian Cassarino[3,4], Marta Morrocchi[3,4], Gabriela Berenice Gomez Gonzalez[1,2], Roberta Parolisi[1,2], Edoardo Sozzi[5], Giacomo Turrini[1,2], Valentina Cerrato[1,2], Simone Diana[1,2], Erika Gallo[1,2], Paola Conforti[3,4], Riccardo Tognato[6], Greta Galeotti[3,4], Chiara Cordiglieri[4], Maria Cristina Crosti[4], Martina Lorenzati[1,2], Linda Ottoboni[7], Malin Parmar[5], Simone Maestri[3,4], Dario Besusso[3,4], Elena Cattaneo[3,4], Annalisa Buffo[1,2]

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Human embryonic stem cell (hESC)-derived neuronal progenitors hold strong potential for treating several neurodegenerative pathologies. To be effective, cell products need to properly restore lost neuronal populations and to functionally integrate in the host tissue and reconstruct damaged circuits.

In this perspective, we examined the therapeutic potential of second generation human striatal progenitors (sg-hSP) derived from hESCs via a morphogen-guided protocol (Conforti et al, Cell Reports 2022) after transplantation in the quinolinic acid-induced HD rat model.

Long term single nuclei-RNA sequencing (snRNAseq) analyses revealed, with unprecedented resolution, that hSP-grafts generate a diverse population of striatal-relevant cell types, including medium spiny neurons, CGE- and MGE-derived interneurons, and regionalized astrocytes, recapitulating key aspects of ventral telencephalic development. Immunohistochemistry confirmed stable graft composition over time, supported a neurogenic-to-gliogenic switch post-transplantation, and identified a subset of graft-derived neurons and glia with migratory behavior within host tissue, in line with a remarkable potential of the grafted cells to integrate into the host environment.

Multimodal connectivity analyses, including virus-based tracing, *in vivo* calcium recording and *ex vivo* MEA recordings, demonstrated anatomical and functional integration of the grafts into host circuitries. Moreover, chemogenetic modulation of graft activity influenced locomotion and fine motor behaviors, including grooming, providing direct evidence of functional engagement with the host striatal circuitry.

Altogether, these findings support the ability of hSP-grafts to contribute to the functional reconstruction of host damaged circuits and offer new insights into the therapeutic potential of stem cell-derived cell replacement approaches.

DB#10 | Molecular lineage tracing of stem cell derived dopamine grafts

Petter Storm[1], Malin Åkerblom[1], Yu Zhang[1], Tomas Björklund[2], Malin Parmar[1]

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Human pluripotent stem cells (PSCs) differentiated into dopamine (DA) neurons provide an unlimited cell source for cell replacement therapy for Parkinson's disease (PD). However, histology and scRNA-seq profiling of resulting grafts in preclinical models have revealed that DA progenitors that are seemingly homogenous at the time of transplantation give rise to heterogenous grafts composed of different mature cell types.

We have used single nuclei RNA-seq combined with molecular barcodes to determine origin and shared lineage of the mature cell types forming in grafts of dopaminergic progenitors currently explored in cell therapy for Parkinson's Disease. Single-cell chromatin accessibility and RNA expression paired maps identified only subtle chromatin accessibility differences among progenitors supporting their homogeneity, and together with the tracing data shows that the PSC-derived floor plate cells used for transplantation are tri-potent, giving rise to dopamine neurons, astrocytes and VLMCs.

In parallel, we have also generated new barcode libraries designs for more efficient capture of the barcode transcript taking advantage of feature barcoding. This will allow for more detailed fate and state mapping in stem cell based regenerative approaches.

DB#11 | Enhancing cell therapy for PD – Assessing clonal expansion and graft diversity

Jana Bonsberger[1]

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Human pluripotent stem cell-based therapies are emerging as a promising treatment for Parkinson's disease (PD), with ongoing clinical trials demonstrating the feasibility of targeted regenerative approaches. However, a key challenge in cell replacement therapies for the central nervous system is the limited initial survival of transplanted cells - less than 10% survive and form the graft. This limited initial survival contributes to variability of therapeutic outcomes and complicates dosing strategies in clinical trials. Understanding which progenitor cells survive and mature into functional grafts is critical for advancing translational cell replacement therapies.

