NECTAR2022

NECTAR

32nd Annual meeting
of the Network for European CNS
Transplantation and Restoration

nectar-athens22.gr

Program - Abstracts

ATHENS GREECE

COTSEN HALL

24-26 OCTOBER 2022

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ORGANIZED BY

Network for European

CNS Transplantation and Restoration

NECTAR

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WELCOME MESSAGE

Dear participants, presenters and sponsors,

A very warm welcome to the 32nd Annual meeting of the Network for European CNS Transplantation and Restoration (NECTAR).

Nectar – athens.2022 has assembled an excellent line-up of speakers from Europe: France, Sweden, Luxembourg, Finland, Switzerland, Greece, and the UK, Israel, the US and more, to share their latest research with us. We have planned 3 plenary lectures on hot topics as well as sessions on the most recent developments in cell transplantation clinical trials for neurodegenerative diseases, immune system and brain cross-talk, advances in CNS disease modeling, mechanisms of neurodegeneration, neuroinflammation and glial cells, and preclinical cell therapies for brain disorders.

As always at NECTAR meetings, we have significant input into the scientific program from young researchers, PhD students and post-docs, through data-blitz oral presentations and e-posters, and a focus session from Industry partners.

This year we also very much look forward to the planned special session on patient advocacy.

Many people have helped towards Nectar – athens.2022 and I thank them all heartily. In particular I wish to extend warm thanks to:

The President of NECTAR, Romina Aron Badin, for her untiring contribution to the program and conference organization;

The Organizing and Program Committee for their brilliant ideas and suggestions;

Tilo Kunath, for transferring his invaluable experience as organizer of last year's INTR-NECTAR 2021 and his vital assistance with funds and sponsors;

The outstanding scientists that agreed to speak at the conference;

Our sponsors Novo Nordisk, AXOL, NEURATRIS, Cure Parkinson's, Zona Microfluidics, ANTISEL for their generous support;

Our professional conference organizers, ERA Ltd, especially Natassa Tourkantoni, George Peppas and Effie Stamena for their excellent management;

My colleagues, students and post-docs in the lab for their willingness and enthusiasm to help. Special thanks to Era Taoufik and Katerina Segklia for sharing responsibilities and walking with me all along this endeavour.

We are all eager to meet and hope that the scientific program will be rewarding with enriching interactions and discussions.

I wish you a great meeting!

Rebecca Matsas

Athens, October 2022

COMMITTEES

Organizing and Program Committee		
Rebecca Matsas	Tilo Kunath	Achille Gravanis
Romina Aron Badin	Lachlan Thompson	Stefano Pluchino
Era Taoufik	Andreas Heuer	Katerina Segklia
Agnete Kirkeby	Marina Romero-Ramos	Charlotte Bridge

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INFORMATION

VENUE - Athens, Cotsen Hall

The American School of Classical Studies at Athens (ASCSA)
9 Anapiron Polemou str.
GR-106 76, Athens, Greece
Phone: (+30) 213 000 2400
Fax: (+30) 210 725 0584
Email: ascsa_info@ascsa.edu.gr

Few Words about Cotsen Hall

Cotsen Hall, which was inaugurated in 2005 and named after its benefactor who was a great Philhellene and School Trustee, Lloyd Cotsen, serves as the main cultural center and embodies the mission of the American School of Classical Studies. The Hall is a landmarked building designed in modern style in the center of Athens. It is located just 5 minutes away, on foot, from the Evangelismos station and ten minutes from the underground parking of the War Museum, Cotsen Hall is literally located in the heart of Athens. It is fully equipped for a wide range of events including lectures, conferences and symposia, to chamber music concerts and theatre performances, and has been recognized as one of the best venues in Athens. Cotsen Hall permits the American School of Classical Studies to build on its outreach to the international community through its seminars, lectures, concerts, and other special programming, most of which are live-streamed by our production team, to the public throughout the world. Cotsen Hall has an overall capacity of 377 seats in order to accomodate the needs of all cultural and educational programs of the School and also welcomes events organized by distinguished groups and individuals from the world of education, culture, and business. Cotsen Hall also has a two-level lobby that is provided for indoor breaks and reception space for gatherings of up to 200 people. For larger gatherings during most months of the year, the Hall Terrace is available alone or in combination with two additional garden terraces.

Speaker's accommodation

EMBASSY HOTEL
22 Timoleontos Vassou Street
11521 Athens – Greece
Tel : 0030 210 6415000
E-mail : info@embassyhotel.gr, Web: http://www.embassyhotel.gr

Official Language

The official language of the conference is English.

Meeting Secretariat

era congresses and events

T.: +30 210 3634944, Mob: +30 699 702 8501
E-mail: info@era.gr, Web Site: www.era.gr

The Meeting Secretariat, ERA Ltd will be located at the entrance of Cotsen Hall and will be open from Monday 24th October 2022 from 12:00 and throughout the Meeting. Please collect your badge at the desk.

Dinner

The conference dinner will be held on **Tuesday October 25 2022** at **Strofi Athenian Restaurant** at 21.00.
It is located in the shadow of the Acropolis with views to the ancient monument and serves traditional Greek and Mediterranean cuisine.
Location: ***Rovertou Galli 25, Athina 117 42***

Price 60€/per person | *Please take your personal invitation from Meeting secretariat.*

Welcome reception

A Welcome reception will be held for all delegates after the end of the Monday scientific program, at the yard of Cotsen Hall.

Lunch & coffee Breaks

Lunch and coffee breaks will be served according the program at the ground floor of Cotsen Hall.

Instructions for speakers

Considering the full timetable of the meeting, we kindly ask speakers to present their talks within the allocated time. PowerPoint projection facilities and technical assistance will be available throughout the meeting. We ask all speakers to bring their talks on a USB-stick.

- **E-posters** presentation duration: **2 min talk + 1min** for questions.
- **Data Blitz** presentation duration: **4 min talk + 1min** for questions.

Information for virtual delegates

All registered delegates have received a link for the Live Streaming of the meeting.

Conference Recordings

Talks will be recorded and available for viewing by both in-person and virtual-only delegates after the end of the event. If you do not wish your presentation to be recorded, please inform the congress secretariat.

SPONSORS

The Organizing Committee of the Meeting and the NECTAR Board would like to thank the following companies and organizations for their support

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Program

MONDAY 24 October 2022

12:00-13:30 Registration

13:15-13:30 Opening - Welcome

13:30-14:30 Plenary I

Chairs: **Romina Aron Badin, Rebecca Matsas**

Nissim Benvenisty, *The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel*
The Essentialome of Human Development and Disease

14:30-16:00 Session 1

Clinical trials

Chairs: **Anders Bjorklund, Achille Gravanis**

Tilo Kunath, *Institute for Stem Cell Research, University of Edinburgh, UK*
Tool kit for dopaminergic progenitor cell production

Jun Takahashi, *CiRA-Kyoto University, Japan*
R&D of iPS cell-based therapy for stroke

Steven Goldman, *Centre for Translational Neuromedicine, University of Rochester & University of Copenhagen Faculty of Health and Medical Sciences, Denmark*
Challenges towards clinical translation of cell therapies for Huntington's disease

16:00-16:30 Coffee

e-Posters session I

Chair: **Anastasios Kollias**

MONDAY 24 October 2022

16:30-18:30 Session 2

Advances in CNS disease modeling

Chairs: **Tilo Kunath, Era Taoufik**

Jens Schwamborn, *Luxembourg Centre for Systems Biomedicine, Luxembourg*
Modeling Parkinson's disease with human midbrain organoids

Achille Gravanis, *School of Medicine University of Crete & Institute of Molecular Biology and Biotechnology, Crete, Greece*
Designing brain-on-chip platforms for simulating human brain function and malfunction'

Evangelos Kiskinis, *Feinberg School of Medicine, Northwestern University, Chicago USA*
Artificial Extracellular Matrix Scaffolds Enhance Maturation and Ageing of Human Stem Cell-Derived Neurons and Enable the Modeling of Neurodegenerative Disease Pathology

Alessandro Fiorenzano, *Lund Stem Cell Center, Sweden*
Modeling dopamine neuron specification and diversity using human stem cell-based technologies

18:30-19:30 Plenary II

Chair: **Evangelos Kiskinis**

Lee Rubin, *Harvard Stem Cell Institute, USA*
Disease modeling and phenotypic drug screening

19:30 Welcome Reception

TUESDAY 25 October 2022

09:00-11:00 Session 3

Neuroinflammation and glial cells in neurodegenerative diseases

Chairs: **Chiara Zurzolo, Florentia Papastefanaki**

Malú Gámez Tansey, *Center for Translational Research in Neurodegenerative Disease, University of Florida College of Medicine, Gainesville, USA*
Inflammation and immune dysfunction in Parkinson disease

Stefano Plucino, *Dept of Clinical Neurosciences, University of Cambridge, UK*
Molecular fuels and drivers of smouldering disease in progressive multiple sclerosis

Era Taoufik, *Hellenic Pasteur Institute, Athens, Greece*
Neuroinflammation and astrocytes in Parkinson's disease

Jari Koistinaho, *Neuroscience Center, Helsinki Institute of Life Science HiLIFE, Finland*
Modelling the impact of glial cells in Alzheimer's disease and schizophrenia by iPSC-derived cells

11:00-11:25 **Coffee**

e-Posters session II Chair: **Olympia Apokotou**

11:25-12:10 Data Blitz session I

Chair: **Charlotte Bridge**

12:10-13:00 Plenary III

Chair: **Malú Gámez Tansey**

Ido Amit, *Weizmann Institute of Science, Israel*
The power of ONE: Immunology in the age of single cell genomics

13:00-14:00 Lunch

TUESDAY 25 October 2022

14:00-16:00 Session 4

Preclinical cell/gene therapies in neurological disorders I

Chairs: **Eilis Dowd, Andreas Heuer**

Olivier Goureau, *Institut de la Vision, Sorbonne Université, INSERM, CNRS Paris, France*
Pluripotent stem cell-based therapy for outer retinal diseases

Sandro Alves *BrainVectis - Askbio, Institut du Cerveau (ICM), Paris, France*
Restoration of brain cholesterol metabolism as gene therapy approach for Huntington's Disease

Luís Pereira de Almeida, *Center for Neuroscience and Cell Biology, University of Coimbra, Portugal*
Therapeutic strategies for spinocerebellar ataxia type 3

Dimitra Thomaidou *Hellenic Pasteur Institute, Greece*
Direct astrocyte to neuron reprograming: towards a therapeutic strategy for brain injuries

16:00-16:35 Data Blitz session II

Chair: **Panagiotis Chandris**

16:35-17:00 Coffee

e-Posters session III

Chair: **Elpiniki Ninou**

17:00-18:00 Special Session

Patient Advocacy

Chairs: **Emma Lane, Cheney Drew**

Benjamin Stecher, *Chair of the Patient Advisory Board (PAB), Rune Labs, San Francisco, USA*
What Patient Advocacy Can Do

18:00-20:00 Session 5

Preclinical cell/gene therapies in neurological disorders II

Chairs: **Stefano Pluchino, Dimitra Thomaidou**

Malin Parmar, *Wallenberg Neuroscience Center & Lund Stem Cell Center, Sweden*
Reprogramming as a regenerative therapy in PD

Sabah Mozafari, *ICM Paris, Sorbonne University France*
Potential of Multiple Sclerosis iPS-derived oligodendroglia to functionally interact with axons and glia in vivo

Clive Svendsen, *Cedars-Sinai Regenerative Medicine Institute, Los Angeles, USA*
Stem cell therapies for amyotrophic lateral sclerosis

Mark Tuszynski, *University of California San Diego, USA*
Neural Stem Cell Therapy for Spinal Cord Injury

21:00 Conference Dinner

WEDNESDAY 26 October 2022

09:00 - 09:30 Data Blitz session III

Chair: **Katerina Segklia**

09:30 - 10:30 Session 6

Companies/Biotech

Chairs: **Tilo Kunath, Clive Svendsen**

XONA Microfluidics

Assay development and improving reproducibility using microfluidic compartmentalized devices

Anne Marion Taylor, *Chief Scientific Officer, Xona Microfluidics, Inc., USA*

Novo NORDISK

How to bring cell therapy from research to patients?

Good collaboration between academia and pharma companies can be the key for bringing cell therapy from research to patients

Ida Stenfeldt Mathiasen, *Project Director Clinical Pharmacology, Novo Nordisk*

Anne Møller Nielsen, *MD, PhD, International Medical Director, Novo Nordisk*

AXOL Biosciences

HiPSC derived Microglia-like cells as a model for Neurodegenerative diseases

Vijitha Sathiaseelan, *Group Leader, Axol Biosciences*

10:30 -11:00 Data Blitz session IV

Chair: **Elsa Papadimitriou**

11:00-11:30 Coffee

e-Posters session IV

Chair: **Elissavet Akrioti**

WEDNESDAY 26 October 2022

11:30-13:30 Session 7

Disease mechanisms

Chair: **Scott Ryan, Rebecca Matsas**

Frederic Saudou, *Institut Neurosciences, Grenoble, France*
Molecular mechanisms in Huntington's disease

Magdalini Polymenidou, *University of Zurich, Switzerland*
RNA-RBP interactions/ phase separation in neurodegenerative diseases

Chiara Zurzolo, *Institut Pasteur, Paris, France*
Disease spreading through tunneling nanotubes

Maria Xylouri, *Biomedical Research Foundation of the Academy of Athens, Greece*
Untangling the mechanisms underlying Multiple System Atrophy pathogenesis

13:30 -14:00 Closing remarks - Departure

DATA BLITZ

Data Blitz session I

1
Peripheral Immune Cells Induce Reactive Phenotype in Human iPSC-derived Midbrain Astrocytes
Mrs Adina MacMahon Copas, Trinity College Dublin, Ireland

2
Investigating the role of alpha-synuclein in neuronal innate immunity and its role in the interferon response pathway
Mr Andrew Chai, University of Edinburgh, United Kingdom

3
Seeding of α-syn pathology in vivo involves the autophagy protein LC3
Prof. Scott Ryan, University Of Guelph, Canada

4
Developing assays for neuronal cell replacement in Parkinson's Disease and advancing next generation therapeutics
Dr Jonathan Niclis, [1]Novo Nordisk, Copenhagen, Denmark

5
Enhanced Production of Mesencephalic Dopaminergic Neurons from Lineage-Restricted Human Undifferentiated Stem Cells
Dr Mark Denham, Aarhus University, Denmark

6
p.A53T-αSyn iPSC-derived astrocytes from Parkinson's disease patients display intrinsic pathology and affect dopamine neuron viability
Christina Paschou, Hellenic Pasteur Institute, Athens, Greece

7
A Three-Dimensional Co-Culture Model of IPSC Derived Human Astrocytes and Interneurons
Andreas Bruzelius, Lund University, Sweden

Data Blitz session II

8
Myelin regeneration as a potential therapeutic target in Alzheimer's Disease
Ioanna Zota, **Konstantina Chanoumidou,** University of Crete- IMBB-FORTH, Heraklion, Greece

9
Neurotrophin-enriched hydrogels improve human iPSC-derived dopaminergic graft survival and differentiation in athymic nude rats, but not in cyclosporine immunosuppressed rats
Tommy Patton, University Of Galway, Ireland

10
Potential of viral-vector delivery of BMP2 as a Neurotrophic Therapy for Parkinson's Disease
Noelia Morales-Prieto, University College Cork, Ireland

11
Exploring direct lineage reprogramming of human glia to neurons in vitro and after transplantation in a rat model
Mette Habekost, Lund University, Sweden

12
Synuclein aggregates drive different microglial responses to host-to-graft pathology in stem cell-derived transplants in a humanized graft model of PD
Sara Corsi, Lund University Sweden

13
Investigation of the functional integration of hESC-derived grafts in models of Huntington's disease
Patricia Garcia Jareño, Cardiff University, United Kingdom

Data Blitz session III

14
Novel AAV Capsid Variants Targeting Human Glia
Jessica Giacomoni, Lund University, Sweden

15
Allogeneic Regenerative Cell Therapy for White Matter Stroke and Vascular Dementia
Irene L. Llorente, UCLA, USA

16
CSF and serum biomarkers in human neural stem cell-treated Secondary Progressive Multiple Sclerosis patients
Giada D'Aloisio, Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy

17
Modelling Familial ALS Pathology In Vivo
Georgia Eleftheriou, Florey Institute of Neuroscience & Mental Health, Melbourne, Australia

18
Glial dysregulation and SVZ neurogenesis disruption following brain chemical lesion
Irini Thanou, Hellenic Pasteur Institute, Athens, Greece

Data Blitz session IV

19
Bacterial DING proteins in blood serum of Alzheimer's disease and Mild Cognitive Impairment patients and correlation with Aβ42 and COX-1/2
Eleni Andreadou, Aristotle University of Thessaloniki, Greece

20
Using biomaterials to provide local immunosuppression for CNS cell transplantation
Emily Atkinson, University College London, United Kingdom

21
Towards the generation of better Parkinson's in vitro models: human LMX1A tracing line for culture enrichment on mDA neural progenitors
Lucia F. Cardo, Dementia Research Institute at Cardiff, UK

22
Can viral-mediated dopamine synthesis alleviate cognitive impairments in Parkinson's Disease?
Charlotte Bridge, Cardiff University, United Kingdom

23
Cholesterol based-strategies lead to a complete and long-standing recovery of cognitive and motor defects in Huntington's disease mouse models
Marta Valenza, University of Milan, Italy

e-POSTERS

e-Poster session I

P1
Peripheral administration of the Class-IIa HDAC inhibitor MC1568 partially protects against nigrostriatal degeneration in the striatal 6-OHDA rat model of Parkinson's disease
Martina Mazzocchi, Ucc, Cork, Ireland

P2
The Edinburgh Human Progenitor Cell Bank: Cryopreserved iPSC-derived progenitor cells for the neuroscience community
Michela I. Barbato, University of Edinburgh, United Kingdom

P3
Human iPSC-derived reactive astrocytes display an altered metabolic profile compared to quiescent-like astrocytes
Sarah F. McComish, Trinity College Dublin, Ireland

P4
Functional characterization of stem cell-derived dopamine neurons for the treatment of Parkinson's Disease
Ryan Smith, BlueRock Therapeutics, New York, United States

P5
The p.A53T α-synuclein mutation causes dopaminergic neuron deficit in patient-derived midbrain organoids
Olympia Apokotou, Hellenic Pasteur Institute, Athens, Greece

e-Poster session II

P6
Secondary progressive multiple sclerosis: in vitro effect of cerebrospinal fluid on human neural stem cells behavior and immunomodulatory molecules production
Lucrezia Abate, Ospedale Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy

P7
Intracerebroventricular transplantation of Neural Stem Cells in an experimental model of Amyotrophic Lateral Sclerosis
Ivan Lombardi, University of Milano-Bicocca, Italy

P8
Defining the consequence of LRRK2 dysregulation in human ESC-derived astrocytes
Áine Heffernan, University of Edinburgh, UK

P9
The hidden roles of SLITRKS in p.A53T-αSyn synaptic dysfunction
E.K. Akrioti, Hellenic Pasteur Institute, Athens, Greece

e-Poster session III

P10
Exploring the neuro cardiac axis - Defining local mechanisms and long-range signals that promote organ regeneration
Vasiliki Tsata, Biomedical Research Foundation of the Academy of Athens, Greece

P11
Age-related neuronal autophagy changes in induced neurons
Mr Balázs Kis, Hcemm-su Neurobiology & Neurodegenerative Diseases Research Group, Budapest, Hungary

P12
The LEARN study: using participant experience to improve trial design for neurodegenerative diseases
Drew C.J.G, Cardiff University, UK

P13
Longitudinal Behavioural Characterisation of a Transgenic Rat Model of Huntington's Disease
Olivia Edwards, Cardiff University, UK

P14
Mirk/Dyrk1B kinase controls the generation and columnar organization of spinal motor neurons in the embryonic chick spinal cord via Sonic hedgehog pathway
Kokkorakis N., Hellenic Pasteur Institute, Athens Greece

e-Poster session IV

P15
Extra Virgin Olive Oil (EVOO) attenuates LPS-induced inflammatory effect on a 2D model of neuroblastoma and glioblastoma murine cells
Tzekaki E., Aristotle University of Thessaloniki, Greece

P16
Alleviating Effect of Curcumin and a V (IV) – Curcumin complex on LPS-Induced Inflammation in Murine Mixed Neuroblastoma-Glioblastoma Cultures
Tzekaki E., Aristotle University of Thessaloniki, Greece

P17
Molecular lineage tracing of stem cell derived dopamine grafts in vivo reveals a shared origin of all graft-derived cells
Petter Storm, Lund University, Sweden

P18
Disrupted hippocampal neurogenesis and increased neuroinflammation following brain chemical lesion
Mirka Fourmouzi,, Hellenic Pasteur Institute, Athens, Greece

P19
The inhibition and activation of Notch signaling upon differentiation of human induced pluripotent stem cells to dopaminergic precursors
Katolikova Nataliia Victorovna, Saint-Petersburg State University, Russia

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Abstracts

Speakers' abstracts

Restoration of brain cholesterol metabolism as gene therapy approach for Huntington's Disease

Sandro Alves[1*], Emilie Rey[1,2], Romina Aron-Badin[3,4], Gaetan Despres[5], Antonin Lamazière[5], Philippe Hantraye[3,4], Nathalie Cartier[1,2]

[1]Asklepios BioPharmaceutical (AskBio), Institut du Cerveau (ICM), 75013, Paris, France;
[2]INSERM U1169/MIRCen CEA 92265 Fontenay aux Roses and Université Paris-Sud, Université Paris Saclay, 91400 Orsay, France;
[3]Commissariat à l'energie atomique, Direction de la Recherche Fondamentale, Institute of Biology François Jacob, Molecular Imaging Research Center, Fontenay-aux-Roses, France
[4]Université Paris-Saclay, Commissariat à l'energie atomique, CNRS, Neurodegenerative Diseases Laboratory (UMR9199), Fontenay-aux-Roses, France.
[5]Laboratory of Mass Spectrometry, INSERM ERL 1157, CNRS UMR 7203 LBM, Sorbonne Universités, Pierre et Marie Curie- Paris 6, Paris, France

Impaired cholesterol homeostasis is commonly identified in severe brain disorders. In Huntington's disease (HD), impaired brain cholesterol metabolism is a consequence of mutant Huntingtin (mHTT) deposition and a primary driver of HD hallmarks. Cholesterol 24-hydroxylase (CYP46A1) is a key neuronal enzyme of cholesterol metabolism that is decreased in HD patients' brains. CYP46A1 is essential for efflux of excess cholesterol from neurons, thus controlling cholesterol synthesis, and is a major neuronal stress response factor in pathological circumstances. In HD, reduced CYP46A1 is associated with extensive cholesterol accumulation in membranes from neurons with several consequences: impaired synaptic transmission and vesicular transport, compromised autophagy/proteasome clearance of misfolded mHTT and impaired energy metabolism.
Preclinical studies have shown that re-establishing CYP46A1 levels and thus restoring cholesterol metabolism in striatal neurons using an AAV vector, significantly mitigated neuronal dysfunction, improved spine density and reduced mHTT accumulation through enhanced autophagy. In addition, it was associated with improved motor function in HD mice models.
Dose-response studies with intra-striatal AAV-CYP46A1 delivery in R6/2 mice and biodistribution-safety studies in non-human primates (NHPs) have been conducted. In NHPs, AAV-CYP46A1 was administered stereotaxically into the caudate nucleus and putamen of normal NHPs. Conditions (different volumes, titers, number of injections) were optimized to obtain sufficient coverage of transgene expression for potential therapeutic application. In particular, biodistribution, local tolerance after vector administration using different volumes and vector concentrations were assessed after local injection in caudate and putamen. In a translational perspective, AAV-CYP46A1 delivery in the NHP caudate and putamen demonstrated that the procedure was tolerated and increased 24-OHC levels, thus confirming target engagement.
Altogether, we believe these results support CYP46A1 as a relevant therapeutic approach that has demonstrated the potential to confer neuroprotection and potentially broad improvement in cellular functions thus providing a proof-of-concept signal for clinical application in HD patients.