To address this, we have developed a molecular barcoding strategy to track the clonal expansion and survival of transplanted mesencephalic dopaminergic (mesDA) progenitor cells. Unique molecular barcodes were introduced via viral transduction into human embryonic stem cell-derived mesDA progenitor cells and subsequently transplanted into 6-hydroxydopamine (6-OHDA)-lesioned nude rats, a preclinical PD model.

Histological and single-nucleus RNA sequencing analyses confirmed efficient barcode integration and recovery. Barcode detection enabled quantification of surviving clones, and using the Chao1 estimator, we calculated that 4,850–9,000 barcoded cells from an initial 300,000 transplanted cells survived and formed the graft.

Our molecular barcoding approach enables a detailed analysis of early graft composition and clonal dynamics. These insights will support the development of strategies to achieve more consistent graft composition and improve initial cell viability. Ultimately, these advances could improve clinical trial outcomes by reducing variability and minimizing the required cell dose, bringing us closer to more reliable and effective cell replacement therapies for PD.

DB#12 | Chemogenetic Modulation of Pluripotent Stem Cell Therapy for Parkinson's disease

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Parkinsons disease is a chronic neurodegenerative disorder caused, in part, by the loss of dopaminergic neurons within the ventral midbrain (vmDA neurons). Cell replacement using pluripotent stem cell (PSC)-derived vmDA progenitors is already demonstrating promise in early-phase clinical trials. However, the ability to enhance the function of these grafts post-transplant could help further improve clinical outcomes.

The current project aims to explore whether the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) could be used to achieve this goal. DREADDs are engineered G protein–coupled receptors that allow ligand-dependent control of neuronal activity and neurotransmitter release.

Since commonly-studied DREADDs respond to clozapine-N-oxide, a ligand widely used in preclinical research but unsuitable for clinical use, we have instead investigated the application of a novel DREADD that instead responds to a more clinically appropriate ligand. Lentiviral delivery of this novel DREADD in PSC-derived vmDA neurons results in a ligand-dependent increase in neuronal activity, as evidenced by calcium imaging.

We are now exploring whether this, in turn, leads to increases in dopamine release using $GRAB_{DA}$ fluorescent reporters. While these initial and upcoming findings are informative, lentiviral delivery is limited in its suitability for clinical translation. Therefore, using CRISPR targeting, we are now also generating a stable PSC line with this DREADD integrated into the AAVS1 safe harbour locus.

Overall, it is hoped that this work will lay the foundation for future in vivo studies to determine whether changes in neuronal activity and dopamine release induced by the activation of this novel DREADD translate into improvements in motor outcomes. By doing so, such work will ultimately help to advance chemogenetic modulation of PSC-derived vmDA neurons towards clinical translation.

DB#13 | Extrinsic modulation of co-grafted neural progenitors enhances dopamine neuron specification and functional maturation

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Parkinson's disease (PD), the second most common neurodegenerative disorder, is characterized by the progressive loss of A9 dopaminergic (DA) neurons in the *substantia nigra*, leading to dopamine depletion in the striatum and subsequent motor symptoms. Transplantation of ventral midbrain DA progenitors derived from human pluripotent stem cells, aiming to restore DA neurotransmission in the striatum, is currently being developed and explored in clinical trials. One factor that may influence the maturation and fate determination of DA neurons is the intercellular communication within the graft environment, ultimately affecting the therapeutic outcome.

In this study, we co-transplanted DA progenitors with either glial progenitors, interneuron progenitors derived from the ventral forebrain, or striatal progenitors into the striatum of a preclinical model of PD to assess their role in influencing the development, maturation and function of therapeutic DA neurons.

Our findings show that co-grafts with forebrain interneurons increase the yield of DA neurons and promotes their functional maturation. Furthermore, we demonstrated that co-grafts with striatal neurons promotes functional maturation as well as the acquisition of DA subtype identity. From this data, we identified *EBF3* and *PBX3* as candidate transcription factors that may direct DA neuron specification towards the A9 subtype.