The power of ONE: Immunology in the age of single cell genomics

Ido Amit
Weizmann Institute of Science

The immune system is a complex, dynamic and plastic network composed of various interacting cell types that are constantly sensing and responding to environmental cues. From very early on, the immunology field has invested great efforts to characterize the various immune cell types and elucidate their functions. However, accumulating evidence indicates that current technologies and classification schemes are limited in their ability to account for the functional heterogeneity of immune processes. Single cell genomics hold the potential to revolutionize the way we characterize complex immune cell assemblies and study their spatial organization, dynamics, clonal distribution, pathways, and crosstalk. This emerging field can greatly affect basic and translational research of the immune system. I will discuss how recent single cell genomic studies are changing our perspective of various immune related pathologies from cancer to autoimmune disease and neurodegeneration. Finally, I will consider recent and forthcoming technological and analytical advances in single cell genomics and their huge potential impact on the future of immunology research and immunotherapy.

The Essentialome of Human Development and Disease

Nissim Benvenisty
The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel

Human pluripotent stem cells (hPSCs) have a major role in investigating human development, and in modeling genetic disorders. We have recently generated haploid hPSCs carrying only one set of chromosomes. Interestingly, we found that a haploid human genome is compatible not only with the undifferentiated pluripotent state, but also with differentiated somatic fates representing all three embryonic germ layers Furthermore, we demonstrated the superior utility of haploid hPSCs for loss-of-function genetic screening. To define the essentialome of hPSCs we generated a genome-wide loss-of-function library in the haploid cells utilizing CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting virtually all coding genes. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. We could also allude to an intrinsic bias of essentiality across cellular compartments, uncover two opposing roles for tumor suppressor genes and link autosomal-recessive disorders with growth retardation phenotypes to early embryogenesis. More recently, we set out to map the essential genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm, defining the essentialome of each germ layer separately and also identified commonly essential genes for the transition from pluripotency stage into differentiated cells. Our data also enabled analysis of all hereditary neurological disorders, uncovering essentiality of a significant fraction of microcephaly-causing genes during neuroectoderm development. In addition to defining the essentialome of early human development, we utilized the genome-wide screening to identify genes that can reverse the phenotype of imprinting and neurological disorders. We focused on chromatin-related genes that their mutation can induce a normal molecular phenotype, and validated their effect in diseased cells, as a way to suggest novel therapies. Overall, our work sheds light on the gene networks regulating pluripotency, early gastrulation, and human genetic disorders by utilizing genome-wide screening in hPSCs.

Pluripotent stem cell-based therapy for outer retinal diseases

Olivier Goureau, PhD
Research Director, Team leader Retinal Development and Repair Institut de la Vision, Sorbonne Université, INSERM, CNRS Paris, France

Inherited and acquired degenerative diseases of the outer retina, characterized by the death of light-sensitive photoreceptors, are a significant cause of incurable vision loss worldwide. Rescuing the degenerated retina is a major challenge for which human induced pluripotent stem cells (iPSCs) offer a wide range of applications from cell transplantation to disease modelling. We elaborated innovative protocols for the production of self-forming retinal organoids from human iPSCs. Initially composed of retinal progenitor cells, these organoids can be maintained in 3D cell cultures to generate all retinal cell types, including photoreceptors. We will present recent strategies based on the use of human iPSC-derived retinal organoids to isolate transplantation-competent cells such as photoreceptor precursors for cell replacement and to develop new strategies for visual restoration by combining stem cell-based therapy and optogenetics. We will discuss how the use of iPSC-derived retinal pigment epithelium (RPE) is also used to target retinal diseases affecting RPE with stem cell-based therapies already in clinic trials.

Designing brain-on-chip platforms to simulate human brain function and malfunction

Achilleas Gravanis
Dept. of Pharmacology, School of Medicine University of Crete, IMBB-FORTH, Heraklion Greece

One of the challenges in developing new therapies for diseases of human brain is the lack of reliable animal models which effectively simulate human brain function. The recent significant advances in human stem cell technologies, polymer and material sciences, and nanobioengineering were an impetus to develop ex vivo microdevices and human cell-on-a-chip platforms, populated with human neuronal and glia cells, as experimental models of human brain pathologies. These microdevices are reliable platforms to study the pathophysiology (cell to cell interactions, cellular and molecular dynamics, proteomic and genomic analysis) of human brain diseases and use them for screening of drugs on a biological system of human origin. We are developing human brain-on-a-chip platforms to study neuroinflammation, blood brain barrier permeability, spinal cord or optic nerve injury. Additionally, we use these platforms to test our proprietary compounds, neurotrophin receptor agonists, with neuroprotective, anti-neuroinflammatory and neurogenic properties and potential therapeutic applications in the above brain pathologies.

Artificial Extracellular Matrix Scaffolds Enhance Maturation and Ageing of Human Stem Cell-Derived Neurons and Enable the Modeling of Neurodegenrative Disease Pathology

Evangelos Kiskinis
Assistant Professor of Neurology, Feinberg School of Medicine, Northwestern University, Chicago USA

Human induced pluripotent stem cell (iPSC) technologies offer a unique resource for modeling neurological diseases. However, iPSC models are fraught with technical limitations including abnormal aggregation and inefficient maturation and ageing of differentiated neurons. These issues are in part due to the absence of synergistic cues derived from the architecture, chemical composition and molecular dynamics of the native extracellular matrix (ECM). To solve these problems we purified the ECM from the spinal cord of early postnatal and adult mice and profiled its protein composition by mass spectrometry (MS)-based proteomics. We identified several candidate proteins that were signficinalty more abundant in aged ECM, including laminin alpha 1 chain. We hypothesized that laminin alpha 1, which engages with neuronal surface receptors through the bioactive pentapeptide sequence IKVAV, might enhance the maturation of neurons. We functionalized IKVAV on three artificial ECMs based on supramolecular nanofibers containing peptide amphiphile (PA) molecules. All nanofibers displayed on their surface the same IKVAV signal but differed in the nature of their non-bioactive domains. We found that nanofibers with greater intensity of internal supramolecular motion had enhanced bioactivity toward iPSC-derived motor and cortical neurons. Proteomic, biochemical and functional assays revealed that scaffolds with highly mobile molecules lead to enhanced b1-integrin pathway activation, reduced aggregation, increased arborization, and mature electrophysiological activity of neurons. Neurons cultured on the ECM mimetic PA for an extended time exhibited an increase in markers of ageing including DNA damage and oxidative stress. Critically, neurons derived from ALS patients harboring a disease-causing mutation in the *SOD1* gene exhibited pronounced neuropathology including aggregation of ubiqutinated SOD1 protein. Our work resolves long-standing limitations of culturing iPSC-derived neurons, and highlights the importance of designing bioactive ECMs to study the development, function and dysfunction of human neurons in vitro.

Potential of Multiple Sclerosis iPS-derived oligodendroglia to functionally interact with axons and glia in vivo

Sabah Mozafari [1] , Laura Starost [2], Blandine Manot-Saillet [3], Beatriz Garcia-Diaz [1], Yu Kang T. Xu [4], Delphine Roussel [1], Marion J. F. Levy [1], Linda Ottoboni [5], Kee-Pyo Kim [2], Hans R. Schöler [2], Timothy E. Kennedy [4], Jack P. Antel [4], Gianvito Martino [5], Maria Cecilia Angulo [3], Tanja Kuhlmann [2], Anne Baron-Van Evercooren[1]

1) ICM-GH Pitié Salpêtrière, INSERM U1127, CNRS UMR 7225, Sorbonne University, Paris, France. 2) Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany. 3) Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM, Université de Paris, France. 4) Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Canada. 5) Institute of Experimental Neurology-DIBIT 2, Division of Neuroscience, IRCCS San Raffaele Hospital and Vita San Raffaele University, Milan, Italy.

Failed remyelination in multiple sclerosis (MS) leads to altered conduction followed by axon degeneration, which, in the long run, results in severe and permanent neurological deficits. Remyelination failure in MS is associated with a migration/differentiation block of oligodendroglia. The reason for this block is highly debated. It may result from adverse environmental (extrinsic) conditions or the failed capacity of oligodendrocyte progenitors/pre-oligodendrocytes to migrate or mature efficiently into myelin-forming cells (intrinsic) or even a combination of these conditions, all of which may worsen with aging. To avoid confounding immune-mediated extrinsic effect, we used an immune-deficient mouse model to compare induced pluripotent stem cell–derived oligodendroglia from MS and healthy donors following engraftment in the developing CNS. We show that the MS-progeny behaves and differentiates into oligodendrocytes to the same extent as controls. They generate equal amounts of myelin, with bona fide nodes of Ranvier, and promote equal restoration of their host slow conduction. The MS-progeny expressed oligodendrocyte- and astrocyte-specific connexins and established functional connections with donor and host glia. Thus, MS oligodendroglia, regardless of major immune manipulators, are intrinsically capable of myelination and making functional axo-glia/glia-glia connections, reinforcing the view that the MS oligodendrocyte differentiation block is not from major intrinsic oligodendroglial deficits. This makes MS oligodendroglia candidates of interest for personalized drug/cell therapies as pluripotency maintains MS oligodendroglial cells in a genuine “non-pathological” state. This work was supported by the Progressive MS Alliance, the National MS Society and the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS).

Molecular fuels and drivers of smouldering brain disease in progressive multiple sclerosis

Stefano Pluchino
Dept of Clinical Neurosciences, University of Cambridge

Advances in healthcare have significantly increased the life expectancy of populations across the globe. As a result, the total number of people over the age of 65 is expected to double by 2050. This will lead to an increase in the number of people developing age-related neurodegenerative disorders, which will result in an enormous financial burden on health facilities to manage and care for these patients. Decades of research and billions of dollars devoted to studying neurodegenerative diseases has had little success. Currently no treatments exist that can reverse or slow the progression of neurodegenerative disease. However, one feature present across all these diseases is smouldering inflammation, which is peculiar type of persistent, diffuse, low level inflammation affecting the whole brain. Smouldering inflammation is confined to the brain, it is not associated to changes in the peripheral immune system, does not resolve, and plays a major role in preventing the repair processes in the brain from operating normally. As such, the underlying (neurodegenerative) disease continues to progress leading to permanent, irreversible tissue degeneration and neuronal loss.
New research into how cells acquire the fuel needed to maintain smouldering inflammation in the brain is ongoing in our lab. Here, I will discuss of how advanced techniques, such as conditional gene editing in animal disease models and human cells, human stem cell-based disease modelling, mini-brain technology, and computer-aided analytical processing are helping to further uncover the main fuels and drivers of smouldering inflammation.
Our goal is to discover new biological pathways towards the development of targeted precision medicines to stop the permanent, irreversible accumulation of brain damage in neurodegenerative diseases in an effort that shall help preserving the neurological reserve of the brain.

Physiopathological TDP-43 transitions and novel RNA targets with key roles in human disease

Magdalini Polymenidou, PhD

Department of Quantitative Biomedicine, University of Zurich, CH
email: magdalini.polymenidou@uzh.ch
website: https://www.polymenidoulab.com

ALS and FTD are fatal and incurable neurodegenerative diseases, characterized by accumulation of pathologic forms of RNA-binding proteins, predominantly TDP-43. The functional consequences and potential neurotoxic effects of the highly heterogeneous TDP-43 aggregates observed in postmortem brains are debated. Combining structural protein analysis with cellular systems we uncovered a novel and unexpected mechanism that counteracts pathologic aggregation of TDP-43 (1). More recently, we described two distinct mechanisms of aggregation resulting in either nuclear of cytoplasmic TDP-43 aggregates, resembling the neuropathological heterogeneity described in FTD patients (2) and potentially unravels the origins of heterogeneous pathological species occurring in TDP-43 proteinopathies. We also showed that FTD heterogeneity is associated with alternate pathological TDP-43 conformations, reminiscent of prion strains (3). We used advanced microscopy techniques to explore the differences in the physical and seeding properties between these pathological TDP-43 aggregates. We found that the subcellular environment and organization of TDP-43 aggregates differs in patient brains in a subtype-specific manner. When isolated and introduced in cells, these distinct TDP-43 assemblies triggered neoaggregate formation with seeding potencies and morphologies that mimicked human brain pathology (4). To study neurodegeneration phenotypes in human neurons, we developed a new methodology for generating human neuronal networks with remarkable maturity, longevity and reproducibility. Combining high density multielectron arrays and single cell RNA sequencing, we demonstrated the potential of these cultures for modeling neurodegeneration. In this new model, we identified novel RNA targets of TDP-43, which we found abnormally accumulated in neurons with TDP-43 pathology in human brains of ALS and FTD patients (5). The implications of these studies for understanding the mechanisms of neurodegeneration and inspiring therapeutic approaches will be discussed.

1. *Afroz T, Hock EM*, Ernst P*, Jambeau M, Foglieni C, Laferriere F, Gilhespy L, Maniecka Z, Pluckthun A, Mittl P, Paganetti P, Allain FHT, Polymenidou M (2017) Functional and dynamic polymerization of the ALS-linked protein TDP-43 antagonizes its pathologic aggregation Nature Communications, 8(1):45*
2. *Pérez-Berlanga M, Wiersma V, Zbinden A, De Vos L, Wagner U, Foglieni C, Mallona I, Betz K M, Cléry A, Weber J, Guo Z, Rigort R, De Rossi P, Manglunia R, Tantardini E, Sahadevan S, Stach O, Hruska-Plochan M, Allain F H.-T, Paganetti P, Polymenidou M, (2022) TDP-43 oligomerization and RNA binding are codependent but their loss elicits distinct pathologies. BioRxiv. https://doi.org/10.1101/2022.05.23.493029*
3. *Laferriere F*, Maniecka Z*, Pérez-Berlanga M, Hruska-Plochan M, Gilhespy L, Wagner U, Afroz T, Boersema PJ, Barmettler G, Foti SC, Asi Y T, Isaacs A, Al-Amoudi A, Lewis A, Stahlberg H, Ravits J, De Giorgi F, Ichas F, Bezard E, Picotti P, Lashley T, Polymenidou M (2018) TDP-43 extracted from FTLD patient brains displays distinct aggregate assemblies and neurotoxic effects reflecting disease progression rates, Nature Neuroscience, 22, 65-77*
4. *De Rossi P, Lewis AJ, Furrer J, De Vos L, Wiersma VI, Demeter T, Zhong W, Zbinden A, Weber J, Guo Z, Scaramuzza S, Di Fabrizio M, Böing C, Castano-Diez D, Alamoudi A, Pérez-Berlanga M, Lashley T, Stahlberg H, Polymenidou M Pathological TDP-43 assemblies extracted from FTLD patient brains seed neoaggregates with distinct properties following a prion-like cascade, in preparation*
5. *Hruska-Plochan M, Hembach KM, Ronchi S, Wiersma V.I, Maniecka Z, Hock EM, Laferrière F, Sahadevan S, Hoop V, Delvendahl I, Panatta M, van der Bourg A, Bohaciakova D, Frontzek K, Aguzzi A, Lashley T, Robinson MD, Karayannis T, Mueller M, Hierlemann A, Polymenidou M. (2021) Human neural networks with sparse TDP-43 pathology reveal NPTX2 misregulation in ALS/FTLD. BioRxiv https://doi.org/10.1101/2021.12.08.471089*

hiPSC derived Microglia-like cells as a model for Neurodegenerative diseases

Vijitha Sathiaseelan
Group Leader, Axol Biosciences

Microglia are the resident immune cells of the central nervous system (CNS). These cells are derived from yolk sac progenitors that arise during the primitive hematopoiesis. Microglia are involved in the immune defence of the brain, regulating inflammation, phagocytosis and repair of brain tissue.
Recent studies have indicated a relationship between microglia and neurodegenerative disease, with several genes identified as playing a role; TREM2, CD33 and GBA. Modelling this cell type *in vitro* may provide important insights into the role that microglia play in neurodegeneration as well as providing a platform for compound testing and drug discovery.
Isolation of human microglia is not common practice and use of this material in drug discovery is limited by quantity, ethics, reproducibility and supply. Many researchers use rat or mouse derived microglial tissue or aim to mature human peripheral monocytes to microglia by the addition of cytokines but models cannot be effectively translated to human pathophysiology.
Using iPSC technology, it is possible to differentiate human iPSCs to microglia-like cells. This cell type provides a robust and reproducible disease model for the characterization of clinically relevant mutations as well as for compound screening and drug discovery. We describe data on cell surface expression profiles, IL1β activation and phagocytosis
Axol bioscience's human Microglia-like cells have been extensively characterised and are available for clients to use in drug discovery projects.

Modeling Parkinson's disease with human midbrain organoids

Jens C. Schwamborn
University of Luxembourg

In Parkinson's disease (PD) patients the dopaminergic neurons of one region in the midbrain, the substantia nigra, are highly vulnerable for degeneration, while the dopaminergic neurons of a neighboring region, the ventral tegmental area, are not. The reasons for this selective vulnerability are largely unknown to date. This lack of knowledge, to a good extend is the consequence of the absence of human specific models for the midbrain.
Here, we demonstrate that three-dimensional (3D) differentiation of expandable human midbrain floor plate neural progenitor cells (mfNPCs) leads to organoids that resemble key features of the human midbrain. These organoids are composed of midbrain dopaminergic neurons (mDANs), which produce and secrete dopamine. Additionally, the midbrain organoids contain other neuronal subtypes, astrocytes and oligodendrocytes. They can be further enriched with induced pluripotent stem cell (iPSC) derived microglia. Patient and disease specific midbrain organoids can be generated through the usage of patient derived iPSCs. Importantly, in these disease specific organoids, key hallmarks of PD including reduced numbers of dopaminergic neurons and appearance of alpha-Synuclein positive protein aggregates are recapitulated. Thus, we provide a robust method to reproducibly generate 3D human midbrain organoids containing mDANs to investigate PD-relevant patho-mechanisms.

R&D of iPS cell-based therapy for stroke

Jun Takahashi, MD,PhD
Center for iPS cell research and application (CiRA), Kyoto University, JAPAN

Many patients suffer from motor and/or speech disturbance after stroke. Although medical treatment and rehabilitation are aggressively implemented, functional impairment often remains as a permanent sequela. In recent years, mesenchymal stem cells have been attempted to treat stroke, but these are expected to have cytokine and anti-inflammatory effects by the grafted cells, not reconstructing neural circuits. Although I do not deny these therapies, they are conceptually the same as conventional therapies. Therefore, we are conducting developmental research to reconstruct corticospinal tracts with transplanted cells. To this end, we performed serum-free three-dimensional floating culture (SFEBq method) using human ES/iPS cells and induced cerebral organoids by inhibiting TGFβand Wnt signals. The 6-week organoids showed better cellular engraftment and more axonal extension along the corticospinal tracts compared to the 10-week organoids. However, the grafts were larger because they contained more proliferating neural progenitor cells, so optimizing the donor cells and host brain environment is a future challenge.

Neuroinflammation and astrocytes in Parkinson's disease

Era Taoufik[1]
1 Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece

Alpha-synuclein (αSyn) dysregulation leads to profound neurodegeneration, however our knowledge of the molecular/biochemical events that initiate disease-related processes remains limited. Therefore there is an imminent need to understand how αSyn triggers degeneration, how pathology evolves, what primary pathways are impaired, and ideally if these mediators can serve as biomarkers or disease intervention points. In our team we integrate knowledge derived from p.A53T αSyn mutant mice and 2D/ 3D cellular systems derived from patients bearing the p.A53T mutation to determine early events in αSyn pathology. We have selected to exploit systems that express the p.A53T-αSyn mutation (Polymeropoulos et al., 1997), as they show accelerated aggregation kinetics and a number of early distorted molecular pathways in neurons. Transcriptomics and proteomics approaches have revealed alterations in core cellular metabolic pathways including RNA metabolism, lipid and protein biosynthesis, proteostasis and synaptogenesis (Kouroupi et al, 2017; Antoniou et al, 2020). In follow-up studies, we examine in detail both mouse and human p.A53T neuronal aberrant connectivity and alterations in the numbers of excitatory and inhibitory synaptic contacts. The contribution of glial cells (astrocytes and microglia) in neuronal dysfunction are also assessed in both mouse models of PD and cell-based systems. Altogether, the combinatorial analysis of cellular and *in vivo* models of p.A53T-αSyn pathology allows us to gain a better insight in the spatiotemporal events αSynuclein mediated dysfunctions.

Acknowledgements: *Funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project Number: 1019; to RM); the G.S.R.I. and the H.F.R.I. under the "1st Call for H.F.R.I. Research Projects to support Postdoctoral Researchers" (PARKINSynapse 899; to GK); the Hellenic General Secretariat for Research and Innovation (G.S.R.I.) Flagship Action for Neurodegenerative Diseases on the basis of Personalized Medicine (2018ΣΕ01300001)*

Assay development and improving reproducibility using microfluidic compartmentalized devices

Anne Marion Taylor, Ph.D.,
Xona Microfluidics, Inc.

Microfluidic compartmentalized devices have facilitated neuroscience research for almost two decades. These devices allow unprecedented access to axons and synapses for measurements and manipulations. Traditionally, these microfluidic devices are made in poly(dimethylsiloxane) (PDMS), but more advanced versions are injection molded in the optically transparent plastic, cyclic olefin copolymer (COC). COC overcomes many of the limitations of PDMS, such as small molecule adsorption and device-to-device reproducibility. Numerous assays have been developed using compartmentalized devices, including assays focused on trans-synaptic propagation of tau, axon regeneration following injury, and mitochondrial transport in axons. In this talk I will discuss our work using these devices to investigate axotomy-induced synaptic remodeling and transport of mitochondria. I will also present our newest device configuration that reduces the footprint of the device, thereby reducing the number of neurons needed and increasing scalability.

Acknowledgements and funding:
A.M.T. acknowledges support from NIH/NINDS (R21 NS109750) and NIH/NIA (R43 AG072985). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. A.M.T. is an inventor of the microfluidic device technology and an owner of Xona Microfluidics, Inc.

Direct astrocyte to neuron reprograming: towards a therapeutic strategy for brain injuries

Dimitra Thomaidou
Hellenic Pasteur Institute, Greece

Direct astrocytic reprogramming to induced-neurons (iNs) is a powerful approach for manipulating cell fate, as it takes advantage of the intrinsic neural stem cell (NSC) potential of reactive astrocytes, while it offers the possibility of reprogramming resident brain cells. To this end astrocytic cell fate conversion to iNs has been well-established in vitro and in vivo using combinations of transcription factors (TFs) or chemical cocktails, while miRNAs have been introduced, supplementary to TFs to instruct direct neuronal reprogramming. The neurogenic miRNA, miR-124 in particular has been utilized along with other TFs and miRNAs to support cell fate conversion, however its independent reprogramming potential and mechanism of action have not been explored. Our *in vitro* analysis indicates that miR-124 is a potent driver of the reprogramming switch of astrocytes towards an immature neuronal fate, by directly targeting the RNA-binding protein Zfp36l1 implicated in ARE-mediated mRNA decay and subsequently de-repressing Zfp36l1 neurogenic interractome. To this end miR-124 contribution in iNs' production largely recapitulates endogenous neurogenesis pathways, being further enhanced upon addition of the neurogenic compound Isoexazole-9 (ISX9), which improves both miR-124-induced reprogramming efficiency and iNs' functional maturation. Importantly, miR-124 is potent to guide direct conversion of reactive astrocytes to immature iNs of cortical identity *in vivo* following cortical trauma, confirming its 'master reprogramming' capacity within the injured cortical microenvironment, while ISX9 supplementation confers a survival advantage to newly produced iNs. However, the synergistic effect of miR-124/ISX9 does not seem to be sufficient to drive iNs' full maturation, indicating that more intrinsic and/or extrinsic cues are required. To this end focusing on the common transcriptional and post-transcriptional downstream effectors of miR-124/ISX9 action in the future, holds promise for the establishment of a more efficient *in vivo* reprogramming protocol to improve maturation of iNs and their functional integration in the host circuit following trauma or neurodegeneration.

Neural Stem Cells Form Neural Relays Across Sites of Spinal Cord Injury: Prospects for Human Translation

Mark H. Tuszynski[1,2], Ephron Rosenzweig[1], Jacob Koffler[1], John Brock[1,2], Paul Lu[1,2]
[1]*Department of Neurosciences, University of California – San Diego, La Jolla CA,*
[2]*Veterans Administration Medical Center, La Jolla, CA*

Neural stem cell grafts to sites of SCI form new neural relays across the injury: host axons regenerate *into* the stem cell graft and form synapses, and neural stem cell-derived axons extend *out from* the lesion site and into the host spinal cord distal to the injury, forming synapses with the denervated host. Conduction across the lesion site is re-established, improving functional outcomes.

Grafts of neural stem cells to sites of spinal cord injury extend extremely large numbers of axons over very long distances. While regeneration of injured adult axons is severely restricted in spinal cord white matter, myelin *stimulates* the growth of axons emerging from neural stem cell grafts, a finding related in part to expression of the cell adhesion molecule *Negr1*.

All host axonal systems that normally innervate the spinal cord also innervate neural stem cell grafts placed in the lesion site, as established by trans-synaptic rabies virus labeling. Moreover, host axons regenerating into the graft are generally *guided* to appropriate neuronal targets, and avoid inappropriate targets within the developing graft.

Extension of this work to non-human primates confirms findings from rodent models, and shows that neural stem cell grafts improve functional outcomes in the rhesus monkey. Based on this collective set of findings, the work is on a translational path to humans.

Future work will combine neural stem cell grafts with neuromodulation and bioengineered scaffolds, testing the hypothesis that additional guidance and training of re-forming neural circuits will further improve functional outcomes.

Supported by the Veterans Administration, NIH, Department of Defense, Craig H. Neilsen Foundation, Wings for Life, the Adelson Foundation and the Spitzer Foundation.

Untangling the mechanisms underlying Multiple System Atrophy pathogenesis

Maria Xylouri
Biomedical Research Foundation of the Academy of Athens, Greece

A growing body of evidence, encompassing neuropathology studies in human brain, animal models of concomitant proteinopathies and studies employing sophisticated methods of probing protein-protein interaction, cumulatively suggest that the neuronal protein alpha-Synuclein is well positioned to exert a strong influence on the pathogenesis of the neurodegenerative comorbidities. Accumulation of alpha-Synuclein-positive inclusions in neurons and oligodendrocytes is the main histopathological hallmark of Parkinson's disease (PD) and multiple system atrophy (MSA), respectively. Up to date, the unique ectopic alpha-synuclein expression in MSA oligodendrocytes remains an enigma in the field, since mature oligodendrocytes are considered to express low to non-detectable levels of the protein under basal conditions. Importantly, our recent published work identified the endogenous oligodendroglial alpha-synuclein and TPPP/p25α as major culprits for the development of MSA pathology *in vitro* and *in vivo*, pinpointing that manipulation of the expression of both proteins in oligodendrocytes, may provide a rationale approach to combat their accumulation and the progression of MSA.
In the current talk, recent advances in regards to the clearance (focusing mostly on the autophagy-lysosome pathway), release (occurring partly via small nanovesicles called exosomes), uptake and seeding of alpha-synuclein and TPPP/p25α, the two main components of the glial cytoplasmic inclusions (GCIs) found in MSA brains, will be discussed.
Elucidation of these pathways and the key culprits underlying MSA initiation and progression could unravel avenues for developing novel therapeutic strategies for neurodegenerative diseases in general.

Data Blitz session I

1

Peripheral Immune Cells Induce Reactive Phenotype in Human iPSC-derived Midbrain Astrocytes

Mrs Adina MacMahon Copas[1,2], Dr Sarah McComish[1,2], Katarzyna Plesniar[1], Daniel Ivers[1], Anna Stricker[1], Harriet O'Leary[1], Dr Jean M. Fletcher[3], Dr Maeve A. Caldwell[1,2]

[1]Department of Physiology, School of Medicine, Trinity College Dublin, Dublin, Ireland, [2]Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland, [3]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

Parkinson's disease (PD) is a chronic neurodegenerative disease characterised by accumulation of misfolded alpha-synuclein protein and the loss of dopaminergic neurons in the *substantia nigra pars compacta* of the midbrain.
Involvement of the inflammatory processes of astrogliosis and microgliosis in the pathogenesis of PD is well established. However, recent studies have demonstrated infiltration of peripheral immune cells into the brain parenchyma in PD, facilitated by a disrupted blood brain barrier. In particular, $CD4^+$ T cells have been observed in the post-mortem brain tissue of PD patients and their infiltration in animal models has been linked to neurodegeneration. However, the implications of this infiltration on astrocytes has yet to be fully elucidated.
We aimed to investigate this utilising iPSC-derived midbrain astrocytes (iPSC-Astros) from healthy controls. $CD4^+$ T cell conditioned media (CD4CM) was collected following stimulation with anti-CD3 and anti-CD28. iPSC-Astros were then stimulated with the CD4CM and their reactivity profile was assessed. Following evidence that CD4CM induced reactivity in the iPSC-Astros we aimed to determine the probable origin of this response.
There is increasing evidence supporting the role of the $CD4^+$ T cell subtype, Th17 in the pathogenesis of PD. IL-17 is the main secreted cytokine of Th17 cells and is demonstrated to synergise with other cytokines, such as TNFα, which is secreted by activated microglia and known to induce a reactive astrocyte phenotype.
In view of this, we stimulated the iPSC-Astros with IL-17, TNFα or a combination of the two. Although IL-17 had a negligible effect, a synergistic relationship between IL-17 and TNFα was observed with regards to astrocytic IL-6 secretion. In order to investigate the potential implications of chronic cytokine exposure on astrocyte reactivity and viability, responses at 72 hrs and 7 days were also assessed. Results suggest that duration of time has no effect on astrocyte reactivity however, cellular integrity may be comprised. Together this data provides a novel insight into the effect of peripheral immune cell infiltration on astrocyte reactivity in PD.

References

Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay J, Duyckaerts C, Flavell R, Hirsch E & Hunot S (2008). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. Journal of Clinical Investigation; DOI: 10.1172/jci36470.
MacMahon Copas A, McComish S, Fletcher J & Caldwell M (2021). The Pathogenesis of Parkinson's Disease: A Complex Interplay Between Astrocytes, Microglia, and T Lymphocytes?. Frontiers in Neurology; DOI: 10.3389/fneur.2021.666737.
Crompton L, McComish S, Stathakos P, Cordero-Llana O, Lane J & Caldwell M (2021). Efficient and Scalable Generation of Human Ventral Midbrain Astrocytes from Human-Induced Pluripotent Stem Cells. Journal of Visualized Experiments; DOI: 10.3791/62095.

Acknowledgements and Funding Health Research Board

2

Investigating the role of alpha-synuclein in neuronal innate immunity and its role in the interferon response pathway

Mr Andrew Chai[1], Dr Matthieu Vermeren[1], Dr Tilo Kunath[1]

[1]*University Of Edinburgh, Edinburgh, United Kingdom*

Alpha synuclein (αSyn), and the modified, oligomeric and fibrillar disease state conformations it adopts, is a major pathological molecular driver of dementia with Lewy bodies (DLB), Parkinson's disease (PD), and other synucleinopathies. However, αSyn's physiological role is undefined, leading to poor understanding of pathophysiological disease mechanisms which could contribute to αSyn-mediated neurodegeneration.

Recent evidence suggests αSyn has innate immune roles in protecting neurons from neurotrophic viruses, with mouse *in vivo* models showing increased susceptibility to viral-induced infection and death when αSyn is knocked-out. However, no functional studies in human disease models have investigated the innate immune role of αSyn in the CNS.

We investigated αSyn transcription, protein expression, and protein localisation, in human pluripotent stem cell (hPSC)-derived cortical neurons and neuroblastoma SH-SY5Y cells stimulated with viral mimics of infection and innate immune activators, such as Poly (I:C) and Type-I interferons. We showed that Type-I interferon (IFN) stimulation in human SH-SY5Y cells showed increased αSyn transcription, as well as increased intracellular αSyn protein concentration and extracellular αSyn secretion into conditioned media. We also showed that Type-I IFN stimulation in hPSC-derived cortical neurons increased nuclear localisation of αSyn in a subset of cortical neurons.

αSyn's changes due to innate immune stimulation may provide mechanistic understanding into αSyn's unique role in CNS innate immunity, and may reveal links between infection and αSyn-mediated neurodegenerative diseases such as DLB and PD.

3

Seeding of α-syn pathology in vivo involves the autophagy protein LC3

Prof. Scott Ryan[1], Natalie Trachsel[1], Dr. Morgan Stykel[1]

[1]*University Of Guelph, Guelph, Canada*

Parkinson's Disease (PD) is the most common movement disorder affecting over 10 million people worldwide. Etiology of PD involves the loss of dopaminergic (DA) neurons projecting from the substantia nigra to the striatum, coupled to the buildup of Lewy bodies consisting, in part, of α-synuclein (α-syn) aggregates. Recent evidence suggests that α-syn behaves in a prion like manner. In keeping with this hypothesis, we have characterized the movement of α-syn pathology from diseased human neurons harboring the α-syn-A53T variant to healthy, isogenic control neurons. To investigate the mechanism of cell-to-cell transmission, we assessed the spread of α-syn from human pluripotent stem cell-derived neurons expressing the α-syn-A53T variant (A53T hiPSC-derived DA neurons) to the striatum of healthy nod scid gamma mice following cell-engraftment into the striatum. Pathology was assessed by immuno-labeling of hyperphosphorylated α-syn (PS129). Analysis shows survival and integration of grafted neurons into the striatum and evidence of α-syn (PS129) transfer to the area immediately adjacent to the graft. We further investigated the mechanism of transfer in vitro, by isolating exosomes from A53T hiPSC-derived DA neurons. We visualized transfer of human α-syn from these exosomes to healthy primary neurons, as assessed by immuno-labelling of aggregated α-syn. Furthermore, we also investigated whether C-terminal truncations that disrupt binding of α-syn to the autophagy protein LC3B, alter the kinetics of secretion and seeding. Overall, our results suggest that human DA neurons seed α-syn pathology to unaffected tissue. These results may inform on the mechanism of α-syn seeding in synucleinopathies such as PD.

Acknowledgements and Funding
This work was supported in part by the Canadian Institutes of Health Research (2014-685 to SDR), the Natural Sciences and Engineering Research Council of Canada

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Developing assays for neuronal cell replacement in Parkinson's Disease and advancing next generation therapeutics

Dr Jonathan Niclis[1], Ms Josefine R Christiansen[1], Ms Veronica Castiglioni[1], Dr Kristian H de Lichtenberg[1], Dr Gunther Galler[1], Ms Simone M Jensen[1], Dr Hanni Willenbrock[1], Dr Maria Pereira[1], Dr Djordje Djordjevic[1], Dr Tess Lu[1], Ms Nuria Antiolin[1], Dr Henning Kempf[1], Dr Christian Honore[1], Dr Camilla Ingvorsen[1], Dr Nicolaj S Christophersen[1]

[1]Novo Nordisk, Copenhagen, Denmark

In collaboration with Prof. Malin Parmar and Assoc. Prof. Agnete Kirkeby at Lund University, Novo Nordisk is developing a cell therapy for the treatment of Parkinson's disease (PD). Specifically, we are using human embryonic stem cells to generate replacement ventral midbrain dopaminergic neurons with an efficient and GMP compliant protocol.
In moving towards clinical translation, this type of advanced cell product is required to undergo scrutinous testing to accommodate regulatory requirements that ensure safety and efficacy. This has led to Novo Nordisk developing an array of robust analytical tools and improving methods for dopamine differentiation. Insights gained from these assays indicate additional generations of PD cell therapies could yield enhanced safety and efficacy outcomes, which the organisation has begun to explore.

5

Enhanced Production of Mesencephalic Dopaminergic Neurons from Lineage-Restricted Human Undifferentiated Stem Cells

Dr Muyesier Maimaitili[1], Dr Muwan Chen[1], Dr Fabia Febbraro[1], Dr Noëmie Mermet-Joret[1], Johanne Lauritsen[1], Dr Ekin Ucuncu[1], Ida Hyllen Klæstrup[1], Dr Per Qvist[1], A/Prof Sadegh Nabavi[1], A/Prof Marina Romero-Ramos[1], **Dr Mark Denham[1]**

[1]Aarhus University, Aarhus, Denmark

The differentiation of human pluripotent stem cells (hPSCs) into mesencephalic dopaminergic (mesDA) neurons requires a precise combination of extrinsic factors that recapitulates the *in vivo* environment and timing. Current methods are capable of generating authentic mesDA neurons after long-term culture *in vitro*; however, when mesDA progenitors are transplanted *in vivo*, the resulting mesDA neurons are only minor components of the graft. In this study, we genetically modified pluripotent stem cells to generate a novel type of stem cells called lineage-restricted undifferentiated stem cells (LR-USCs), which robustly generate mesDA neurons. By knocking out genes that are critical for the specification of cells of alternate lineages, LR-USCs are prevented from differentiating into a broad range of nondopaminergic cell types. Specifically, we target transcription factors involved in the production of spinal cord and posterior hindbrain cell types. When LR-USCs are differentiated under caudalizing condition, which normally give rise to hindbrain cell types, a large proportion adopt a midbrain identity and develop into authentic mesDA neurons. We show that the mesDA neurons are electrophysiologically active, and due to their higher purity, are capable of restoring motor behavior eight weeks after transplantation into 6-hydroxydopamine (6-OHDA)-lesioned rats. This novel strategy improves the reliability and scalability of mesDA neuron generation for clinical use.

6

p.A53T-αSyn iPSC-derived astrocytes from Parkinson's disease patients display intrinsic pathology and affect dopamine neuron viability

Ms Christina Paschou[1], Ms Olympia Apokotou[2], Mr Anastasios Kollias[1], Dr Era Taoufik[1], Dr Rebecca Matsas[1], Dr Florentia Papastefanaki[1,2]

[1]Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, Athens, Greece, [2]Human Embryonic and Induced Pluripotent Stem Cell Unit, Hellenic Pasteur Institute, Athens, Greece

Parkinson's disease (PD) is a complex neurodegenerative disorder of 1-2% worldwide prevalence over the age of 60, with motor and non-motor manifestations[1]. Most PD cases are sporadic; however, 5-10% of cases have been linked with mutations in specific genes, such as the SNCA gene encoding for α-synuclein (αSyn). The best-characterized mutation is pA53T-αSyn (G209A in SNCA), causing a familial form of PD with early onset and severe phenotype[2]. Although the disease mechanisms remain largely unresolved, cell reprogramming technologies provide a unique human setting for the identification and interpretation of PD phenotypes. We have previously established an induced pluripotent stem cell (iPSC)-based neuronal model of PD from patients harboring the p.A53T-αSyn mutation, which displayed disease-associated phenotypes, including intraneuronal protein aggregates, axonal pathology, and reduced synaptic connectivity[3]. Here we sought to extend our studies and investigate the potential role of astrocytes in PD. Contrary to intensive research on neuron-intrinsic dysfunction, the contribution of other cell types in PD, particularly astrocytes, has lagged behind. Astrocytes, the most abundant cells in the human brain play critical roles in maintaining neuronal health whereas they may also exert neuroprotective or neurotoxic effects upon disease. In this context, we generated ventral midbrain-patterned astrocytes from p.A53T-αSyn patient and control iPSCs. We found that p.A53T-αSyn astrocytes distinctively displayed cytoplasmic vacuoles suggestive of ER-stress, as revealed by upregulation of UPR-associated gene expression. This was accompanied by increased levels of p62 and prominent protein aggregates, pointing towards dysregulation of the cellular protein quality control machinery. Further, we established a co-culture system to examine reciprocal interactions between p.A53T-αSyn astrocytes and dopaminergic neurons. Notably, we observed compromised neuronal viability of both control and p.A53T-αSyn neurons when co-cultured with p.A53T-αSyn astrocytes, indicating that the latter either fail to provide neuronal support or become characteristically neurotoxic. In addition, MAP2 protein levels were reduced in p.A53T-αSyn neurons when co-cultured with p.A53T-αSyn astrocytes. Reversely, p.A53T-αSyn neurons triggered a reactive response in co-cultured astrocytes. Overall, our data support the presence of intrinsic pathological features in p.A53T-αSyn astrocytes, as well as the existence of reciprocal effects between neurons and astrocytes indicating an important non-cell-autonomous component in PD pathology.

References

1. Tysnes, O.B. and Storstein, A. (2017) 'Epidemiology of Parkinson's disease', *Journal of Neural Transmission*, 124(8), pp. 901–905. Available at: https://doi.org/10.1007/s00702-017-1686-y.
2. Polymeropoulos, M.H. *et al.* (1997) 'Mutation in the α-synuclein gene identified in families with Parkinson's disease', *Science*, 276(5321), pp. 2045–2047. Available at: https://doi.org/10.1126/science.276.5321.2045.
3. Kouroupi, G. *et al.* (2017) 'Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease', *Proceedings of the National Academy of Sciences of the United States of America*, 114(18), pp. E3679–E3688. Available at: https://doi.org/10.1073/pnas.1617259114.