Taken together, our data highlight that the cellular microenvironment, including specific interactions with surrounding cells, influences *in vivo* dopaminergic neuron specification and maturation. These findings can be used to develop more refined and effective cell preparations for replacement therapy in Parkinson's disease.

DB#14 | Unravelling the central breathing circuitry to study CNS-related breathing disorders

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The signal to breathe originates from the preBotzinger complex (preBotC), the core of the breathing centre in the brainstem. The preBotC have has an intrinsic central pattern generator activity to maintain a rhythm for autonomous breathing. Opioid overdose fatalities arise due to respiratory depression, seen in the US opioid crisis and growing outbreaks in Europe. Despite breathing's fundamental role, research on the central breathing circuitry has mostly relied on rodents and largely unexplored in humans.

To model human central breathing circuitry, we developed a regionally-specified hindbrain differentiation protocol using human pluripotent stem cells (hPSCs) to generate preBotC neurons in vitro, and in vivo via neural transplantation.

After neural induction of hPSCs, we finetuned the rostro-caudal and dorso-ventral patterning by titrating a series of small molecules and using reporter cell lines to specify respiratory progenitors and mature preBotC neurons. Function was assessed on multi-electrode arrays (MEA) with opioid treatment to model opioid overdose. For in vivo analysis, we performed intracerebral injections of our transplantable progenitors.

Differentiated hPSCs acquired HOXB4+HOXB5– rhombomere 7 hindbrain and DBX1+ V0 subplate identity, consistent with the developmental origin of preBotC. Mature MAP2+ neurons expressed markers appropriate for also preBotC, including HOXB4+ and NK1R+. Consistent with the intrinsic central pattern generator function of preBotC, neurons exhibited regular, spontaneous bursting activity on the MEA, which was disrupted by opioids. Transplanted preBotC progenitors produced HOXB4+NK1R+HuD+ neurons that survived and innervated ipsilateral and contralateral host brain structures, showing capacity to form a bilateral network, which is important for coordinating bilateral breathing movements.

We demonstrated hour PSC- derived preBotC neurons can model CNS breathing disorders like opioid overdose *in vitro* and, for the first time, be transplanted for *in vivo* disease modelling.

DB#15 | In vitro Parkinson's disease modelling with NPC-derived dopaminergic neurons

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Parkinson's disease (PD) has been widely modelled *in vitro* to enable mechanistic studies and therapeutic screening. Many existing PD models use induced pluripotent stem cells (iPSCs) differentiated into dopaminergic (DA) neurons, a process that often takes upwards of 40 days. While effective, this long differentiation period can limit experimental throughput and scalability.

This project aims to establish a less time-consuming and more efficient *in vitro* PD model using neural progenitor cells (NPCs), which can differentiate into DA neurons in around 2 weeks, followed by 6-hydroxydopamine (6-OHDA)-induced neurodegeneration. In addition to establishing the degeneration model, this project compared the differentiation efficiency of NPCs cultured in monolayer versus collagen gel matrices, quantifying the proportion of DA neurons generated under each condition.

The NPCs were cultured and differentiated into DA neurons under specific growth factor stimulation. The yield and identity of DA neurons were assessed by immunocytochemistry for tyrosine hydroxylase (TH) and other lineage markers. After differentiation, cells were treated with a range of 6-OHDA concentrations to evaluate dose-dependent neurotoxicity. Cell viability was subsequently measured to obtain the extent of neuronal loss, which was then used to establish an optimal degeneration model.

This provides a time-efficient and scalable alternative to traditional iPSC-based PD models. The NPCs-based model supports investigation of neuroprotective strategies and evaluating candidate therapeutics for Parkinson's disease in a controlled and reproducible setting.

DB#16 | Assessment of cryogel microcarriers for improving the engraftment of iPSC-DAPs

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Cellular based brain repair is a potential therapeutic option to treat Parkinson's disease (PD) but engraftment remains limited with poor survival and differentiation of Induced Pluripotent Stem cells (iPSCs) *in situ* in the brain.