Funding

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project 1019-DiseasePhenoTarget).

7

A Three-Dimensional Co-Culture Model of IPSC Derived Human Astrocytes and Interneurons

Mr Andreas Bruzelius[1], Mrs Christina-Anastasia Stamouli[1], Mrs Jessica Giacomoni[2], Dr Daniella Ottosson[1]

[1]Group of Regenerative Neurophysiology, Lund University, Lund, Sweden, [2]Group of Developmental and Regenerative Neurobiology, Lund University, Lund, Sweden

Interneuron and astrocytic interplay has a central role in circuit dynamics, where damage or alteration in either cell type can lead to neurological and neuropsychiatric disorders. One example being the astrocytic regulation of synaptic potassium homeostasis could contribute to a significantly lower firing threshold for interneurons resulting in network desynchronization. As a tool to study human brain cells *in vitro* reprogramming strategies have evolved where patient specific cells (e.g. skin cell) can be reprogrammed to a neuron with the same genetic component as the starting cell. Both human interneurons and astrocytes are notoriously difficult to derive in vitro and model systems to study their interaction are lacking.
We propose a co-culture 3D system in which both human interneurons and astrocytes can be derived from the same induced pluripotent stem cells line (iPSC). We have established this co-culture model in the lab where cells are derived independently from healthy donor iPSC and combined in a 3D spheroid manner at a progenitor state to mature together which allows for easy to maintain long-term culture compared to their 2D counterpart. In these spheroids we can generate subtype specific interneurons such as somatostatin and calretinin at day 35, with increasing number at later time point.
We are currently investigating the interplay between interneurons and astrocytes and potential deficits present for diseased donors (e.g. Hungtington's or Schizophrenic patients) with aspect to their functional parameters, gene regulation and morphology. Our studies could identify molecular targets allowing for new drug or treatment applications for neurological disorders affecting interneurons and astrocytes.

Data Blitz session II

8

Myelin regeneration as a potential therapeutic target in Alzheimer's Disease

Ioanna Zota [1,2], **Konstantina Chanoumidou** [1,2], Ioannis Charalampopoulos [1,2], Achilleas Gravanis [1,2]
[1] *Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece*
[2] *Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology-Hellas (IMBB-FORTH), Heraklion, Greece*

Recent studies in mouse models and human patients highlight myelin breakdown and loss of myelin sheath as an early stage event in Alzheimer's Disease (AD). However, it is still unknown whether myelin loss is attributed to increased oligodendrocyte vulnerability, reduced repairing capacity or toxic stimuli. In the present study, we compared neuronal myelination in 12 months old wild type (wt) and 5xFAD (animal model of AD) mice, revealing a significant decrease of myelin in the hippocampal regions of dentate gyrus, CA1 and CA3 of 5xFAD mice, compared to wild type littermates. Further immunohistochemical analysis showed decreased number of PDGFRa^{+} oligodendrocyte progenitor cells (OPCs) supporting the hypothesis of defective oligodendrogenesis. Signaling of the neurotrophin receptor TrkB, which is expressed in the cell membrane of oligodendrocytes, seems to play an important role in central nervous system myelination (Xiao et al., 2012; Wong et al., 2013). BDNF, acting through this receptor, enhances myelination in the CNS and is tending to increase the density of OPCs, both *in vitro* and *in vivo* (Wong et al., 2014). However, natural neurotrophins are characterized by short half-life, inability to access the blood-brain barrier and a low pharmacokinetic profile. Therefore, we attempted to target OPCs and study the oligodendrogenic properties of two novel small-sized, non-steroid, synthetic neurotrophin analogs, the microneurotrophins TC508 and TC509, which selectively activate the TrkB receptor. Our results indicate that both analogs highly promote primary OPCs differentiation to oligodendrocytes and accelerated adult neural stem cells (NSCs) differentiation to PDGFRa+ OPCs under both physiological and AD related conditions (presence of Amyloid-β). In summary, our study shows that myelination process constitutes an appealing therapeutic target against neuronal loss in AD and suggests two novel BDNF-mimetics as promising lead therapeutic agents in the field of myelin regeneration and restoration.

References

Wong, A. W. et al. TDP6, a brain-derived neurotrophic factor-based trkB peptide mimetic, promotes oligodendrocyte myelination. Mol Cell Neurosci 63, 132-140, (2014).
Wong, A. W.et al., Oligodendroglial expression of TrkB independently regulates myelination and progenitor cell proliferation. J Neurosci (2013).
Xiao J et al. Extracellular signal-regulated kinase 1/2 signaling promotes oligodendrocyte myelination *in vitro*. J Neurochem (2012)

Acknowledgements and Funding

IZ is supported by DINNESMIN (T1EΔK - 03186) co-financed by Greece and EU in the context of Operational Program "Competitiveness, Entrepreneurship and Innovation" (ΕΠΑΝΕΚ) of the NSRF 2014–2020

9

Neurotrophin-enriched hydrogels improve human iPSC-derived dopaminergic graft survival and differentiation in athymic nude rats, but not in cyclosporine immunosuppressed rats

Dr Rachel Kelly[1], Dr Sarah Jarrin[1], **Tommy Patton[1]**, Kaushik Narasimhan[1], Giulia Comini[1], Saoirse Ryan[1], Nicola Drummond[2], Tilo Kunath[2], Prof. Eilis Dowd[1]

[1]University Of Galway, Galway, Ireland, [2]University of Edinburgh, Edinburgh, Scotland

Although human iPSC-derived cell replacement for Parkinson's has considerable reparative potential, the full therapeutic benefit of these cells can be limited by poor survival and dopaminergic maturation in the brain due to the necessity for transplantation at an early progenitor state. Injectable biomaterial scaffolds, such as collagen hydrogels, have the potential to address these issues as they provide a plethora of supportive benefits to transplanted cells including provision of the necessary trophic support for progenitor survival and dopaminergic maturation[1,2]. Thus, the aim of this project was to assess the effect of a neurotrophin-enriched collagen hydrogel on the long-term survival and maturation of dopaminergic progenitors derived from human iPSCs (iPSC-DAPs) after transplantation into the rat Parkinsonian brain. This was assessed in two separate studies: one in 6-hydroxydopamine-lesioned, cyclosporine-immunosuppressed Sprague-Dawley rats and another in 6-hydroxydopamine-lesioned athymic nude rats. In each study, cryopreserved human iPSC-DAPs[4] were recovered and transplanted into the lesioned striatum either alone, in an unloaded collagen hydrogel, with the neurotrophins GDNF & BDNF, or in a neurotrophin-loaded collagen hydrogel. Post-mortem, human nuclear immunostaining was used to identify surviving iPSC-DAPs while tyrosine hydroxylase immunostaining was used to identify iPSC-DAPs that had differentiated into mature dopaminergic neurons. In the cyclosporine-immunosuppressed rats, surviving iPSC-DAPs and mature dopaminergic neurons were found in all groups but neither the collagen hydrogel nor the neurotrophins, either alone or in combination, had any significant effect on graft survival or maturation. In the nude rats, the iPSC-DAPs also survived and matured in all groups, but in contrast to the study in the cyclosporine-immunosuppressed rats, graft survival and maturation was dramatically enhanced by transplantation of the cells in the neurotrophin-enriched hydrogel. In conclusion, this study shows that neurotrophin-enriched collagen hydrogels have the potential to enhance iPSC-derived brain repair for Parkinson's. However, given the striking differences in the outcomes between immunosuppressed and nude rats, this could have implications for allogenic versus autologous transplantation where the former requires pharmacological immunosuppression while the latter does not. Ultimately, further investigation of this approach is required to identify the mechanisms of the beneficial effects and to determine the optimal makeup of these biomaterials for supporting iPSC-DAPs to be tested in clinical trials.

References

1. Moriarty N, Pandit A, Dowd E. (2019). Encapsulation of primary dopaminergic neurons in a GDNF-loaded collagen hydrogel increases their survival, re-innervation and function after intra-striatal transplantation. Scientific Reports. 7(1):16033.
2. Moriarty N, Cabré S, Alamilla V, Pandit A, Dowd E. (2019). Encapsulation of young donor age dopaminergic grafts in a GDNF-loaded collagen hydrogel further increases their survival, reinnervation, and functional efficacy after intrastriatal transplantation in hemi-Parkinsonian rats. European Journal of Neuroscience. 49:487-496.
3. Drummond NJ, Singh Dolt K, Canham MA, Kilbride P, Morris GJ, & Kunath T. (2020). Cryopreservation of human midbrain dopaminergic neural progenitor cells poised for neuronal differentiation. Frontiers in Cell and Developmental Biology. 8, 578907.

Acknowledgements and Funding: This research was funded through the Michael J. Fox Foundation for Parkinson's Research (MJFF-009201) and Science Foundation Ireland (19/FFP/6554).

10

Potential of viral-vector delivery of BMP2 as a Neurotrophic Therapy for Parkinson's Disease

Dr Noelia Morales-Prieto[1], Ms Natasha Leeuwendaal[1], Dr Louise Collins[2], Prof Aideen Sullivan[1], Prof Gerard O'Keeffe[1]

[1]Department of Anatomy and Neuroscience. Universtity College Cork, Cork, Ireland, [2]Department of Physiology. University College Cork, Cork, Ireland

Due to the lack of disease-modifying therapies for Parkinson's Disease (PD), the discovery of new treatments that show neuroprotective and/or neuroregenerative properties in dopaminergic (DA) neurons is crucial. Neurotrophic factors have shown great potential in vitro and in animal models of PD, such as GDNF and neurturin. However, they have been have failed to meet the predetermined primary endpoints when they have been tested in clinical trials. BMP2 is distinct from these neurotrophic factors in that it has a different signalling mechanism that can be targeted to protect dopaminergic neurons (1). This project seeks to test the hypothesis that viral delivery of the neurotrophic factor bone morphogenetic 2 (BMP2) to the substantia nigra (SN) would protect DA neurons from degeneration and motor impairments induced by the overexpression alpha-synuclein (αSyn) in a rat model of Parkinson's Disease (PD). Adult male and female Sprague-Dawley rats received unilateral injection of AAV-A53T-αSyn to the SN. Four weeks later, they received unilateral injection of AAV-BMP2 or of AAV-Null Control vector to the same SN. Behavioural testing specific to detect unilateral motor impairments (Cylinder test) was carried out at 12-, 16- and 20-weeks post-surgery to analyse the degree of contralateral motor impairments. Nigrostriatal integrity was assessed at 24 weeks post-surgery. Longitudinal behavioural testing revealed that 4 weeks after injection of AAV-A53T-αSyn there were significant impairments in contralateral paw use compared to the ipsilateral paw in the cylinder test. This indicated that there was some degree of nigrostriatal damage at the time of AAV-BMP2 delivery. Longitudinal behavioural testing after AAV-BMP2 or AAV-Null (Cont) treatment showed significant improvements in contralateral paw use after AAV-BMP2 compared to controls. This is the first report showing that AAV-BMP2 can improve behavioural outcomes in the rat AAV-A53T-αSyn model of PD. BMP2 has previously been shown to have neurotrophic effects on DA neurons in vitro (2). Moreover, it is a member of the same protein family as GDF5, which has been shown to have beneficial effects in the AAV-αSyn rat model (3). Further analysis of transgene expression and of other markers is currently underway to better determine the potential of AAV-BMP2 treatment.

References

1. Goulding et al. (2020) The potential of bone morphogenetic protein 2 as a neurotrophic factor for Parkinson's disease. Neural Regen Res. 15(8): 1432–1436.
2. Goulding et al. (2019) Gene co-expression analysis of the human *substantia nigra* identifies BMP2 as a neurotrophic factor that can promote neurite growth in cells overexpressing wild-type or A53T α-synuclein. Parkinsonism Relat Disord. pii: S1353-8020(19)30203-2.
3. Goulding et al. (2021) Growth differentiation factor 5 exerts neuroprotection in an α-synuclein rat model of Parkinson's disease. Brain, 144(2): e14.

Acknowledgements and Funding

This project is funded by the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie grant agreement No 890290.

11

Exploring direct lineage reprogramming of human glia to neurons in vitro and after transplantation in a rat model

Mette Habekost[1,2], Jessica Giacomoni[1], Deidre Hoban[1], Steven A. Goldman[2,3], Malin Parmar[1]

[1]Developmental and Regenerative Neurobiology, Wallenberg Neuroscience Center, and Lund Stem Cell Centre, Department of Experimental Medical Science, Lund University, 221 84 Lund, Sweden.
[2]Center for Neuroscience, University of Copenhagen Faculty of Health and Medical Sciences, 2200 Copenhagen N, Denmark
[3]Department of Neurology and Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY 14642, USA

Parkinson's Disease (PD) is a neurodegenerative disorder, which is associated with focal loss of dopaminergic neurons in the substantia nigra. The therapeutic prospective of cell replacement therapy for treating PD has been established through clinical trials transplanting human fetal tissue and stem cell therapies are under development. Direct lineage reprogramming of resident glia into neurons *in situ* emerges as a novel approach for cell replacement that circumvents the need for cell transplantation. Recent studies have provided proof-of-principle evidence for efficient reprogramming of cell identity in the rodent brain, which is usually accomplished through forced expression of transcription factors or downregulation of key barriers such as *Ptbp1* (Qian et al, 2020). However, some of the reports have been met with skepticism, revealing discrepancies and technical concerns in glia-to-neuron reprogramming (Wang et al, 2021).

To tackle current challenges and move towards pre-clinical studies, we use human stem cell-derived glia progenitor cells (hGPCs) as reprogramming model *in vitro* (Nolbrant et al, 2020) and after transplantation into the striatum of 6-OHDA lesioned immunodeficient rats. The engrafted hGPCs expand within the rodent brain while maintaining their GPC identity (PDGFRa+) and generating astrocytes (GFAP+). We have engineered the hGPCs to express CRE recombinase, which allows us to specifically target the human cells *in vivo* upon stereotactic injection of viral vectors containing CRE-dependent reprogramming factors. We are currently using both the *in vitro* and *in vivo* models to explore *Ascl1*, *Lmx1a* and *Nurr1*-mediated and *PTBP1* knockdown-mediated reprogramming with the aim to select reprogramming factors that specify the generation of dopaminergic neurons from human glia directly in the brain.

References

1. Nolbrant S, Giacomoni J, Hoban DB, Bruzelius A, Birtele M, Chandler-Militello D, Pereira M, Ottosson DR, Goldman SA, Parmar M. Direct Reprogramming of Human Fetal- and Stem Cell-Derived Glial Progenitor Cells into Midbrain Dopaminergic Neurons. Stem Cell Reports. 2020
2. Qian H, Kang X, Hu J, Zhang D, Liang Z, Meng F, Zhang X, Xue Y, Maimon R, Dowdy SF, Devaraj NK, Zhou Z, Mobley WC, Cleveland DW, Fu XD. Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature. 2020
3. Wang LL, Serrano C, Zhong X, Ma S, Zou Y, Zhang CL. Revisiting astrocyte to neuron conversion with lineage tracing in vivo. Cell. 2021

Acknowledgements and Funding
Lundbeckfonden, Danmarks Frie Forskningfond, Swedish Research Council, Knut och Alice Wallenberg Foundation

12

Synuclein aggregates drive different microglial responses to host-to-graft pathology in stem cell-derived transplants in a humanized graft model of PD

Dr Sara Corsi[1,2], Shelby Shrigley[1,2], Bengt Mattsson[1], Fredrik Nilsson[1,2], Jenny Nelander Wahlestedt[1,2], Kelvin C. Luk[3], Deirdre B. Hoban[1,2], Anders Björklund[1], Malin Parmar[1,2]
[1]Developmental and Regenerative Neurobiology, Lund University, Lund, Sweden, [2]Lund Stem Cell Center, Lund University, Lund, Sweden, [3]3Center for Neurodegenerative Disease Research, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

Microglia are important mediators of the inflammatory process and have a crucial role in Parkinson's disease (PD) pathology. Indeed, they have been shown to contribute to the ongoing neurodegeneration by spreading αSynuclein (αSyn) aggregates [1].
Exploiting our fully humanized xenograft PD model (SynFib model), we previously showed that dopamine (DA) neuron transplants derived from human embryonic stem cells (hESC) eventually develop phosphorylated pathological αSyn inclusions (p-Syn) in a small fraction of cells [2].
In the present study, we assessed the spread of p-Syn from host to graft in an αSyn-enriched environment, resembling the clinical setting. The model combines adeno-associated viral vectors overexpressing the human □Syn gene and human preformed □Syn fibrils directly infused into the substantia nigra of nude rats. In this model, we investigated how αSyn pathology affects the microglial response, and their relation to the p-Syn aggregates transfer in grafts of either a human-induced pluripotent stem cell (hiPSC) line, from a patient with an αSyn gene triplication (AST18), or a clinical grade hESC line (RC17) with no known PD-mutations, transplanted 4 weeks after the establishment of the SyFib model. To this aim, the pathology was assessed in human DA neurons by staining for TH, p-Syn, and huNu, while microglial response was assessed by Iba1 immunoreactivity in three different areas: the transplant, the grafted host striatum, and the contralateral intact striatum at 18- and 24-weeks post-transplantation. We identified 5 subtypes of microglia based on their morphological features: resting (R), hypertrophic round (HR), hypertrophic elongated (HE), dystrophic (D), and small and round cells (SR). R cells were strongly reduced ($p<0.05$) in the AST18 grafts, whereas a significant increase ($p<0.05$) was found in the amount of SR cells compared to the RC17 controls. By contrast, no changes were observed in the contralateral or in the grafted host striatum. Interestingly, the percentage of SR cells was directly proportional to the amount of p-Syn inclusions in the transplant, while the opposite appeared for the R microglia. Thus, our findings suggest that the Syn-enriched environment might lead to a different expression of microglial phenotypes, although their beneficial or detrimental effects need to be clarified.

References

1. Guo M, Wang J, Zhao Y, Feng Y, Han S, Dong Q, Cui M, Tieu K. Microglial exosomes facilitate α-synuclein transmission in Parkinson's disease. Brain. 2020 May 1;143(5):1476-1497. doi: 10.1093/brain/awaa090. PMID: 32355963; PMCID: PMC7241957.
2. Hoban DB, Shrigley S, Mattsson B, Breger LS, Jarl U, Cardoso T, Nelander Wahlestedt J, Luk KC, Björklund A, Parmar M. Impact of α-synuclein pathology on transplanted hESC-derived dopaminergic neurons in a humanized α-synuclein rat model of PD. Proc Natl Acad Sci U S A. 2020 Jun 30;117(26):15209-15220. doi: 10.1073/pnas.2001305117. Epub 2020 Jun 15. PMID: 32541058; PMCID: PMC7334514.

Acknowledgments and Fundings

This work was supported by the European Union's funded ERC (ERC treat PD - "Patient-specific treatment for Parkinson's disease using reprogrammed skin cells", Grant nr. 771427), and VR fundings (VR 3R: 2021-02967 - Circuitry repair in Parkinson's disease: from stem cell replacement to axonal targeting using stem cell models; VR Distinguished professor grant (2021-00661): Stem cell-based treatments for Parkinson's disease: Novel concepts and future therapies).

13

Investigation of the functional integration of hESC-derived grafts in models of Huntington's disease

Mrs Patricia Garcia Jareño[1], Olivia Edwards[1], PhD Marija Fjodorova[2], Professor Meng Li[2], PhD Mariah Lelos[1], Professor Anne Rosser[1]

[1]Brain Repair Group; Sir Martin Evans Building, School of Biosciences, Cardiff University, Cardiff, United Kingdom, [2]Stem Cell Neurogenesis Group; Neuroscience and Mental Health Research Institute; School of Medicine and School of Bioscience; Hadyn Ellis Building, Cardiff University, Cardiff, United Kingdom

Huntington's disease (HD) is an autosomal dominant genetic disorder in which striatal medium spiny neuron (MSN) degeneration is predominant and occurs early in the course of the disease.HD is associated with progressive deterioration of movement, cognition and behaviour.. Currently, there are no approved disease-modifying interventionss and symptomatic treatments are limited, although there are a number of potential disease-modifiers currently in early stage clinical trials. Cell replacement therapy, in which striatal MSN progenitors are transplanted directly into the adult striatum, is in development as a potential therapeutic intervention for HD patients. Preclinical data, using foetal-derived and pluripotent stem cell (PSC)-derived donor cells and proof-of-concept clinical studies, using foetal-derived donor cells, suggest that striatal grafts containing mature MSNs can alleviate behavioral deficits. It is hypothesized that functional recovery relies largely on reconstruction of the basal ganglia circuitry that is disrupted in the HD brain. The aim of this project is to determine whether grafts of human embryonic stem cell (hESC)-derived MSN-like cells form functional synapses with host brain neurons. We address this by mapping efferent and afferent graft-brain projections using monosynaptic rabies technology. We have created a hESC line expressing the TVA receptor and demonstrate that MSN progenitors differentiated from this cell line result in surviving grafts containing mature MSNs, following transplantation into two different HD animal models; the quinolinic acid (QA)-lesioned rat and the F344tgHD transgenic rat model. Our preliminary data demonstrates that host-to-graft connectivity is established with appropriate brain regions, such as the globus pallidus, cortex, thalamus and substantia nigra. Further exploration of host-to-graft and graft-to-host connectivity is ongoing. These data will be important in establishing the ability of hESC-derived MSNs grafts to reconstruct damaged neural circuitry, and to facilitate our understanding of the mechanisms underlying recovery following hESC-derived cell transplantation.