The aim of this research is to determine if PEGDA-SPA cryogel microcarriers loaded with the neurotrophic factors GDNF and BDNF, can aid engraftment of iPSC-derived dopaminergic progenitors (iPSC-DAPs). The microcarriers bind GDNF and BDNF, releasing them in a controlled manner to aid the growth of cells. The cytocompatibility of the microcarriers with iPSC-DAPs must be assessed.

Therefore an *in vivo* study was conducted with the iPSC-DAPs and the microcarriers without neurotrophic factors. Female cyclosporine-immunosuppressed Sprague Dawley rats received bilateral intrastriatal transplants. The treatment groups were Day 16 RM3.5 iPSC-DAPs alone or the iPSC-DAPs with unloaded microcarriers or the iPSC-DAPs with unloaded fluorescently labelled microcarriers. The rats were sacrificed by transcardial perfusion fixation at Day 4, Day 7 and Day 14 post transplantation (n=9 per day) and histological analysis was carried out.

Results from Human Nuclei staining which assesses cell survival show that there are no significant differences between the cells alone group and the microcarrier groups at any of the timepoints. STEM121 staining was carried out to assess cell engraftment and the results were similar to the Human Nuclei staining.

Therefore, the microcarriers are compatible with the iPSC-DAPs. Further long-term studies are needed to evaluate the potential of the neuroptrophin loaded microcarriers and whether they can aid with engraftment and differentiation of iPSC-DAPs.

DB#17 | A 3D model to characterize the maturation of an iPSC-derived mDA progenitor cell therapy

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Human pluripotent stem cells (hPSCs) can be leveraged to generate midbrain floorplate progenitor cell therapies to replace the dopaminergic (DA) neurons lost to Parkinson's Disease (PD). The efficacy of these therapies is dependent on the successful differentiation of the floorplate progenitor cells into adult DA neurons that acquire physiological maturity over several months in the *in vivo* environment.

To study this protracted transition from progenitors to neurons, it is important to have *in vitro* culture systems that support long-term neuronal maturation and mimic the *in vivo* environment. Conventional *in vitro* models have relied on monolayer (2D) cell culture systems, which do not accurately recapitulate the architectural complexity of the *in vivo* environment and can be confounded by cell detachment.

Here we describe a 3D cell culture model developed and leveraged to characterize the maturation of an hiPSC-derived midbrain dopaminergic progenitor cell therapy. Ventral midbrain DA (mDA) progenitors were cultured long-term in 2D and 3D *in vitro* environments in parallel and submitted for molecular (qPCR, flow cytometry, single nuclei RNA sequencing) and immunohistochemical assays at various timepoints across maturation to understand cell fate and function. To assess the relevance of the 3D modeling environment, results were compared to the immunohistochemical characterization of iPSC-mDA cells grafted into the 6-OHDA rodent model of PD.

These findings provide insights into the transition of midbrain floorplate progenitors to DA neurons in the context of a cell therapy for PD and demonstrate the potential of a 3D culture system for modeling the dynamics of iPSC-derived cell therapies upon transplantation.

DB#18 | Strategies to enhance stem cell migration and differentiation upon cerebellar transplantation

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Stem cell transplantation is a promising approach for neurodegenerative diseases, but limited migration and differentiation hinder success. In this work, we tested small-molecule drugs to enhance hNESC migration and differentiation. Human induced pluripotent stem cells (iPSC)-derived cerebellar neuronal progenitors (CNPs) were also established as a more specialized cell source for cerebellar regeneration.

From the 8 drug candidates tested to enhance cell migration, 2 mM Ligustrazine treatment for 6 days led to enhanced cell migration by 66.89%. Six weeks after hNESC cerebellar transplantation, 75 mg/kg Ligustrazine-treated mice showed higher percentage of DCX-positive cells compared to controls.

From the 8 drug candidates tested to enhance hNESC differentiation into neurons, 50 nM Smoothened agonist (SAG) led to a 2-fold increase in neuronal markers MAP2 and β3-tubulin protein levels. Six weeks after hNESC cerebellar transplantation in NOD/SCID mice, 30 mg/Kg SAG-treated mice presented a 20-fold increase in NeuN-positive graft-derived neurons. Additionally, 10 nM Crenigacestat (CRE) increased MAP2 and β3-tubulin protein by 93.94% and 83.54%, respectively. NeuN-positive graft-derived neurons showed a significant rise from 35.66% in control mice, to 77.28% in 3 mg/kg CRE-treated mice. Thus, both SAG and CRE enhanced hNESC neuronal differentiation.

The patterning protocol to derive CNPs from iPSCs resulted in significant upregulation of CNPs markers including a 10,033.70-fold increase in *ZIC1* mRNA levels, and flow cytometry showed a population of 97.70% ATOH1-positive and 89.00% ZIC1-positive cells. Two months post-transplantation into mice cerebella, CNPs grafts showed 87.73% NeuN-positive neurons and higher ZIC1-positive cells compared with hNESC indicating improved cerebellar commitment.

These findings support Ligustrazine, SAG, and CRE for enhancing hNESC engraftment success, and CNPs as a promising cell source for cerebellar regeneration.

DB#19 | The gut microbiome influences nigrostriatal degeneration in vivo.

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Parkinson's disease (PD) is a neurodegenerative disorder characterised by degeneration of midbrain dopaminergic neurons. Evidence indicates that PD may begin in the gut, with gastrointestinal dysfunction and dysbiosis experienced by many patients. Antibiotic-induced microbiota depletion (AIMD) model is used to study the microbiota's role in neurodegeneration. AIMD has been found to worsen PD symptoms in α-synuclein-overexpressing mice, but to improve them in 6-OHDA-lesioned rats.

We examined the effects of AIMD on nigrostriatal integrity and motor function in a brain-first 6-OHDA-leisoned rat model of PD. Adult Sprague-Dawley rats received unilateral stereotactic injections of 6-OHDA, and were treated with a broad-spectrum antibiotic cocktail for two weeks before and two weeks after surgery. Motor tests and faecal collections (at day 0, 7, and 14), immunohistochemistry on brain cryosections, and 16S-sequencing for faecal microbiota profiling were performed.

Immunohistochemistry for tyrosine hydroxylase (a dopaminergic neuronal marker) confirmed that antibiotics partially prevented loss of striatal dopaminergic fibres and cell bodies, as well as total neuronal numbers, in the substantia nigra in 6-OHDA-lesioned animals. Furthermore, antibiotics improved motor performance and decreased nigrostriatal microglial activation in 6-OHDA-lesioned animals. 6-OHDA-lesioned animals had upregulation of histone deacetylase 5 expression, which is known to cause dopaminergic neurodegeneration; this was prevented by antibiotic treatment.

These findings showed that AIMD partially preserved nigrostriatal integrity following intrastriatal 6-OHDA-induced insult. Since gut dysbiosis is a common symptom in PD patients, these findings highlight the potential of gut microbiota modulation for treatment of PD.

DB#20 | Generation of multi-regional neural spheroids to model brain circuitry in vitro and for multi-circuit cell replacement in vivo

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Current induced pluripotent stem cell (iPSC) derived neural cell replacement therapies generally rely on the transplantation of a single regionalised cell type. However, many neurological conditions affect multiple brain regions, so that replacing a single cell type may not address the requirements for functional circuit repair.

Recently, we have explored combining progenitors patterned with specific neural identity in 2D into multi-regional 3D spheroids, which we have termed “combinoids”. Using iPSC lines engineered to constitutively express fluorescent reporters and live cell imaging, we have shown that mixed preparations of striatal, ventral midbrain and cortical progenitor types robustly self-organise into 3D structures with a predictable spatial arrangement of regional cell types. We have also observed that the combination of progenitors drives maturation of the respective cell types.

Preliminary results show that these combinoids can also be transplanted and survive and integrate as multi-regional neural grafts in athymic mice. Spheroids containing cortical and striatal progenitors mature as neural grafts composed of cells that retain cortical and striatal identity, and notably including specific patterns of innervation of the host corresponding to these distinct neuronal populations.

In conclusion, we describe a multi-regionalised neural spheroid model as a promising transplantation avenue to enable the repair of multiple damaged areas with a single injection of a single combinoid into the affected brain region. This approach offers a new strategy for how cell replacement therapies could be used to treat neurodegenerative diseases.