Acknowledgements and Funding
This project is part of the ITN ASCTN Training, funded by the European Union Horizon 2020 Programme (H2020-MSCA-ITN-2018) under the Marie Skłodowska-Curie Initial Training Network.

Data Blitz session III

14

Novel AAV Capsid Variants Targeting Human Glia

Mrs Jessica Giacomoni[1], Malin Åkerblom[2], Mette Habekost[1], Marcus Davidsson[2], Malin Parmar[1], Tomas Björklund[2]

[1]Developmental and Regenerative Neurobiology, Lund Stem Cell Centre,Department of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden, [2]Molecular Neuromodulation, Lund Stem Cell Centre, Department of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden

Direct neural conversion of resident glia to therapeutic neurons is an emerging alternative strategy for brain repair. Proof-of-concept has been obtained from animal studies and AAV-based therapeutics offer a suitable option for the clinical translation of this approach. However, the lack of selective AAVs targeting human glia cell types with low off-target expression remains an unsolved challenge.
In this study we used the hESC-derived glial progenitor cell-model (Nolbrant et al., 2020; Wang et al., 2013), *in vitro* and after transplantation into the adult rat brain, as a screening system for engineering the AAV capsid surface with the goal to achieve selective infection of human glia. We identified and validated several novel AAVs with higher transduction efficiency and/or specificity for human glia that could improve the delivery of neural reprogramming factors for *in vivo* experimental studies and future therapeutic applications.

References

Nolbrant, S., Giacomoni, J., Hoban, D.B., Bruzelius, A., Birtele, M., Chandler-Militello, D., Pereira, M., Ottosson, D.R., Goldman, S.A., and Parmar, M. (2020). Direct Reprogramming of Human Fetal- and Stem Cell-Derived Glial Progenitor Cells into Midbrain Dopaminergic Neurons. Stem Cell Reports *15*, 869-882.

Wang, S., Bates, J., Li, X., Schanz, S., Chandler-Militello, D., Levine, C., Maherali, N., Studer, L., Hochedlinger, K., Windrem, M., and Goldman, S.A. (2013). Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell *12*, 252-264.

Acknowledgements and Funding

The Swedish research council (VR) and Knut and Alice Wallenberg Foundation (KAW).

15

Allogeneic Regenerative Cell Therapy for White Matter Stroke and Vascular Dementia

Irene L. Llorente[1], William E. Lowry[2] and S. Thomas Carmichael[1].

1, Department of Neurology, David Geffen School of Medicine at UCLA, 90095
2, Department of Molecular, Cell and Developmental Biology, UCLA, 90095

Subcortical white matter stroke (WMS) constitutes up to 30% of all stroke subtypes. The progressive deterioration of the WM produces hemiparesis with incomplete recovery, gait abnormalities, cognitive decline, and difficulties in executive functioning that present as vascular dementia (VaD). There is no specific therapy for WMS. Therefore, a cell-based therapy that can replace lost glia and induce structural repair in WMS or VaD is of great promise. We have characterized the cellular properties and in vivo tissue repair activity of glial enriched progenitor cells differentiated from human induced pluripotent stem cells, termed hiPSCs-GEPs. hiPSC-GEPs are derived from hiPSC-Neural Progenitor cells (hiPSC-NPC) via an experimental manipulation of hypoxia inducible factor (HIF) activity by brief treatment with a prolyl hydroxylase inhibitor, deferoxamine. This treatment permanently biases these cells to further differentiate towards a “pro-repair” astrocyte fate. We have demonstrated that hiPSC-GEPs transplanted into the brain in the subacute period after WMS migrated throughout the injured brain and stimulated endogenous OPC differentiation, promoted myelination of damaged brain tissue, and drove the formation of cortical connections after stroke. hiPSC-GEPs transplant promoted recovery of motor and cognitive deficits in WMS. The ability to differentiate patient-derived hiPSCs into hiPSC-GEPs provides a strong candidate for a cell-based therapy to target WMS, VaD and other neurodegenerative diseases. We have also demonstrated reproducible production of the hiPSC-GEP, having produced them from at least five independent donors. To date, we have successfully developed safety, identity, purity, activity, and stability qualification assays of the intended therapeutic candidate, hiPSC-GEPs. We have established the safety and efficacy of hiPSC-GEPs transplantation and determined the appropriate therapeutic time window, location, and dose of hiPS-GEPs needed to induce motor and cognitive improvement after WMS. This study demonstrates the reliable and safe generation of patient derived hiPSC-GEPs that are clinically ready as a cell-based therapeutic approach for WMS and VaD.

16

CSF and serum biomarkers in human neural stem cell-treated Secondary Progressive Multiple Sclerosis patients

Mrs Giada D'Aloisio[1], Mr. Maurizio Gelati[1], Mrs Lucrezia Abate[1], Mrs Valentina Grespi[2], Mrs Elisabetta Mormone[1], Mrs Maria Ripoli[1], Mr Massimiliano Copetti[1], Mr Andrea Fontana[1], Mr Angelo Luigi Vescovi[1], Mrs Daniela Celeste Profico[1]

[1]Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy, [2]S. Maria Hospital, Terni, Italy

In the study of multiple sclerosis (MS), one of the most important challenges is finding specific biomarkers, as they are essential for predicting the progression of disability and assessing the response to treatment. The aim of this study is the evaluation of potential biomarkers in the cerebrospinal fluid (CSF) and serum of patients with Secondary Progressive MS (SPMS), enrolled in the clinical trial EudraCT 2015-004855-37 (NCT03282760). The intent is to visualize biomarkers alterations following the implantation of human Neural Stem Cells (hNSCs) in the cerebral-ventricle to evaluate the response to treatment. The study was performed on 15 patients treated with four different hNSC dosages, CSF and Serum samples were collected before treatment, at follow up (FU) 1, 3, 6, 9 and 12 months following implantation and were analyzed by microfluidic ultrasensitive ELISA (Biotechne, USA). The results shown that in the CSF at every time point, IL-2, IL-17a and TNF-a were below the detection limit: these molecules are indices of acute inflammation and are probably not significantly detectable due to the chronicity of the disease on the contrary, TNF-a was detected in serum therefore indicating a systemic inflammatory condition. OPN, CHI3L, Fractalkine and CCL2 were found in high levels in both serum and CSF. CCL3 and VEGF instead were more present in serum than in CSF but in healthy patients had the lowest concentration of CCL3. Fractalkine a powerful neuroprotector, has an unclear trend in serum but in the CSF has a drastic decrease in FU1, following a sudden rise to FU6 and this could be an index of plaque ignition, data that would also justify the simultaneous raising of IL-8. OPN has an opposite trend: it presents peaks in correspondence of FU1 in the CSF and FU6 in serum. Its demonstrate anti-inflammatory activity making suppose an increase in relation to the treatment. For these elements, we suppose that the treatment with hNSCs may contribute to the decrease of biomarkers related to demyelination and chronic inflammation. The data obtained of course require further analysis, so we will perform a statistical analysis and comparisons with clinical data after their full collection.

References

Belenguer, G., Duart-Abadia, P., Jordán-Pla, A., Domingo-Muelas, A., Blasco-Chamarro, L., Ferrón, S. R., Morante-Redolat, J. M., & Fariñas, I. (2021a). Adult Neural Stem Cells Are Alerted by Systemic Inflammation through TNF-α Receptor Signaling. Cell Stem Cell, 28(2), 285-299.e9. https://doi.org/10.1016/j.stem.2020.10.016

Housley, W. J., Pitt, D., & Hafler, D. A. (2015). Biomarkers in multiple sclerosis. Clinical Immunology, 161(1), 51–58. https://doi.org/10.1016/j.clim.2015.06.015

Funding

This study has been founded by IRCCS Casa Sollievo della Sofferenza and by Italian Ministry of Health “Ricerca

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Modelling Familial ALS Pathology In Vivo

Mrs Georgia Eleftheriou[1,2], Dr Stefano Frausin[1], Professor Lachlan Thompson[1]

[1]Florey Institute Of Neuroscience And Mental Health, Melbourne, Australia, [2]University of Melbourne, Melbourne, Australia

Amyotrophic Lateral Sclerosis (ALS) is characterised by the dysfunction of upper and lower motor neurons. However, the origin of motor neuron degeneration is unknown and the disease's pathogenic pathway remains elusive. Stem cell technologies have become increasingly recognised tools for the investigation of ALS *in vitro*, however there are many limitations, including the failure to replicate conditions in a living organism.
This project aims to model pathogenic hallmarks across familial forms of ALS *in vivo* via the transplantation of patient derived motor neuron progenitors into a host central nervous system. C9orf72 iPSC patient lines and their corresponding isogenic controls were differentiated towards lower motor neuron progenitors (MNPs), and sterotaxically injected into the lumbar spinal cord of Athymic rats. Preliminary results confirmed the successful differentiation of patient-derived lines and long term survival of grafted neurons exhibiting typical motor neuron characteristics.
This study is built on the premise that patient-derived neurons studied in the environment of a living organism, will provide a unique paradigm to reveal temporal, spatial and cellular hallmarks of ALS pathophysiology over extended timeframes. Further investigation of potential pathogenic biomarkers using this model may extend our knowledge of the various molecular mechanics that contribute towards ALS aetiology.
How the support will help my research:
I started my journey as a young researcher at the height of the pandemic in 2020 as an honours student at the Florey Institute. I have since continued with the same lab to commence my PhD in 2021. Unfortunately, due to the pandemic, I haven't had an opportunity to present my research to an audience or network with researchers within the same field. It's been a very isolating experience and I would truly appreciate the opportunity and assistance to get my research communicated to a wider audience, gain experience presenting, and gain potential collaborative partners and connections. I believe attending an international conference for the first time would be an incredible learning experience and an opportunity to accelerate my research.

18

Glial dysregulation and SVZ neurogenesis disruption following brain chemical lesion

Irini Thanou[1], Paraskevi N. Koutsoudaki[2], Anna Haroniti[1], Mary Margariti[1], Sophia Havaki[2], Vassilis G. Gorgoulis[2], Dimitra Thomaidou[1]

[1]Department of Neurobiology, Hellenic Pasteur Institute, 127 Vas. Sofias Avenue, 11521 Athens, Greece, [2]Laboratory of Histology-Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Advert side effect of chemotherapy that impair brain function, has been only recently anticipated and several mechanisms are proposed to be involved in changes regarding the structure and function of the brain following systematic use of chemotherapeutic agents. These include reduction of Neural Stem Cell (NSC) proliferation rates in adult brain neurogenic zones, white matter degeneration and brain inflammation. This phenomenon is particularly pronounced in cancers, such as glioblastoma, which are inherently resistant to chemotherapy and require high doses of chemotherapy to eliminate them, resulting in a high concentration in the cerebrospinal fluid.

Here we aimed to study the effect of brain chemical lesion induced by multiple stereotaxic intraventricular injections of the chemotherapeutic mito-toxic agent arabinoside-C (Ara-C) on adjacent neurogenic and non-neurogenic brain areas fine cytoarchitecture and their relation to the functional outputs of 'Chemobrain'. Characterization of the spatio-temporal distribution of neuronal and glial cells at multiple time points following the chemical lesion (4, 15 days and 6 weeks) revealed that Ara-C infusion leads to ependymal cell layer disruption and impaired neurogenesis in the Subventricular Zone (SVZ) neurogenic niche. Moreover, it triggers doublecortin+ (DCX+) neuroblasts' ectopic presence in the adjacent non-neurogenic striatal parenchyma, the majority of which cluster inside myelinated white matter tracts. Our data also point to a strong neuroinflammatory response with extensive astro- and microgliosis around ventricular and septal walls and adjacent parenchyma and the presence of CD3+ T cells, in the SVZ and choroid plexus, suggesting a disruption of the blood –CSF barrier in that site.

Our studies are ongoing to follow the lineage trajectories of ectopic neuroblasts, characterize the protein expression profile in the disrupted striatum and evaluate striatal myelin bundles' micro-structure and migrating neuroblasts' localization and morphology by using transmission electron microscopy (TEM). We anticipate that the analysis of the brain cyto-architecture, as well as the lineage, molecular phenotype and proliferation properties of SVZ and striatal cell populations will provide us with answers linking brain morphology to function following chemotherapy.

Bibliography

1. Wigmore, P. (2012). doi:10.1007/7854_2012_235
2. Gibson, Erin M et al. (2019) doi:10.1016/j.cell.2018.10.049

Supported by Greek Ministry of Education BIOIMAGING GR- Grant (MIS 5002486), funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund); Stavros Niarchos Foundation Grant.

Data Blitz session IV

19

Bacterial DING proteins in blood serum of Alzheimer's disease and Mild Cognitive Impairment patients and correlation with Aβ42 and COX-1/2

Dr Eleni Andreadou[1,2], Mr Georgios Katsipis[1,2], Dr Magda Tsolaki[2,3,4], Dr Anastasia Pantazaki[1,2,4]

[1]Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki , Greece, [2]Center for Interdisciplinary Research and Innovation, Laboratory of Neurodegenerative Diseases (LND), Aristotle University of Thessaloniki, Thermi/Thesssaloniki, Greece, [3]First Neurology Department, "AHEPA" University General Hospital of Thessaloniki, Aristotle University of Thessaloniki, Thessaloniki, Greece, [4]Greek Association of Alzheimer's Disease and Related Disorders – GAADRD , Thessaloniki, Greece
e-mail: eandreadou@chem.auth.gr, natasa@chem.auth.gr

Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI) have been linked to inflammation as their pathology is partially attributed to common bacterial infections, while the enormous economic burden of dementia imposes the discovery of new biomarkers in biological fluids other than cerebrospinal fluid (CSF). DING proteins identified mainly by their N-terminal sequences are common in *Pseudomonas* and other bacterial species, have potential roles in phosphate acquisition and pathogenicity and they have been implicated with many human diseases. The aim of this research was to investigate the levels of the bacterial DING proteins in blood serum, in MCI and AD patients compared to healthy cognitively individuals, and verify if DING could be employed as potential biomarkers in MCI or AD. Correlation and multilinear regression analysis of DING levels with the inflammation markers COX-1, COX-2, the neurodegeneration markers $A\beta_{42}$ and tau and the mental state marker Mini-Mental State Examination (MMSE) score have also been performed. Levels of DING were measured with indirect ELISA method in serum of eighteen AD (N=18) and twenty-three MCI patients (N=23) in comparison with thirteen cognitively healthy individuals (N=13). The biomarkers of inflammation COX-1 and COX-2, and the established in CSF AD biomarkers, $A\beta_{42}$ and tau, were measured as well. DING levels were found significantly increased in serum of AD patients in comparison with cognitively healthy subjects and MCI patients. AUC analysis proves that serum DING could be used as a possible biomarker in serum to discriminate AD patients from both cognitively healthy individuals and especially MCI patients. Serum DING proteins positively correlate with $A\beta_{42}$, COX-1 and especially with COX-2 levels. Also, serum DING levels negatively correlate with the MMSE (mental state) of patients strengthening the notion that neuroinflammation is a crucial event in cognitive decline in patients with MCI or AD. Multi-linear regression analysis proves that DING levels in serum are significantly determined by the mental state of patients and COX-2 levels, as well as the age of patients. Further studies should be performed for enlightening the pathological pathways of the infectious etiology of AD. To our knowledge, this is the first time such analyses are reported.

References

[1] Ifuku M. et al. (2020) *Acta Neuropathologica Communications* 8: 159
[2] Collombet J-M et al. (2010) *PLoS ONE 5: e9099*

Acknowledgements and Funding
The investigators express their appreciation at the medicinal and scientific personnel of the 1st Neurological Clinic of "AHEPA" - University Hospital of Thessaloniki, for their assistance during the conduction of this study

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Using biomaterials to provide local immunosuppression for CNS cell transplantation

Ms Emily Atkinson[1,2], Miss Despoina Eleftheriadou[1,2], Miss Neringa Cypaite[2], Dr Audrey Mercer[2], Prof James B. Phillips[1,2], Dr Victoria Roberton[1,2]

[1]UCL Centre for Nerve Engineering, University College London, London, United Kingdom, [2]UCL School of Pharmacy, University College London, London, United Kingdom

Therapeutic cells can act as a vehicle for the release of dopamine and neurotrophic factors to the brain for the treatment of Parkinson's Disease (PD). Transplantation of cells for their secretome alone may therefore be beneficial even in the absence of synaptic integration, so an encapsulation approach in which donor cells are physically separated from the host tissue may be useful. Previously a dual-layer alginate bead approach was developed to facilitate long-term encapsulation of therapeutic cells, enhanced by the presence of nanoparticles comprising an immunomodulatory compound, tacrolimus (FK506), for local immunosuppression. The inner part of the alginate bead incorporates the therapeutic cells, then an alginate-hyaluronic acid blend containing immunosuppressive nanoparticles surrounds the bead.[1]
The nanoparticles were synthesised via electrospraying, encapsulating a tacrolimus core within a poly-ε-caprolactone (PCL) shell. The release profile and modulation of T-cell proliferation was measured *in vitro*. The sustained-release particles were incorporated into a second hydrogel layer containing alginate and hyaluronic acid. In vitro results indicated that this dual-layer alginate encapsulation system has the potential for use in therapeutic cell delivery to protect transplanted cells from host cell responses, whilst having the appropriate porosity for the diffusion of nutrients and elements of the secretome.
Subsequent work has focused on further development of the local immunosuppression technology. The PCL particle formulation has been modified to enable GMP-ready production of larger microparticles that could be mixed with a cell suspension for a more versatile approach that does not require alginate encapsulation and is compatible with synaptic integration of transplanted cells. Initial in vivo studies indicate that particles remain at the injection site following stereotaxic delivery into the striatum of rats with low levels of astrocyte and microglial response observed at 1 and 3 weeks.
Local delivery of FK506 using nano- and micro-scale particles therefore provides a potential tool for modulating host tissue immune responses to improve survival of transplanted cells. This could be developed for use alongside an encapsulation system or mixed with a cell suspension, providing a versatile biomaterial approach to enhance CNS cell transplantation.

References

1. Eleftheriadou, D. et al. An alginate-based encapsulation system for delivery of therapeutic cells to the CNS. *RSC Advances* **12**, 4005-4015 (2022).

Acknowledgements and Funding Privacy statement

This work was supported by funding from the Engineering and Physical Sciences Research Council (EPSRC) (EP/R004463/1) and the UCL Rosetrees Stoneygate Prize 2018. EA received funding from EPSRC through the award of PhD studentship in the Centre for Doctoral Training in Transformative Pharmaceutical Technologies (EP/S023054/1).

21

Towards the generation of better Parkinson's in vitro models: human LMX1A tracing line for culture enrichment on mDA neural progenitors

Dr Lucia F. Cardo[1], Dr Jimena Monzón-Sandoval[1], Professor Caleb Webber[1], Professor Meng Li[2]

[1]UK Dementia Research Institute at Cardiff, , , [2]Neuroscience and Mental Health Innovation Institute, ,

The study of neuronal disorders using iPSCs-derived neurons has an outstanding value especially when access to human tissue is only available post-mortem. The use of fluorescent reporter lines allows to sort the cells using Fluorescent activated cell sorting (FACS) enriching the cultures in the desired cell type. It serves also as a tool to follow up the neural progenitor cells (NPCs) after transplantation in animal models and study both integration and migration into other brain areas. The most common reporter line used in mDA in vitro models involves targeting the Tyrosine hydroxylase (TH) with the caveat that dopaminergic (DA) neurons based on TH expression is not only limited to SNc (A9), and other markers should be assessed to characterize bonafide SNc neurons.
LMX1A gene is one of the earliest transcription factors expressed in mDA development, playing an important role in both specification and differentiation of mDA neurons. Generating a human iPSC reporter line for LMX1A would allow for early tracking and monitoring of mDA progenitors population, as well as enrichment of the cultures in LMX1A+ NPCs earlier in the differentiation process via FACS. In the present study, we provide with an engineered human iPSC line to trace LMX1A+ derivatives through the expression of a Blue Fluorescent Protein (BFP), and present ample data of its utility to study mDA neurogenesis, enrichment of mDA cultures via FACS and model PD-related drug toxicity. Current sc-RNAseq methods allowed grasping the complexity and heterogeneity of mDA cultures at the population and single cell level. Our time course scRNA-seq data spanning 4 differentiation stages supports the recapitulation of in vivo midbrain development in our in vitro model and provides in depth characterization of the temporal cell type composition of our cultures. We also explored the damage infringed by MPP+ toxicity at single cell level in basal and FACS cultures.

22

Can viral-mediated dopamine synthesis alleviate cognitive impairments in Parkinson's Disease?