DB#22 | Exploring glucocorticoid receptor function in dopaminergic neuron protection

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After extensive research efforts, human pluripotent stem cells (hPSCs) are now a well-established resource for generating dopaminergic neurons in both 2D and 3D cultures, with the goal of modeling related pathologies such as Parkinson’s disease (PD). Identifying the molecular mechanisms underlying human dopaminergic (DA) neurons differentiation is essential for advancing in vitro models that more faithfully recapitulate human brain development and neurodegenerative disorders not fully represented in rodent systems.

By using single-cell transcriptomics analysis, we identified NR3C1 as one of the most significantly enriched genes in functionally mature DA neurons. NR3C1 encodes for the glucocorticoid receptor (GR), a nuclear receptor protein that functions as a ligand-dependent transcription factor mediating different actions of glucocorticoids.

After employing hPSCs to generate DA neurons using both monolayer and brain organoid strategies, our findings suggest that GR plays a role in the acquisition of postmitotic molecular features during differentiation, and furthermore, that it could have a protective role during neurodegenerative phenomena. In vivo studies on rodent models of PD and epidemiological studies in PD patients have suggested that glucocorticoids may exert a neuroprotective effect.

However, the underlying mechanisms remain poorly understood, including whether these effects are cell-autonomous or mediated by non-cell-autonomous pathways. Human midbrain organoid-based PD models offer a valuable and more physiologically relevant platform to investigate this question, potentially yielding important insights for future therapeutic strategies.

DB#23 | Exploring pathology in directly reprogrammed iDANs from PD patient cells

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Current approaches to cell replacement therapy for Parkinson's disease (PD) focus on stem cell-derived dopamine progenitors for transplantation. However, future therapies might use patient cells that are directly reprogrammed into induced dopaminergic neurons, either *in vitro* for transplantation using dermal fibroblasts as starting cells, or directly *in vivo* reprogramming brain resident cells such as glia progenitor cells (GPCs). It is known that transplanted dopamine neurons from fetal tissue can acquire pathology in a PD patient over time and unpublished data from our lab shows that dopamine neurons derived from patient induced pluripotent stem cells (iPSCs) acquire pathology faster in pre-clinal PD models compared to those differentiated from human embryonic stem cells (hESCs).

In this study we want to investigate whether the same is true for reprogrammed induced dopamine neurons (iDANs). Over the past five years, we have developed both two- and three-dimensional (2D, 3D) cell culture protocols to directly reprogram human adult dermal fibroblasts (hDFs) and human GPCs *in vitro*. Transitioning into 3D cultures has improved post-transplantation survival and allowed for extended maintenance of reprogrammed neurons *in vitro.* In these previous studies we have shown that we can successfully reprogram hDFs from healthy controls and GPCs derived from hESCs. This study includes hDFs and iPSC derived GPCs from healthy controls as well as genetic and sporadic PD patients, enabling us to explore disease-specific pathology development.

By using our previously established protocols we will reprogram hDFs and GPCs to iDANs to assess dopamine identity and investigate whether reprogrammed patient cells are more susceptible to acquire pathology compared to those from healthy controls. We will also compare outcomes between hDFs, which retain environment and age-associated features, and iPSC derived GPCs, where these signatures have been reset. Understanding how patient-specific disease backgrounds influence reprogramming and vulnerability to pathology is critical for advancing patient-specific cell replacement therapies using direct reprogramming.

DB#24 | Neurorestoration by AAV-GDF5 ± GDNF in a rat model of Parkinson's

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Parkinson's disease (PD) is characterised by midbrain dopaminergic neuron degeneration associated with a-synuclein (aSyn) accumulation. Adeno-associated viral vector (AAV)-mediated delivery to the brain of neurotrophic factors (NTFs), including GDNF, has disease-modifying potential in PD. However, clinical trials to date have failed to meet their primary endpoints. One hypothesised reason for this is that aSyn downregulates GDNF's receptor, RET. Growth/differentiation factor 5 (GDF5) is an NTF that operates through Bone Morphogenic Protein receptors (BMPRs), and has neuroprotective effects in *in vivo* PD models.

This study investigated effects of delayed administration of GDF5, and of combined GDF5 and GDNF therapy, in the AAV-aSyn rat model of PD. Following baseline behavioural testing, Sprague-Dawley rats received unilateral intranigral injection of AAV-aSyn, followed after 13 weeks by unilateral intranigral injection of either AAV-Null, AAV-GDF5, or AAV-GDF5 + AAV-GDNF. Behavioural testing was carried out at weeks 12, 16, 20 and 24. Treatment with either AAV-GDF5 or combination of AAV-GDF5 + AAV-GDNF partially rescued motor impairments associated with AAV-aSyn. Animals were perfused after 25 weeks for immunohistochemistry, which showed that both AAV-GDF5 and AAV-GDF5 + AAV-GDNF rescued nigral dopaminergic neurons and striatal terminals from AAV-aSyn-induced degeneration. These data show that intranigral administration of AAV-GDF5, or combination of AAV-GDF5 and AAV-GDNF, has neurorestorative effects in the AAV-aSyn PD model.

We also examined the expression of NTF receptors in paraffin-embedded sections of substantia nigra from PD patients and age-matched controls. RET receptor expression was lower in PD than in healthy controls, but BMPR expression was unaltered in PD. This preservation of GDF5 receptors in the PD brain supports its potential therapeutic application.

DB#25 | The effect of a loaded collagen hydrogel on engraftment of RM3.5 iPSC-DAPs

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Cell-based brain repair using induced pluripotent stem cells (iPSCs) is a promising avenue for the treatment of Parkinson's disease, however, engraftment of these cells in the neurotrophin-depleted environment of Parkinsonian brains remains a hurdle. Biomaterials, such as hydrogels can be loaded with neurotrophins (NTFs) which has been shown to improve engraftment and differentiation of iPSC-derived dopaminergic progenitors (iPSC-DAPs) in athymic rats but has not yet been replicated in immunosuppressed rats.

Therefore, the aim of this study was to determine the effect of the neurotrophin-loaded hydrogels on engraftment of RM3.5 iPSC-DAPs in cyclosporine preconditioned rats.

Hemi-parkinsonism was induced by a 6-hydroxydopamine lesion followed by intra-striatal transplants one month later. There were 4 transplant groups: Cells alone, Cells + NTFs (BDNF and GDNF), Cells + Hydrogel and Cells + NTFs + Hydrogel. All animals received daily immunosuppression and motor function was assessed using Apomorphine-induced rotations. All animals were sacrificed 4 weeks post-transplant and Immunohistochemistry was performed to assess graft survival (HuNu+ and STEM121+ staining) and differentiation (TH+ staining).

The results of the rotations show that the lesion successfully induced parkinsonism in all animals, but no functional recovery was noted in this short study. The HuNu+ and STEM121+ staining revealed surviving grafts in the majority of rats, indicating good overall cell survival but with no improvement with the use of the neurotrophin-loaded hydrogel. There was some evidence of cell differentiation to TH+ dopaminergic neurons across all groups, but this was low, as would be expected at the early post-transplantation timepoint of 4 weeks.

Longer studies would be necessary to ascertain the differentiation capacity of these cells in vivo. Further investigation is warranted to see if the results from the athymic rat study can be replicated in immunosuppressed animals, perhaps investigating different immunosuppressants.

DB#26 | Evaluation of Novel Anti-Aggregation Compounds Against α-Synuclein

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Parkinson's disease (PD) is a devastating neurological condition, where current treatments remain purely symptomatic. Developing effective neuroprotective drugs have been impeded by the overreliance on replacing a single neurochemical target, dopamine. The misfolding and aggregation of a key protein α-synuclein has been linked to the PD pathogenesis. Targeting pathogenic α-synuclein could potentially slow disease progression.

In this study, we aimed to evaluate five novel compounds directed at preventing the formation of α-synuclein aggregates thereby ameliorating its toxic effect.

We first sought to establish the effect of α-synuclein aggregator FN075 in SH-SY5Y cells, and SH-SY5Y cells overexpressing the protein, to simulate the pathological characteristics of PD. This was conducted by alamarBlue viability assays as well as its effects on aggregation by western blot & immunocytochemistry. We next determine safe dose range for our five compounds, before assessing their ability to prevent FN075 induced toxicity in vitro.