Charlotte Bridge[1], Rachel Hills[1], Gareth Williams[1], Dr. Stephen Paisey[2], Dr. Emma Lane[3] & Dr. Mariah J. Lelos[1]

[1]Brain Repair Group, School of Biosciences, Cardiff University, Cardiff, United Kingdom, [2]PETIC, School of Medicine, Cardiff University, Cardiff, United Kingdom, [3]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom

Gene therapy is a promising therapeutic intervention for Parkinson's Disease (PD). Through the delivery of key enzymes in the dopamine (DA) synthesis pathway, we can replace lost DA to the brain. Pre-clinical studies using viral vectors expressing TH and GCH1 have reported motor recovery in rodent and non-human primate models of PD, leading to clinical trials of this form of dopamine replacement therapy. Outcome measures of pre-clinical studies primarily focus on motor dysfunction, however, dopamine-dependant cognitive impairments such as visuospatial dysfunction and incentive motivation have a significant impact on quality of life for patients. We previously demonstrated alleviation of cognitive deficits utilising human fetal tissue in the 6OHDA rat model of PD, however it remains unknown whether gene therapy can alleviate these cognitive impairments. To explore this, lister-hooded rats received operant training on the lateralised choice reaction time task (LCRT), before receiving a unilateral 6OHDA medial forebrain bundle (MFB) lesion. In Experiment 1, rats received a unilateral intrastriatal infusion of an AAV1 viral vector containing human truncated TH and human GCH1 into the ventrolateral striatum. Motor recovery was observed from 5 weeks post-infusion on the adjusting steps task, however virus-treated rats demonstrated a significant impairment in cognitive and neuropsychiatric function (performance accuracy and incentive motivation) compared to untreated rats. In Experiment 2, the viral vector was distributed across the whole striatal nucleus and initial analysis indicates significant improvement in both motor and cognitive function. Finally, to determine whether gene therapy might show differential impact in different striatal subregions, in Experiment 3, rats received an intrastriatal infusion targeted to either the dorsolateral or dorsomedial striatum only. Motor recovery was observed in both groups and interestingly, initial analysis shows rats that received dorsolateral striatal infusions had greater cognitive performance to untreated rats, while dorsomedial striatal infusions did not show improvements in cognitive function. A full post-mortem histological analysis is currently underway. These data demonstrate the potential for impaired cognitive performance after expression in the ventrolateral striatum. They also highlight the potential of using selective targeting of gene therapy within the striatum to improve both motor and cognitive function.

References

LELOS, M. J., MORGAN, R. J., KELLY, C. M., TORRES, E. M., ROSSER, A. E. & DUNNETT, S. B. 2016. Amelioration of non-motor dysfunctions after transplantation of human dopamine neurons in a model of Parkinson's disease. *Experimental Neurology,* 278**,** 54-61.

ROSENBLAD, C., LI, Q., PIOLI, E. Y., DOVERO, S., ANTUNES, A. S., AGÚNDEZ, L., BARDELLI, M., LINDEN, R. M., HENCKAERTS, E., BJÖRKLUND, A., BEZARD, E. & BJÖRKLUND, T. 2019. Vector-mediated l-3,4 dihydroxyphenylalanine delivery reverses motor impairments in a primate model of Parkinson's disease. *Brain,* 142**,** 2402-2416.

23

Cholesterol based-strategies lead to a complete and long-standing recovery of cognitive and motor defects in Huntington's disease mouse models

Prof. Marta Valenza[1,2], Giulia Birolini[1,2], Ilaria Ottonelli[3], Francesca Talpo[4], Lucia Minoli[5,7], Andrea Cappelleri[6,7], Mauro Bombaci[2], Claudio Caccia[8], Valerio Leoni[9], Alice Passoni[10], Monica Favagrossa[10], Maria Rosaria Nucera[1,2], Laura Colombo[10], Saverio Paltrinieri[11], Renzo Bagnati[10], Jason Thomas Duskey[3], Riccardo Caraffi[3], Maria Angela Vandelli[3], Franco Taroni[8], Mario Salmona[10], Eugenio Scanziani[6], Gerardo Biella[4], Barbara Ruozi[3], Giovanni Tosi[3], Elena Cattaneo[1,2]

[1]Department of Biosciences, University of Milan, Milan, Italy, [2]Istituto Nazionale di Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy, [3]Nanotech Lab, Te.Far.T.I. Center, Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy, [4]Department of Biology and Biotechnologies, University of Pavia, , Italy, [5]Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Grugliasco, Italy, [6]Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy, [7]Mouse & Animal Pathology Lab (MAPLab), Fondazione UniMi, Milano, Italy, [8]Unit of Medical Genetics and Neurogenetics, Istituto Neurologico Carlo Besta, Milan, Italy, [9]Department of Medicine and Surgery, University of Milano Bicocca, Monza, Italy, [10]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, , Italy, [11]Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy

Cholesterol (chol) in the brain is produced locally by astrocytes and used by neurons. Perturbation of its metabolism is linked to brain diseases. Data from the last 20 years have shown that activity of this pathway is reduced in the brain of 7 rodent models of Huntington's Disease (HD).
Consistently, strategies based on delivering chol to the brain through brain-permeable nanoparticles (NPs) (1, 2) or directly into striatum through osmotic mini-pumps, have been shown to be beneficial in the R6/2 mouse model, a fast-progressing transgenic mouse model of HD (3). A third strategy used gene therapy to increase chol biosynthesis through forced expression of SREBP2 -a key trigger of cholesterol biosynthesis- in striatal astrocytes, leading to reversal of disease phenotypes in R6/2 mice (4).
In our new effort (submitted), we used a recent formulation of brain-permeable NPs with increased chol loading capacity to explore the long-term therapeutic potential of chol administration to the brain of the slow-progressing zQ175DN HD mice. Pre-symptomatic or symptomatic treatment normalized cognitive behavior for up to 5 months (mo), and improved motor and neuropathological defects. Repeated treatment produced long-lasting benefits for 12 mo without serious side-effects. The distribution of data from the cognitive tests administered to HD mice treated with any of the treatments mentioned above, for a total of 381 mice (comprising wt, HD and HD-treated mice) showed that regardless of treatment, timing, and animal model, increased chol biosynthesis in the brain of HD mice is a therapeutically viable option against cognitive deficits, highlighting its translational potential in patients.

References

1) Valenza M, Chen JY, di Paolo E, et al. Cholesterol-loaded nanoparticles ameliorate synaptic and cognitive function in H untington's disease mice. *EMBO Molecular Medicine*. 2015;7(12):1547-1564. doi:10.15252/emmm.201505413
2) Birolini G, Valenza M, Ottonelli I, et al. Insights into kinetics, release, and behavioral effects of brain-targeted hybrid nanoparticles for cholesterol delivery in Huntington's disease. *Journal of Controlled Release*. 2021;330:587-598. doi:10.1016/j.jconrel.2020.12.051
3) Birolini G, Valenza M, di Paolo E, et al. Striatal infusion of cholesterol promotes dose- dependent behavioral benefits and exerts disease-modifying effects in Huntington's disease mice. *EMBO Molecular Medicine*. 2020;12(10). doi:10.15252/emmm.202012519
4) Birolini G, Verlengia G, Talpo F, et al. SREBP2 gene therapy targeting striatal astrocytes ameliorates Huntington's disease phenotypes. *Brain*. 2021;144(10):3175-3190. doi:10.1093/brain/awab186/6273579

Acknowledgements and Funding
Fondazione Telethon - Italy (Grant no. GGP17102). This work was also partially supported by European Union Circ Prot (n. 643417) and by the 2021 Leslie Gehry Brenner Prize for Innovation in Science of the Hereditary Disease Foundation (USA).

e-Poster session I

P1

Peripheral administration of the Class-IIa HDAC inhibitor MC1568 partially protects against nigrostriatal degeneration in the striatal 6-OHDA rat model of Parkinson's disease

Dr Martina Mazzocchi[1]

[1]*Ucc, Cork, Ireland*

Parkinson's disease (PD) is a neurodegenerative disorder characterized by nigrostriatal dopaminergic (DA) degeneration. There is a critical need for neuroprotective therapies, particularly those that do not require direct intracranial administration. Small molecule inhibitors of histone deacetylases (HDAC) (HDIs) are neuroprotective in in vitro and in vivo models of PD. We show that 6-hydroxydopamine (6-OHDA) treatment induces protein kinase C (PKC)-dependent nuclear accumulation of Class IIa HDAC5 in SH-SY5Y cells and cultured DA neurons. Treatment of these cultures with the Class-IIa specific HDI, MC1568, partially protected against 6-OHDA- induced cell death. In the intrastriatal 6-OHDA lesion in vivo rat model of PD, MC1568 treatment (0.5 mg/kg i.p.) for 7 days reduced forelimb akinesia and partially protected nigral DA neurons and their striatal terminals. MC1568 prevented 6-OHDA-induced increases in microglia in the striatum and nigra, and in nuclear HDAC5 levels in nigral DA neurons. These data rationalize the study of peripheral administration of Class-IIa specific HDIs as a potential neuroprotective therapy for PD.

P2

The Edinburgh Human Progenitor Cell Bank: Cryopreserved iPSC-derived progenitor cells for the neuroscience community

Michela I. Barbato[1], Nicola J. Drummond[1], Karamjit Singh Dolt[1], Maurice A. Canham[1], Peter Kilbride[2], G. John Morris[2], Andrew Chai[1] and Tilo Kunath[1].

(1) Centre for Regenerative Medicine, Institute for Stem Cell Research, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom, (2) Cytiva, Cambridge, United Kingdom.

Human induced pluripotent stem cells (hiPSCs) can be differentiated into diverse types of cells for disease modelling and cell replacement therapy. Although hiPSCs are widely used, they can be difficult to reliably differentiate within the same laboratory, and across different laboratories. Furthermore, there are many laboratories with cell culture capabilities, but lack expertise in hiPSC differentiation. This restricts the use of the large collection of hiPSC lines to select laboratories with differentiation expertise.
One solution to this problem is to differentiate hiPSCs into progenitor cells and cryopreserve them in a state where they are committed and poised for differentiation. Cells can be frozen after passing the early critical stages of the differentiation, and test thaws of the cryopreserved vials are performed before sharing with other labs. Furthermore, an allelic series of isogenic CRISPR-engineered iPSC lines can be simultaneously differentiated and provided as sets of cryopreserved progenitor cells. Quality-controlled progenitors are already committed to their differentiation path and are ready to be applied for experimental studies in any laboratory with cell culture facilities.
We have initially focused on perfecting the protocol to differentiate and cryopreserve hiPSC-derived midbrain dopaminergic (mDA) neurons. These mDA neural progenitors provide a source of cells for transplantation in pre-clinical animal models or for mature human dopaminergic neurons for different applications, such as Parkinson's disease modelling. We have established the Edinburgh Human Progenitor Cell Bank to provide easy access to cryopreserved human progenitor cells to the broader neuroscience community to accelerate research in neurodegenerative diseases.

References

https://www.frontiersin.org/articles/10.3389/fcell.2020.578907/full

Acknowledgements and Funding

This work was funded by MRC RMRC grant (MR/K017276/1), MRC Confidence in Concept award, and Cure Parkinson's Trust grant to Tilo Kunath.

P3

Human iPSC-derived reactive astrocytes display an altered metabolic profile compared to quiescent-like astrocytes

Sarah F. McComish[1, 2], Rebecca Mansfield[1], Myles Corrigan[1], Lucy A. Crompton[3, 4], Jon D. Lane[4], Maeve A. Caldwell[1, 2]

[1]*Department of Physiology, School of Medicine, Trinity College Dublin, Dublin, Ireland*
[2]*Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland*
[3]*Regenerative Medicine Laboratory, School of Clinical Sciences, University of Bristol, Bristol, UK*
[4]*Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, UK*

Astrocytes are the most abundant cell type in the CNS and play a central role in the development and homeostasis of the healthy brain. However, astrocytes have the potential to become activated and reactive in neurodegenerative diseases such as Alzheimer's disease. Our study aimed to investigate the impact of astrocyte reactivity on gene and protein expression, and function using human induced pluripotent stem cell (iPSC)-derived astrocytes.
Human iPSC were patterned towards a neural fate using dual SMAD inhibition. Astrocytes were derived using epidermal growth and human leukemia inhibitory factors to produce mature astrocytes after 90 days. They were characterised then stimulated with IL-1□, TNF□ and C1q to induce a reactive phenotype.
iPSC-derived astrocytes were positive for astrocyte markers GFAP, S100β, Connexin-43 and EAAT1 confirming cell fate. Stimulation resulted in increased IL-6 secretion, coupled with increased expression of genes associated with reactive astrocytes (e.g., *C3*, *CXCL10*, *PTX3*). Reactive astrocytes were also shown to have an altered metabolic profile with significantly elevated glycolysis and glycolytic capacity compared to controls as assessed by Seahorse Assay.
In conclusion, the ability to produce reactive astrocytes from human cells *in vitro* provides a powerful model for investigating the mechanisms that underpin the role of astrocytes in neurodegenerative diseases.

Acknowledgements and Funding
Health Research Board

P4

Functional characterization of stem cell-derived dopamine neurons for the treatment of Parkinson's Disease

Dr Ryan Smith[1], Mr Edmund Tam[1], Ms Haley Clark[1], Mr Mark Ebel[1], Ms Evelyin Suman[2], Dr Elizabeth Pereira[1], Dr Dan Wilkinson[1], Dr Prasad Purohit[1], Dr Maya Srinivas[1], Dr Thomas Hudzik[1], Dr Alyssa Petko[1]

[1]BlueRock Therapeutics, New York, United States, [2]BlueRock Therapeutics, Toronto, Canada

Human pluripotent stem cell-derived dopamine (DA) neuron progenitors show great promise for the applications in regenerative medicine, including authentic cell replacement for the treatment of Parkinson's disease. DA neurons generated from pluripotent stem cells are currently being tested for safety and tolerability in clinical trials (https://clinicaltrials.gov/ct2/show/study/NCT04802733). Here, we characterized a research-grade human stem cell-derived DA neuron cell therapy product, DA01, applying molecular and functional approaches over the course of *in vitro* neuronal cell maturation. Using a multi-electrode array (MEA) platform to measure population activity, we found that DA01 cells reach a mean firing rate of 2-8 Hz, typical of midbrain DA neurons, after extended maturation. Using the patch clamp technique to analyze activity from single cells, DA01 cells begin to show spontaneous spiking activity early in maturation, while IH currents are observed later in maturation. We used additional, complementary approaches to further characterize DA01 *in vitro*, profiling transcriptomic changes, ionic composition, and activity-dependent calcium signaling, as well as the release of DA. Importantly, DA01 cells showing significant *in vitro* functional activity express the gene encoding tyrosine hydroxylase (TH), in addition to other genes associated with midbrain DA neuron specification and function. In order to test the integrity of DA receptor function in our cell product, we found that quinpirole, a D2 receptor agonist, decreases population activity as expected. Finally, we extended our analysis to multiple independently produced lots of research-grade DA01 cell product, in order to understand batch-to-batch reproducibility of these characteristics. Taken together, these data confirm that DA01 neurons molecularly and functionally resemble canonical DA neurons over the course of their maturation.

P5

The p.A53T α-synuclein mutation causes dopaminergic neuron deficit in patient-derived midbrain organoids

Olympia Apokotou[1]

[1]*Hellenic Pasteur Institute, Athens, Greece*

Brain organoids that recapitulate the physiology and complexity of human brain have great potential for in vitro disease modeling, including neurodegenerative diseases. Parkinson's disease (PD) is a complex neurodegenerative disorder with varying motor and non-motor manifestations, characterized by loss of midbrain dopaminergic neurons. The SNCA gene encoding α-synuclein (αSyn) protein is the major gene associated with sporadic PD, while point mutations and multiplications of the locus cause autosomal dominant PD forms. The best-characterized mutation is p.A53T-αSyn (G209A *SNCA*) that causes a familial form of the disease with early onset and severe phenotype. We have previously established a 2-D neuronal model of p.A53T pathology that displays disease-associated features, including protein aggregation, axonal neuropathology, compromised neuritic outgrowth and impaired synaptic connectivity (1). To study further the role of p.A53T-αSyn in early PD pathogenesis, we generated midbrain organoids (MLOs) using iPSCs from patients carrying the p.A53T-αSyn mutation and healthy donors as well as an isogenic pair of a p.A53T patient and its gene-corrected counterpart. MLOs were maintained up to 90 days in vitro (DIV), at which time they encompassed MAP2+ neurons displaying widespread synapsin immunoreactivity, and GFAP+ astrocytes. Immunofluorescence analysis as early as 15 DIV revealed numerous neuroepithelial rosettes positive for NESTIN and FOXA2, indicating correct ventral midbrain patterning, as well as extensive LMX1A immunoreactivity that marks midbrain dopaminergic progenitors. Some TH+ dopamine neurons were already visible in control cells at 15 DIV and their numbers rapidly increased at 30 and 60 DIV. Notably, p.A53T MLOs displayed significantly reduced numbers of TH+ neurons at all times tested, indicating a retardation in dopaminergic differentiation or increased vulnerability of these cells over time. In addition, α-Syn accumulation, a typical pathological manifestation in PD, was predominantly detected in p.A53T organoids. Further analysis is on-going to address if the p.A53T mutation causes early neurodevelopmental defects, as recently shown for *LRRK2* and *DNAJC6* PD-associated mutations (2, 3) or triggers cell death of differentiated dopamine neurons due to increased vulnerabilty, with these events being non-mutually exclusive.

References

(1) Kouroupi G, Taoufik E, Vlachos IS, et al. Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease. *Proc Natl Acad Sci U S A*. 2017;114(18):E3679-E3688. doi:10.1073/pnas.1617259114

(2) Zagare A, Barmpa K, Smajic S, et al. Midbrain organoids mimic early embryonic neurodevelopment and recapitulate LRRK2-p.Gly2019Ser-associated gene expression. Am J Hum Genet. 2022;109(2):311-327. doi:10.1016/j.ajhg.2021.12.009

(3) Wulansari N, Darsono WHW, Woo HJ, et al. Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson's disease-linked DNAJC6 mutations. Sci Adv. 2021;7(8):eabb1540. Published 2021 Feb 17. doi:10.1126/sciadv.abb1540

Funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project 1019-DiseasePhenoTarget).

e-Poster session II

P6

Secondary progressive multiple sclerosis: in vitro effect of cerebrospinal fluid on human neural stem cells behavior and immunomodulatory molecules production

Mrs Lucrezia Abate[1], Mr Maurizio Gelati[1], Mrs Giada D'Aloisio[1], Mrs Valentina Grespi[2], Mrs Elisabetta Mormone[1], Mrs Maria Ripoli[1], Mr Massimiliano Copetti[1], Mr Andrea Fontana[1], Mr Angelo Luigi Vescovi[1], Mr Daniela Celeste Profico[1]

[1]Ospedale Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy, [2]S.Maria Hospital, Terni, Italy

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by demyelination. Several studies have attempted to identify soluble factors present in the cerebrospinal fluid (CSF) of MS patients related to course of the pathology. Human neural stem cells (hNSCs) obtained from foetal tissue following spontaneous abortion or in utero death were injected into cerebral lateral ventricle of 15 patients affected by secondary progressive MS (SPMS) recruited in phase I clinical trial (EudraCT 2015-004855-37 NCT03282760). CSF was collected from these patients before and six months after being treated with hNSCs. The aim of this project is to measure the expression of inflammatory factors released in the CSF of MS patients, verify their variation after hNCS therapy and evaluate the effect of CSF on hNSCs behaviour. We cultured hNSC in standard differentiation condition, in presence of 5% CSF. Culture supernatant, serum and CSF were used for ELISA assays to measure the content of B lymphocyte chemoattractant (CXCL13) and Aquaporin-4 (AQP4). mRNA from cultures was analysed with RT-PCR to evaluate the expression of osteopontin (OPN), neurotrophin 3 (NT3), Brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and CXCL13; CSF of healthy patients and supernatant from standard differentiation and undifferentiated cells were used as controls. The quantitative analysis of AQP-4 and CXCL-13 showed that they decrease in CSF and serum six months after treatment compared to pre-treatment and to control and, in the same way there was a reduction of their production in cell cultures treated with CSF. NT3 and CXCL13 genes appear to be poorly expressed in all the samples analysed, while VEGF shows greater expression in the undifferentiated control compared to the other controls and patients. Our preliminary results suggest that there may be a slightly decrease in brain inflammation due to the high expression of VEGF, that modulates the production of CXCL13 and AQP-4. We will present complete results of the study also comparing those with the data obtained by the clinical team in order to evaluate the differences between diverse time points, if any, and the potential role of the biomarkers involved in neuroinflammation.

References

Cristofanilli, M., Cymring, B., Lu, A., Rosenthal, H., & Sadiq, S. A. (2013). Cerebrospinal fluid derived from progressive multiple sclerosis patients promotes neuronal and oligodendroglial differentiation of human neural precursor cells in vitro. Neuroscience, 250, 614–621. https://doi.org/10.1016/j.neuroscience.2013.07.022

Novakova, L., Axelsson, M., Malmeström, C., Zetterberg, H., Blennow, K., Svenningsson, A., & Lycke, J. (2020). NFL and CXCL13 may reveal disease activity in clinically and radiologically stable MS. Multiple Sclerosis and Related Disorders, 46. https://doi.org/10.1016/j.msard.2020.102463

Ikeshima-Kataoka, H. (2016). Neuroimmunological implications of AQP4 in astrocytes. In International Journal of Molecular Sciences (Vol. 17, Issue 8). MDPI AG. https://doi.org/10.3390/ijms17081306

Funding

This study has been founded by IRCCS Casa Sollievo della Sofferenza and by Italian Ministry of Health “Ricerca Corrente” project code 2763688

P7

Intracerebroventricular transplantation of Neural Stem Cells in an experimental model of Amyotrophic Lateral Sclerosis

Ivan Lombardi (1), Silvia de la Morena (2), Diego Pastor Campos (3), Rose Mary Carletti (4), Maurizio Gelati (5), Daniela Celeste Profico (5), Valentina Grespi (6), Gianmarco Muzi (6), Daniela M Rasà (2, 7), Jessica Rosati (8), Elisa Perciballi (1), Letizia Mazzini (9), Fabiola De Marchi (9), Alessandro Vercelli (2, 7), Salvador Martinez Perez (3), Angelo L Vescovi (1, 4), Marina Boido (2, 7) and Daniela Ferrari (1).