Viability assays demonstrated 50-100µm of FN075 resulted in significant SH-SY5Y cell loss, however overexpressing cells exhibited a resistance to FN075 toxicity. Western blot revealed an increase trend in α-synuclein in response to FN075 in overexpressing cells. None of the five novel compounds had a significant protective effect against FN075 induced toxicity in SH-SY5Y cells.

Although the novel compounds did not display an ability to prevent cell death in response to FN075, further testing is required to verify these compounds can inhibit α-synuclein aggregation in vitro. Preventing the formation of toxic aggregates or breaking down these misfolded proteins might be the answer to developing a disease modifying therapy for PD.

DB#27 | Oligodendrocyte maturation & myelination defects in PD due to oxidative stress

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Traditionally, Parkinson's disease (PD) has been considered a purely neuronal disorder due to it being primarily characterized by the degeneration of dopaminergic neurons in the substantia nigra. However, emerging evidence suggests that glial cells, particularly oligodendrocytes (OLs), may also play a critical role in PD pathogenesis. Recent studies have identified PD risk factors linked not only to dopaminergic neurons but also to OLs. However, how the molecular signature of OLs differs between PD and healthy control brains, and which OL functions and cellular pathways may be involved in midbrain neuron vulnerability remains unknown.

To explore the possibility of a causal role for OLs in PD etiology, we utilized PD-patient-derived and CRISPR-engineered OLs generated from induced pluripotent stem cells (iPSCs) with mutations in the PD-linked genes *DJ-1* or *Parkin*. In these, we uncovered a reduction in both early and mature OLs, accompanied by oxidative stress and mitochondrial dysfunction, which was reversible through antioxidant treatment. Analysis of PD patient brain tissue confirmed decreased myelination, and also transplantation of DJ-1-deficient OLs into *ex vivo* mouse brain slices resulted in impaired myelination capacity.

Collectively, our study sheds light on how loss of functional OLs impact their maturation, cellular stress and myelin formation. These findings highlight the critical role of OLs in PD pathology, and suggest that targeting OL function and restoration may offer new therapeutic avenues for PD.

DB#28 | Strain-specific α-Synuclein effects on brain function and pathology in non-human primates

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Synucleinopathies are defined by the pathological aggregation of alpha-synuclein (α-syn), a normally monomeric presynaptic protein. In Parkinson's disease (PD), these aggregates form Lewy bodies and neurites, driving neurodegeneration. Evidence supports a prion-like propagation of α-syn by recruiting monomers and spreading between cells. Experimental models using viral vectors to overexpress wild-type or mutant α-syn, inoculation of brain homogenates from transgenic mice or synucleinopathy patients, or synthetic α-syn preformed fibrils (PFF), have demonstrated α-syn propagation and dopaminergic degeneration.

Importantly, α-syn can form structurally distinct fibrillar "strains" with unique biochemical and pathological properties. In vivo, these strains induce distinct patterns of α-syn pathology and neurotoxicity, suggesting they may underlie clinical heterogeneity in synucleinopathies.

Here we performed bilateral inoculation of 18 primates both in the substantia nigra (AAV-CBA-aSynA53T or PBS) and putamen (PFF, patient-derived α-syn or PBS). All animals underwent motor and cognitive testing as well as PET imaging at baseline, 6, and 12 months post-injection. Post-mortem analysis included TH immunocytochemistry to detect nigrostriatal alterations. We also assessed the seeding propensity of the various α-syn inocula by quantifying phosphorylated and aggregated α-syn.

Results show motor deficits varying according to the inocula, and suggest that the combination of AAV injection in the SN and PFF or patient α-syn significantly exacerbates the phenotype. This was further confirmed by PET imaging, looking at the activity of the DAT transporter (18F-FE-PE2I). Post-mortem and biomarker studies are currently ongoing.

Our findings highlight the critical role of α-syn strain diversity and dual-hit models in reproducing the complexity of synucleinopathies and offer a valuable platform for therapeutic and biomarker evaluation.

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