1: *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy*
2: Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy
3: Instituto de Neurociencias de Alicante, Alicante, Spain
4: Fondazione IRCCS, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
5: Fondazione IRCCS Casa Sollievo della Sofferenza, Production Unit of Advanced Therapies (UPTA), Institute for Stem-Cell Biology, Regenerative Medicine and Innovative Therapies (ISBReMIT), San Giovanni Rotondo, Italy
6: Cell Factory and Biobank Santa Maria Hospital, Terni, Italy
7: Department of Neuroscience "Rita Levi Montalcini", University of Turin, Italy
8: Cellular Reprogramming Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
9: Centro Regionale Esperto SLA Azienda Ospedaliero-Universitaria "Maggiore della Carità", Novara, Italy

Human Neural Stem Cells (hNSCs) treatment for neurodegenerative and neuroinflammatory diseases might exert antagonizing effects on inflammation and neurodegeneration (1). We showed that hNSCs transplantation in the spinal cord of $SOD1^{G93A}$ rat can delay disease progression, motor functions deterioration and significantly extends animals survival (2). These clinical improvements were associated with a reduction of ALS histopathological markers (SOD1 deposits, reactive astro/microgliosis) and motor neurons preservation in the transplanted areas.
By the phase I trial NCT01640067, we demonstrated that the procedure is feasible in ALS patients (3). The patient cohort was too small to draw final conclusions, however we observed a significant transitory decline of the ALS-Functional Rating Scale score progression within the first month after transplantation that continued for up to 4 months, in accordance with another comparable trial (NCT01348451).

To improve the hNSCs efficacy and extend the duration of the putative beneficial outcomes, a conceivable hypothesis is to increase cell dosage. This objective has been tackled by increasing the number of spinal cord injections (NCT01730716), however, this approach is limited due to the backbone destabilization consequent to the surgery.
In this study, we are evaluating the implementation of intracerebroventricular delivery of hNSCs, as an effective strategy to increase cell dosage, favor a broader spread of transplanted cells and of their secreted healing factors throughout the motor neuraxis by exploiting the liquor circulation. Our results show that hNSCs (300,000 cells/mice) transplanted into the lateral ventricle of immunodeficient mice are well tolerated and not tumorigenic after 6 months, can extensively migrate and adhere to the ventricle wall occasionally migrating into the parenchyma.
The same dosage was injected into the brain of $SOD1^{G93A}$ mice using a transient immunosuppression protocol. hNSCs survived for at least 2 months and our preliminary data suggest that the treatment improved mice motor performances. However, cell survival was not optimal, thus, at this stage, the reduced sample size prohibits any conclusions on survival.

We are currently evaluating the safety and efficacy of increased dosage of hNSCs (up to $1x10^6$ cells) in nude and SOD1 ^{G93A} mice.

References

(1) Willis CM, Nicaise AM, Peruzzotti-Jametti L, Pluchino S. The neural stem cell secretome and its role in brain repair. Brain Res. 2020 Feb 15;1729:146615. doi: 10.1016/j.brainres.2019.146615. Epub 2019 Dec 19. PMID: 31863730.

(2) Zalfa, C., Rota Nodari, L., Vacchi, E. *et al.* Transplantation of clinical-grade human neural stem cells reduces neuroinflammation, prolongs survival and delays disease progression in the SOD1 rats. *Cell Death Dis* 10, 345 (2019). https://doi.org/10.1038/s41419-019-1582-5

(3) Mazzini L, Gelati M, Profico DC, Sorarù G, Ferrari D, Copetti M, Muzi G, Ricciolini C, Carletti S, Giorgi C, Spera C, Frondizi D, Masiero S, Stecco A, Cisari C, Bersano E, De Marchi F, Sarnelli MF, Querin G, Cantello R, Petruzzelli F, Maglione A, Zalfa C, Binda E, Visioli A, Trombetta D, Torres B, Bernardini L, Gaiani A, Massara M, Paolucci S, Boulis NM, Vescovi AL; ALS-NSCs Trial Study Group. Results from Phase I Clinical Trial with Intraspinal Injection of Neural Stem Cells in Amyotrophic Lateral Sclerosis: A Long-Term Outcome. Stem Cells Transl Med. 2019 Sep;8(9):887-897. doi: 10.1002/sctm.18-0154. Epub 2019 May 18. PMID: 31104357; PMCID: PMC6708070.

P8

Defining the consequence of LRRK2 dysregulation in human ESC-derived astrocytes

Áine Heffernan[*1], James Cooper[1], Dario Alessi[2], Bhuvaneish Selvaraj[1] and Siddharthan Chandran[1]

[1]*Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, EH16 4SB, UK*
[2]*Medical Research Council (MRC) Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, UK*

Parkinson's disease (PD) is the fastest growing neurodegenerative disorder with no treatment available to cure or alter its progression. Mutations in *leucine-rich repeat kinase 2* (*LRRK2*) are one of the most common causes of familial PD which closely resembles idiopathic PD, both neuropathologically and clinically. LRRK2 is a large multidomain protein kinase whose activity is aberrantly increased in PD patients carrying *LRRK2* mutations and in individuals with idiopathic PD[1]. This supports the study of *LRRK2* mutations to better understand PD pathogenic mechanisms. Most research has focused on the consequences of *LRRK2* mutations in non-physiologically relevant cell lines, with few studies investigating the importance of these mutations in a human disease context. Astrocytes are the most abundant glial cell in the human brain and are essential for maintaining brain homeostasis. Multiple preclinical and clinical studies have demonstrated that these homeostatic functions are disrupted in PD, but the exact mechanisms underlying how *LRRK2* mutations in astrocytes may contribute to PD pathogenesis have yet to be established.
To investigate this, a suite of *LRRK2* mutant lines generated in a human embryonic stem cell line (LRRK2$^{G2019S/G2019S}$, *LRRK2* knockout and LRRK2$^{D2017A/D2017A}$ kinase dead) have been differentiated into functional astrocytes. To explore the relationship of *LRRK2* genotype with LRRK2 expression level and kinase activity, qPCR and western blot were carried out at baseline and following treatment with stimuli such as a triple cytokine cocktail (TNF□, IL1□ and C1q), IFN□, nigericin and MLi-2, which either increased or decreased LRRK2 expression levels or kinase activity. To delineate the consequences of the changes recorded, an inflammatory profile of the astrocytes was established at baseline and in response to these stimuli, as it has been suggested that an inflammatory trigger is needed for pathogenic *LRRK2* mutations to induce a PD phenotype[2]. Preliminary findings show increased expression of proinflammatory markers such as *CXCL10* and MX1 following triple cytokine cocktail and IFN□ stimulation, while a protective genotype effect was noted in the knockout line. Future work aims to establish the importance of these mutations via unbiased proteomic evaluation and co-culture experiments with microglia will investigate the non-cell autonomous consequences of these astrocytes.

References

[1]Di Maio, R., Hoffman, E. K., Rocha, E. M., Keeney, M. T., Sanders, L. H., De Miranda, B. R., . . . Greenamyre, J. T. (2018). LRRK2 activation in idiopathic Parkinson's disease. *Sci Transl Med, 10*(451). doi:10.1126/scitranslmed.aar5429

[2]Cabezudo, D., Baekelandt, V., & Lobbestael, E. (2020). Multiple-Hit Hypothesis in Parkinson's Disease: LRRK2 and Inflammation. *Front Neurosci, 14*, 376. doi:10.3389/fnins.2020.00376

P9

The hidden roles of SLITRKS in p.A53T-αSyn synaptic dysfunction

E.K. Akrioti[1], K. Segklia[1], P. Chandris[1], A. Stavrou[1], K. Charbi[1], R. Matsas[1], E. Taoufik[1]

[1]*Laboratory of Cellular & Molecular Neurobiology- Stem Cells, Hellenic Pasteur Institute, Athens, Greece*

Parkinson's disease (PD) and related synucleinopathies are a group of incurable neurodegenerative disorders associated with alpha-synuclein (αSyn) pathology, with the best-characterized mutation, G209A, in the αSyn gene SNCA resulting in the pathological p.A53T-αSyn protein. An analysis of a human induced pluripotent stem cell (iPSC)-based model from patients that bear the p.A53T mutation showed defects in synapse formation and function and dysregulation of genes involved in synaptic signaling (Kouroupi et al., 2017). Characteristically, three members belonging to the SLITRK family of postsynaptic adhesion proteins that form trans-synaptic complexes with the presynaptic LAR-RPTPs molecules, were significantly downregulated in mutant neurons. Currently it is known that these molecules regulate neurite outgrowth, promotion of neuron survival, and synapse formation (Proenca et al., 2011), while they have been linked to neurodevelopmental disorders including autism and neuropsychiatric syndromes. Nevertheless, a link between dysregulation of SLITRKs and αSyn mediated dysfunction has not been previously described. To investigate the role of SLITRK1/2/4 in p.A53T-αSyn mediated synaptic pathology we used both a transgenic mouse model that expresses the human p.A53T-αSyn under the control of the PrP promoter (Prnp-SNCA*A53T) (Giasson et al., 2001) and hiPSC-derived neurons harboring the p.A53T mutation. We detected a significant reduction in the expression of all three SLITRKs, evident already at 4-months, that is further enhanced at 12-months in the cortex, hippocampus, and striatum. Additional studies in both p.A53T-αSyn mouse hippocampal and hiPSC-derived neurons also showed reduction of SLITRKs. Examination of their localization in fully formed synapses *in vitro* revealed diminished SLITRK-containing Bassoon/ PSD95 synaptic puncta. Additionally, the sub-neuronal localization of SLITRKS was also affected by p.A53T-aSyn expression, indicating a dysfunction in the cellular trafficking machinery, which is currently being analyzed in detail using SH-5YSY cells that express stably the human p.A53T-αSyn protein. Overall, our work has identified three new mediators of p.A53T-αSyn-induced synaptopathy, linking the expression of pathological αSyn to trans-synaptic adhesion.

References

1. Kouroupi, G., Taoufik, E., Vlachos, I.S., Tsioras, K., Antoniou, N., Papastefanaki, F., Chroni-Tzartou, D., Wrasidlo, W., Bohl, D., Stellas, D., et al. (2017). Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease. Proceedings of the National Academy of Sciences of the United States of America 114, E3679-E3688.
2. Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., and Lee, V.M. (2002). Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. Neuron 34, 521-533.
3. Proenca, C.C., Gao, K.P., Shmelkov, S.V., Rafii, S., and Lee, F.S. (2011). Slitrks as emerging candidate genes involved in neuropsychiatric disorders. Trends in neuroscience*s* *34*, 143-153.

Acknowledgements and Funding: Funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Project Number: 1019; to RM); the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T2ΕΔΚ-02813, AlphaSyn); the Hellenic Pasteur Institute Excellence Scholarship -Nostos Foundation.

e-Poster session III

P10

Exploring the neuro cardiac axis - Defining local mechanisms and long-range signals that promote organ regeneration

Vasiliki Tsata[1], Stelios Psarras[2], Dimitris Beis[1]

[1] Center of Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece.
[2] Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece.

Diseases of the heart and central nervous system (CNS)/brain are the 1st and 2nd leading causes of death worldwide resulting in disability-adjusted life for more than half of surviving individuals each year. Heart and brain disorders share various risk factors, and damage to the heart is often accompanied by CNS pathologies and vice versa, suggesting a causal link between conditions that affect the CNS and the heart. Yet, the underlying mechanisms regulating such inter-organ communication in both homeostatic and post-injury conditions are poorly understood. This poses a major challenge to effective disease prognosis and medical treatment. In contrast to mammals, zebrafish exhibit an elevated regenerative capacity for a variety of organs, including the brain and heart. This enables the investigation of the injury responses and interactions between these organs in a regenerating context. In our approach we utilize i) diverse models of CNS and heart injury, ii) advanced imaging to map cellular responses in the distant organ and iii) RNAseq to acquire a mechanistic understanding of the brain-heart axis after injury and during regeneration in an adult vertebrate. Successfully identified brain-heart mediators will be validated *in vivo* by generating novel transgenic lines enabling conditional, cell type-specific manipulation of selected genes. *in vivo* work will be complemented using primary murine cultures in which injury conditions will be mimicked *in vitro*. We propose that identifying the cues that orchestrate brain-heart interactions in zebrafish, could provide targets with implications to regenerative medicine.

P11

Age-related neuronal autophagy changes in induced neurons

Mr Balázs Kis[1,2], Abbas Anna[1,2], Lea Danics[1,2], Anikó Göblös[3], Roger A. Baker[4], Johann Jakobsson[5], Lajos Kemény[3,6], Karolina Pircs[1,3,5]

[1]Hcemm-su Neurobiology And Neurodegenerative Diseases Research Group, Budapest, Hungary, [2]Institute of Translational Medicine, Semmelweis University, Budapest, Budapest, Hungary, [3]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary, [4]Wellcome-MRC Cambridge Stem Cell Institute & John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom, [5]Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, Lund University, Lund, Sweden, [6]HCEMM-USZ Skin Research Group, Szeged, Hungary

Human lifespan continues to climb, this will result in an ageing society with its own problems. One of them is the increase of neurodegenerative diseases (ND) due to age, however we know little about the connection of age to these diseases. One of the promising targets of interest is autophagy in neurons. It was shown that autophagy declines with age in neurons.
Autophagy is an evolutionary conserved lysosomal degradation pathway that ensures the cytoplasmic homeostasis. Deficits in autophagy have been linked to decreased mitochondrial biogenesis and trafficking, oxidative stress, increased apoptosis and ATP deficit, which have been implicated in neurodegenerative diseases like Parkinson's and Alzheimer's disease.
Modeling human ageing in a lab so far was challenging, especially neuronal ageing as donor neurons were not readily available. iPSC-s solved some of the availability issue, but however due to the process of rejuvenation they are not the best candidate to model ageing.
In this project we will use an induced neuronal model (iN) to follow the changes in autophagy during neuronal ageing. We will use an all-in-one self-inactivating lentivirus to directly reprogram donor fibroblasts into neurons. The advantage of this methodology is that iNs maintain age (and disease)-related signatures of the donor cells as direct reprogramming bypasses the intermediary cell rejuvenation step inevitable in other approaches such as iPSC.
We will convert 50 healthy sex matched human dermal fibroblasts iNs from different ages. We will use immunostaining combined with high content automated microscopy to monitor the conversion efficiency, purity, cell number, neuronal morphology using neuronal markers like MAP2 and TAU. Afterwards, we will measure the expression of different autophagy markers during basal and starvation conditions (LC3, p62, LAMP1, BECN1). We will also monitor proteomic and transcriptional changes of autophagy using mass spectrometry and RNA-seq. We will combine our data to establish the relation of age to autophagy.

P12

The LEARN study: using participant experience to improve trial design for neurodegenerative diseases

[1]Drew C.J.G, [1]Smallman K., [2]Shahmoon S., [3]Lane E.L.

[1]Centre for Trials Research, Cardiff University, UK, [2]Institute of Neurology, University College London, UK [3]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, UK

Introduction: A number of advanced medicinal therapeutic products (ATMPS) for the treatment of neurodegenerative diseases are moving to clinical trials. The nature of ATMPs (cell, gene and protein products) and the challenge of circumventing the blood brain barrier mean that many require direct cranial administration. Combined with the ongoing management of the disease, the requirement for direct central nervous system administration and complex multi-component assessments results in a number of unique challenges. Understanding the patient journey during AMTP investigations is important for both mitigating against these challenges and to aid recruitment and retention in clinical trials as candidate ATMPs move forward.
We set out to understand the experiences of participants from a trial in which people with Parkinson's disease received infusions of a growth factor (GDNF) via permanently implanted intra-cranial drug delivery catheters.
Methods: We conducted semi-structured interviews with GDNF trial participants and their family members/ care partners using topic guides informed by our participant consultation group. We also attempted to interview people who had discussed taking part in the trial but declined participation. Interviews were conducted on-line alone or in dyads, and observed by a second non-participatory researcher. Interviews were transcribed verbatim and analysed thematically using NVivo software.
Results: The experiences of participants in the GDNF trial were largely positive, expressing strong feelings of collegiality and working towards the greater good. Specific issues surrounding the nature and timing of assessments and post operative care were identified. These related to the importance participants put on additional support in attending study visits being beneficial to their overall experience. A common thread relating to negative experiences lay with the difficulties participants faced once the trial had ended, which included learning about the negative outcome of the trial and the abrupt lack of interaction following an intensive study period.
Discussion: Listening to the voice of lived experience from novel complex trials is vital to inform the design of efficient, patient-centred studies to maximise recruitment and retention. Simple modifications to trial conduct can significantly enhance the participant experience and advocates for the inclusion of broad and diverse patient experience in the development of future ATMP studies.

Acknowledgements and funding
This study was funded by Parkinson's UK.

P13

Longitudinal Behavioural Characterisation of a Transgenic Rat Model of Huntington's Disease

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

[1]*Brain Repair Group; Sir Martin Evans Building, School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK.*

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that is caused by an expansion of a CAG repeat sequence in the huntingtin (*HTT*) gene, resulting in production of a mutant form of the HTT protein (mHTT). The most prominent neuropathological feature of HD is the degeneration of striatal medium spiny neurons (MSNs), leading to progressive motor, cognitive and neuropsychiatric symptoms. Preclinical HD models are critical tools for understanding disease pathology and developing therapeutics, but existing mouse models possess excessively long CAG repeat expansions, which may not fully recapitulate the human disease. A more clinically relevant model with a lower CAG repeat expansion is necessary to advance therapeutic intervention in preclinical studies. The recently developed F344tgHD transgenic rat model of HD expresses a fragment of human mHTT with 51 CAG repeats, and an initial characterisation suggests that it manifests behavioural deficits and pathology relevant to HD. The goal of this project was to conduct a comprehensive, longitudinal study of cognitive and neuropsychiatric impairments in the F344tgHD model, to establish a baseline upon which therapeutics can be tested. To this end, a wide range of behavioural tasks have been performed, including the elevated plus maze, 5-Choice Serial Reaction Time Task, open field, locomotor activity, social interaction and light/dark box. The results thus far suggest that the transgenic rat exhibits changes in motivation, as demonstrated by performance in the 5-Choice Serial Reaction Time Task, and disinhibition of anxiety-related behaviours, which have been identified in the elevated plus maze task. Data from this study will form the baseline for future assessment of therapeutic interventions in the F344tgHD rat model.

Acknowledgements and Funding
This project is funded by the Medical Research Council (MRC).

P14

Mirk/Dyrk1B kinase controls the generation and columnar organization of spinal motor neurons in the embryonic chick spinal cord via Sonic hedgehog pathway

Kokkorakis N.[1], Politis PK.[2], Zagoraiou L.,[2] Matsas R.[1] and Gaitanou M.[1]*

1. *Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute*
2. Center of Basic Research, Biomedical Research Foundation of the Academy of Athens
** e-mail: mgaitanou@pasteur.gr*

Functional integration and cross-talk between Mirk/Dyrk1B kinase and Sonic hedgehog/Gli, PI3K/mTOR/AKT and MEK/ERK signaling pathways, affects cellular and molecular processes in development, physiology, and pathology[1]. We have first demonstrated Dyrk1B implication in neurogenesis[2,3]. Recently, we have revealed a novel role of Dyrk1B in the generation of spinal motor neurons (SpMNs) in the embryonic chick spinal cord via regulation of Sonic hedgehog (Shh) pathway. Using *in vivo gain-and-loss-of-function and rescue phenotype* approaches in E2 chick spinal cord, and subsequent analysis at E4, we found that Dyrk1B regulates the number of p2, pMNs and p3 progenitors, as well as MNs and V2a populations. Especially, Dyrk1B overexpression at E2 promoted at E4 cell cycle exit and neuronal differentiation in a cell-autonomous manner, while in a non-cell autonomous manner Dyrk1B overexpression promoted increased apoptosis in the MN domain, and dramatic loss of p2, pMNs, and p3 progenitors, as well as of MNs and V2a post-mitotic neurons. This intense ventral phenotype of Dyrk1B overexpression suggested the involvement of Shh signaling. In agreement, real-time RT-qPCR analysis revealed that Dyrk1B overexpression reduces dramatically Shh and Gli3 mRNA levels. At E6, the loss of MNs is selectively reflected, in reduced LMCm MNs population, that innervate the muscle limbs ventrally. In rescue phenotype experiments, the compromised Shh signaling, due to Dyrk1B overexpression, was restored, when we used AZ191 compound, a specific DyrK1B kinase inhibitor, or SAG agonist of Smoothened (SMO), which it activates, at a down-stream level, the Shh pathway. Both compounds resulted in the restoration of progenitors populations, as well as of LMCm MNs. The specific effect of Dyrk1B in LMCm MNs could be explained by our remarkable finding that Shh is expressed exclusively by LMCm MNs at E6 chick spinal cord. In conclusion, Dyrk1B acts as a potent up-steam transcriptional suppressor of Shh and Gli3, during spinal cord development, regulating thus the number of all progenitor's populations that are under the strong influence of Shh gradient, and especially controls the generation and survival of SpMNs as well as their columnar organization.

References

1. Kokkorakis N, Gaitanou M. (2020). Minibrain-related kinase/dual-specificity tyrosine-regulated kinase 1B implication in stem/cancer stem cells biology. World J Stem Cells. 2020 Dec 26;12(12):1553-1575. doi: 10.4252/wjsc.v12.i12.1553. PMID: 33505600

2. Gaitanou M, Segklia K, Matsas R. (2019). Cend1, a Story with Many Tales: From Regulation of Cell Cycle Progression/Exit of Neural Stem Cells to Brain Structure and Function. Stem Cells Int. 2019 May 2;2019:2054783. doi: 10.1155/2019/2054783. eCollection 2019. PMID: 31191667

3.Tsioras K, Papastefanaki F, Politis PK, Matsas R, Gaitanou M. (2013). Functional Interactions between BM88/Cend1, Ran-binding protein M and Dyrk1B kinase affect cyclin D1 levels and cell cycle progression/exit in mouse neuroblastoma cells. PLoS One. 2013 Nov 28;8(11):e82172. doi: 10.1371/journal.pone.0082172. eCollection 2013. PMID: 24312406

Acknowledgements and Funding
Nostos Foundation Funding.

e-Poster session IV

P15

Extra Virgin Olive Oil (EVOO) attenuates LPS-induced inflammatory effect on a 2D model of neuroblastoma and glioblastoma murine cells

Tzekaki E. [1,2], Katsipis G. [1,2], Lavrentiadou S.N. [3*], Pantazaki A. A. [1,2*]

(1) Laboratory of Biochemistry, Department of Chemistry, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece
(2) Center for Interdisciplinary Research and Innovation, Laboratory of Neurodegenerative Diseases (LND), Aristotle University of Thessaloniki, Thermi 57001, Greece
(3) Laboratory of Physiology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece

Murine neuroblastoma and glioblastoma cells were co-cultured in a 10:1 ratio, in order to generate a 2D-model for the LPS-induced inflammation in the CNS. LPS present in the outer membranes of Gram-negative bacteria induces pro-inflammatory, pro-apoptotic, and anti-apoptotic pathways through binding to macrophage TLR4 receptor. Upon binding, LPS activates macrophages, which increase the release of cytokines (IL-1β, TNFα), upregulate inducible nitric oxide synthase 2 (iNOS/NOS2), and provoke oxidative stress due to the release of various reactive oxygen species (ROS). *In vivo* and *in vitro* studies have demonstrated the detrimental effects of LPSs on the Central Nervous System (CNS), leading to neuroinflammatory effects.
Glial cells have a pivotal role on the induction of these effects, as they are the main cells reacting to LPSs. Extra virgin olive oil (EVOO) is a natural product with antioxidant properties, and its consumption is explicitly connected to refinement of overall health. The foremost component of EVOO are phenolic compounds, which the potent neuroprotective, antioxidant and anti-inflammatory properties of EVOO have been attributed to. In this study, we explored the effect of EVOO on LPS-exposed N2A-GL261 cell co-cultures.LPSs increased IL-1β, TNFα, and iNOS intracellular protein levels in N2A-GL261 cell co-cultures but decreased superoxide dismutase 1 (SOD1) levels .EVOO inhibited the LPS-induced increase of intracellular IL-1β levels by 46%, TNFα levels by 11% and iNOS levels by 63%. In addition, EVOO enhanced cellular antioxidant defenses by increasing SOD1 protein levels by 4,3-fold , compared to cells treated only with LPSs, and by 1,3-fold compared to untreated cells. These results demonstrate the anti-inflammatory and antioxidant effects of EVOO and thus advocate for further research towards its applicationsfor the treatment of neuroinflammatory disorders.

Acknowledgements and Funding
Murine N2A neuroblastoma cells and murine GL261 glioblastoma cells were a kind gift from Professor Gaetano Donofrio, Department of Medical Veterinary Science, University of Parma, Italy

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Alleviating Effect of Curcumin and a V (IV) – Curcumin complex on LPS-Induced Inflammation in Murine Mixed Neuroblastoma-Glioblastoma Cultures

Katsipis G. [1,2], **Tzekaki E.** [1,2], Lavrentiadou S.N. [3*], Pantazaki A. A. [1,2*]

(1) Laboratory of Biochemistry, Department of Chemistry, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece,
(2) Center for Interdisciplinary Research and Innovation, Laboratory of Neurodegenerative Diseases (LND), Aristotle University of Thessaloniki, Thermi, 57001, Greece
(3) Laboratory of Physiology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece

Lipopolysaccharides (LPSs) are endotoxins on the cell wall of gram-negative bacteria. Lymphocytes (e.g. monocytes, dendritic cells, macrophages and B cells) recognize LPSs and upon their encounter they induce the release of cytokines (IL-1β, TNFα) and upregulate the inducible nitric oxide synthase 2 (iNOS/NOS2). LPSs can cause deleterious effects on the health of Central Nervous System (CNS) both in vivo and in vitro, leading to neuroinflammatory effects similar to those found in AD and Parkinson's disease. The main cells that react to LPSs and thus play a pivotal role to the mediated effects, are glial cells. Curcumin is a natural product derived from the turmeric seed (root?). It possesses a plethora of medicinal effects, including its anti-amyloid, antioxidant and anti-inflammatory role. However, curcumin employment in medicine is hindered by its low solubility, bioavailability and crossing through the blood-brain barrier. To circumvent this several approaches have been tested, including complexation of curcumin with metals. We have previously created a complex of V (IV) – Curcumin (V-Cur), which is cell- and blood-tolerable and significantly more soluble than curcumin. In the current study, the inflammatory effect of LPSs on murine mixed blastoma cultures (N2A neuroblastoma and GL261 glioblastoma cells) and the beneficial effects of curcumin and V-Cur have been examined. Treatment of mixed blastoma cells with LPSs has been found to lead to significant increase of IL-1β, TNFα, and iNOS, in a dose-dependent manner. Curcumin was able to inhibit the inflammatory effect of LPSs by reducing IL-1β levels up to -42%, TNFα levels up to -33% and iNOS levels up to -26%. V-Cur reduced the levels of IL-1β in a similar manner (up to -38%) but mediated a more potent reduction of TNFα and total iNOS (up to -40% and -58%, respectively). These results demonstrate the inflammatory effect of LPSs on a novel neuroglia system and provide proof that curcumin and V-Cur can effectively alleviate these effects. The anti-inflammatory role of these substances in the nervous system should be further investigated.

Acknowledgements and Funding
Murine N2A neuroblastoma cells and murine GL261 glioblastoma cells were a kind gift by Professor Gaetano Donofrio, Department of Medical Veterinary Science, University of Parma, Italy

P17

Molecular lineage tracing of stem cell derived dopamine grafts in vivo reveals a shared origin of all graft-derived cells

Dr Petter Storm[1], Zhang Yu, Fredrik Nilsson, Alessandro Fiorenzano, Niklas Krausse, Malin Åkerblom, Marcus Davidsson, Joan Yuan, Agnete Kirkeby, Tomas Björklund, Malin Parmar

[1]*Lund University, Lund, Sweden*

Human pluripotent stem cells (PSCs) differentiated into dopamine (DA) neurons via a ventral floor plate intermediate provide an unlimited cell source for cell replacement therapy for Parkinson's disease (PD). However, histology and scRNA-seq profiling of resulting grafts in *in vivo* models have revealed that seemingly homogenous cell preparations give rise to heterogenous grafts not only made up of dopamine neurons. Here, we used single nuclei RNA-seq combined with molecular barcode tracing delivered to the PSC-derived DA progenitors both *in vitro* and *in vivo* to show that three cell types (neurons, astrocytes and vascular leptomeningeal cells, VLMCs) make up the cellular composition of the grafts and that these cell types all share a common progenitor. Additionally, barcoding proliferative cells *in vivo* one month after transplantation showed that a fraction of the cells retained their multipotent potential also after transplantation. Single-cell chromatin accessibility and RNA expression paired maps identified seemingly 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.

P18

Disrupted hippocampal neurogenesis and increased neuroinflammation following brain chemical lesion

Mirka Fourmouzi[1]*, Eleni Makarouni[1]*, Irini Thanou[1] , Mary Margariti 1, Dimitra Thomaidou1

[1]Department of Neurobiology, Hellenic Pasteur Institute, 127 Vas. Sofias Avenue, 11521 Athens, Greece
**equal contribution*

Long-term adverse side effects of chemotherapy have been only recently anticipated and several mechanisms are proposed to be involved in changes regarding brain structure and function. The disturbance of the homeostasis and cellular architecture of the neurogenic zones in particular is having a wider impact on the entire brain and along this line hippocampal NSCs disturbance after chemotherapy is considered a determining factor for the cognitive deficits that patients present.
Our data indicate that intraventricular administration of a chemotherapeutic agent, Ara-C, lead to the disruption in neuronal cell lineage dynamics in the dentate gyrus (DG) of the hippocampus accompanied by an abnormal dendritic morphology of the dendritic projections of newborn DCX+ neuroblasts into the granule cell layer. Moreover, our experimental observations indicate an extensive astro- and microgliosis but we did not detect any infiltrating T-cells in the DG of the hippocampus.
Our study is ongoing o further elucidate the cellular and molecular alterations imposed by Ara-C and its effect on dendritic arborization and overall glial dysregulation.

P19

The inhibition and activation of Notch signaling upon differentiation of human induced pluripotent stem cells to dopaminergic precursors

Katolikova Nataliia Victorovna (1,2), Shafranskaya Daria Dmitrievna (3), Prjibelski Andrey Dmitrievich (3), Efimova Evgeniya Victorovna (1), Malashicheva Anna Borisonva (2,4), Gainetdinov Raul Radikovich (1,5)

1 Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia
2 Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia
3 Center for Algorithmic Biotechnology, Institute of Translational Biomedicine, Saint-Petersburg State University, Saint-Petersburg, Russia
4 Almazov National Medical Research Centre, Saint-Petersburg, Russia
5 Pirogov Clinic of High Medical Technologies, Saint-Petersburg State University, Saint-Petersburg, Russia

Notch signaling plays an essential role in embryonic development of the central nervous system where it plays role in the regulation of cellular migration, controls the balance between self-renewal and differentiation, is important for the maintanance of the growth of axons and dendrites, is involved in synaptic plasticity, and also in neuronal death [1]. Postnatally Notch is used to maintain the pool of stem cells, plays a role in regulation of proliferation and differentiation of the cells, is important for the functionality of adult neurogenesis zones, and is also critical for activation of latent neurogenic programs.
It is known, that during the neuronal differentiation of pluripotent stem cells proteins of Notch signaling cascade are essential for neuronal outgrowth and play the role in specification and regionalization of differentiating cells [2]. But the effects of exposure to Notch signaling is not always unambiguous, they are extremely dependent on the cellular context and on the states of a spectrum of signal cascades with which Notch signaling interacts [3].
In our work we inhibited the Notch signaling by 1) short hairpin RNA (shRNA) to its effector protein RPBJ-k, 2) by gamma-secretase inhibitor DAPT, and activated of Notch signaling by overexpression of Notch intracellular domain (NICD), during the differentiation of human induced pluripotent stem cells to dopaminergic neurons. We analyzed transcriptomic profiles of the cells in the indicated conditions by RNA sequencing at day 7 of differentiation.
We found no reliable difference between Notch inhibition by shRNA to RBPJ-k versus control, which presumably suggests that induction of neuronal differentiation itself leads to inhibition of Notch signaling. At the same time we found that inhibition of Notch signaling by DAPT has common features but is not identical to more specific inhibition by shRNA to RPBJ-k.
Activation of Notch signaling during differentiation led to some bias of gene expression in a more caudal direction, expression of activator of the Wnt signaling pathway and inhibitor of SMAD proteins, as well as an increase in the expression of Glial cell line-derived neurotrophic factor and CORIN.

References

1. Son, A. I. *et al.* Dual Role of Rbpj in the Maintenance of Neural Progenitor Cells and Neuronal Migration in Cortical Development. *Cereb. Cortex* 30, 6444–6457 (2020).
2. Tieng, V. *et al.* Engineering of Midbrain Organoids Containing Long-Lived Dopaminergic Neurons. *Stem Cells Dev.* 23, 1535–1547 (2014).
3. Qi, Y. *et al.* Combined small-molecule inhibition accelerates the derivation of functional cortical neurons from human pluripotent stem cells. *Nat. Biotechnol.* 35, 154–163 (2017).

Funding This work was supported by the Russia

Speaker Biographies

Sandro Alves

Director of Preclinical Research BrainVectis-Askbio, France

Sandro Alves, PhD, is Director of Preclinical Research at Brainvectis/AskBio Europe (Paris, France), leading the preclinical R&D activity since 2016, in particular developing therapeutic strategies in preclinical animal models of neurodegenerative diseases. Prior to joining Brainvectis/AskBio Europe, Sandro experienced two post-doctoral positions: he first conducted his research at the Paris Brain Institute (Paris, France), where he developed disease animal models, investigated disease mechanisms and evaluated therapeutic strategies in animal models of Spinocerebellar ataxia type 7 (2008-2013). After that, he joined the team of Pr. Nathalie Cartier at INSERM (MIRCen, Fontenay-aux-Roses, France), again as post-doctoral fellow, developing preclinical therapeutic strategies based on gene transfer (Brain cholesterol metabolism and immunomodulation) in animal models of Alzheimer's Disease and Huntington's disease, as well as, participating in the generation of rat and mouse models of Alzheimer's Disease (2013-2016). Sandro obtained his PhD in Health Sciences Technology (Neuroscience) at the Faculty of Pharmacy of the University of Coimbra (Portugal)/Center of Neuroscience and Cell Biology of Coimbra (CNC), in 2008, where he developed an animal model of Spinocerebellar ataxia type 3 and evaluated preclinical strategies of allele-specific/non-allele specific gene silencing in a lentiviral-based animal model of SCA3.

Ido Amit

Weizmann Institute of Science, Israel

Born in Kibbutz Hazor, Prof. Ido Amit earned his PhD in biological regulation at the Weizmann Institute of Science in 2007. He conducted a four-year postdoctoral fellowship at the Broad Institute of Harvard University and the Massachusetts Institute of Technology, before joining the Weizmann Institute in 2011. He is the incumbent of the Eden and Steven Romick Professorial Chair.

Prof. Amit is a world leader and pioneer in the fields of single-cell genomics and clinical big-data analytics. He has spearheaded the application of these technologies to generate a comprehensive "google map" of the entire immune system and how it is perturbed in disease. Prof. Amit's research addresses some of the most fundamental questions in immunology. His discoveries are enabling the development of new immunotherapy strategies,—wotking with the leading biopharma his discoveries are driving immense innovation in targeted immunotherapy for autoimmune disorders, neurodegenerative diseases, and cancer – which will lead to the next generation of immunoitherpahy treatments. In addition, Prof. Amit is renowned in the science community as a leader in immunogenomics, a new field aimed at detecting and engineering specific immune activity to combat disease.

Nissim Benvenisty

Director, The Azrieli Center For Stem Cells And Genetic Research Hebrew University of Jerusalem, Israel

Prof. Nissim Benvenisty is the Herbert Cohn Chair in Cancer Research and the Director of The Azrieli Center for Stem Cells and Genetic Research at the Hebrew University. He earned his M.D. and Ph.D. degrees from the Hebrew University, and conducted postdoctoral studies at Harvard University. Prof. Benvenisty's research projects focus on stem cell biology, tissue engineering, human genetics, and cancer research. He published numerous original and review papers on human pluripotent stem cells, and serves on the editorial board of various stem cell related journals. He is a member of the steering committee of the International Stem Cell Initiative (ISCI), the Programme Board of the UK–Regenerative Medical Platform (MRC), and serves as the academic advisor for the International Symposia of the International Society for Stem Cell Research (ISSCR). Prof. Benvenisty presents the issue of human embryonic stem cells in many international conferences, and gave testimonies before the US Senate and the European Union. He was awarded several prizes among them the Foulkes Prize (London), the Hestrin Prize, the Teva Prize, the Kaye Prize, the Milken Prize, the ACTO Award and the Katzir Prize.

Anders Björklund

Senior Professor Developmental and Regenerative Neurobiology

Anders Björklund is Senior Professor of neuroscience at Lund University (Sweden). He research career at Lund University spans over 50 years, with a publication list that comprises over 650 original articles and reviews. His major achievements are in the field of cell transplantation and brain repair. He started this line of research in the mid 1970ies, based on the idea that immature neurons can be used to replace lost neurons in the brain, restore brain circuitry and promote functional recovery in animal models of neurodegenerative diseases. During this period, 1975-1985, his group pioneered the use of immature, fetal neurons dopamine neuron replacement in rodent models of Parkinson´s disease (PD) and in 1987, Björklund and his clinical partners obtained permission to use tissue from aborted human fetuses in a series of open-label clinical trials in PD patients. Although the outcome of these trials has been quite variable, they have given proof-of-principle that immature dopamine neurons can survive and mature in the striatum in advanced PD patients and restore dopamine neurotransmission in the area of the striatum re-innervated by the grafted neurons.
This pioneering work has formed the basis for the current efforts to develop a restorative stem cell based therapy for patients with PD, performed at centers across the world. Over more that 40 years the Lund neural transplantation program has been a leader in the development of restorative therapies in Parkinson´s disease and other neurodegenerative disorders. Current efforts at the Wallenberg Neuroscience center are focused on the development of stem cell-derived dopamine neurons for transplantation from human pluripotent stem cells to be used in an exploratory clinical trial that is planned to start later this year.
In parallel lines of work Björklund´s group has explored the use of viral vector-mediated gene transfer for neuroprotection and brain repair, with the aim to develop new therapeutic approaches for Parkinson´s disease and other neurodegenerative disorders. In addition, he has pioneered the use of AAV vectors for overexpression of human a-synuclein for induction of Parkinson-like neurodegeneration in the nigrostriatal system. This approach has provided a new tool for modeling of the progressive synuclein-induced disease process in animal models of PD.

Björklund is the recipient of numerous international prizes and awards, and is member of two scientific academies, the National Academy of Sciences in USA and the Royal Swedish Academy of Sciences.

Alessandro Fiorenzano

Assistant Professor Lund Stem Cell Center, Sweden

Dr. Alessandro Fiorenzano holds a senior scientist position at the Department of Developmental and Regenerative Neurobiology, Lund University, Sweden. Alessandro obtained his PhD in molecular and cellular biotechnology after training at the Italian National Research Council, where he consolidated his background in stem cell biology. During his PhD program, he studied the impact of the extracellular microenvironment on pluripotency and differentiation of pluripotent stem cells (PSCs), with particular emphasis on extrinsic and intrinsic pathways including PSC self-renewal regulation by noncoding RNAs. His current focus is on investigating the molecular mechanisms underlying neuronal specification that efficiently drive controlled differentiation of human stem cells into specific neuronal subtypes for use in stem cell-based therapies for brain repair in neurodegenerative diseases.

Steven Goldman

Universities of Rochester and Copenhagen, Denmark

Dr. Steven A. Goldman is the URMC Distinguished Professor of Neuroscience and Neurology, and co-Director of Rochester's Center for Translational Neuromedicine. He has a concurrent appointment as Professor of Neuroscience at the University of Copenhagen in Denmark, and as co-director of its sister Center for Translational Neuromedicine. He also serves as Senior Vice President and Head of CNS Therapy at Sana Biotechnology, in Cambridge, Massachusetts. Goldman moved to Rochester in 2003 from the Weill Medical College of Cornell University, where he was the Nathan Cummings Professor of Neurology, and Attending Neurologist at New York Presbyterian Hospital. A summa cum laude graduate of the University of Pennsylvania, he obtained his PhD with Fernando Nottebohm at the Rockefeller University in 1983, and his MD from Cornell in 1984. Dr. Goldman interned in Medicine and did his residency in Neurology at both New York Hospital-Cornell and the Memorial Sloan-Kettering Cancer Center, before joining the Cornell faculty. Goldman moved to Rochester as the Dean Zutes Chair in Biology of the Aging Brain, and as Chief of the Neurology department's Division of Cell and Gene Therapy; he subsequently served as the founding director for Rochester's neuro-oncology training program, and served as Chairman of the Department (2008-12). Dr. Goldman remains active clinically, with subspecialty interests in myelin disease and neuro-oncology, and additional research interests in stem cell-based treatment of neurological disease.

Research

Goldman is interested in cell genesis and regeneration in the adult brain, with a focus on the use of stem and progenitor cells in both modeling and treating demyelinating and neurodegenerative diseases, including targets as diverse as progressive multiple sclerosis, the leukodystrophies, Huntington Disease and schizophrenia. He also has a strong interest in the conversion of resident cells into brain cancers, and is board certified in neuro-oncology. He has published over 250 papers, over 100 as first or senior author, and holds over 30 patents in his field. Dr. Goldman is a recipient of the Novo Nordisk Foundation Laureate Award, the Jacob Javits Neuroscience Investigator Award of the NIH, and has been elected to Academia Europaea, the Association of American Physicians, the American Society for Clinical Investigation, and the American Neurological Association, and he is a fellow of the American Academy of Neurology. He serves on standing committees of the NIH, and has served as a voting member of the FDA Cellular, Tissue and Gene Therapy Advisory Committee. He is a co-founder of Oscine Therapeutics, and his work is supported by NIH/NINDS, the Adelson Medical Research Foundation, the Mathers Charitable Foundation, the Novo Nordisk Foundation, and the Lundbeck Foundation.

Olivier Goureau

Research Director, Institut de la Vision, Sorbonne Université, INSERM, CNRSParis, France

Olivier Goureau is a tenured research director at INSERM, France and leads the team "retinal development and repair" at the Vision Institute in Paris. Through his career, he acquired a broad background in cellular and molecular biology and basic neurosciences with a strong focus on the last 15 years on stem cell biology in the field of ophthalmology. He was trained at the University of Paris-Orsay and after his PhD in 1991, he developed different molecular and cellular biology strategies to study the retinal pathophysiology at the French National Institute of Health and Medical Research (INSERM) where he got a tenured research associate position. In 1993, he pursued his research at Jules Stein Eye Institute at UC Los Angeles, where he focused his research on retinal development. Back in France, he started his own research group and joined in 2003 the laboratory created by Prof. Sahel which became in 2008 to the Vision Institute. His work includes the development of stem cell-based approaches as a therapeutic option for the treatment of retinal degenerative diseases, affecting light-sensitive photoreceptors or retinal pigmented epithelial cells. His team has become a reference in the field of ocular regenerative medicine, participating in different international research projects.

Achille Gravanis

Professor of Pharmacology, School of Medicine University of Crete, IMBB-FORTH, Greece

Professor of Pharmacology Medical School of University of Crete, Researcher Institute of Molecular Biology & Biotechnology, Foundation of Research & Technology-Hellas. Affiliated Research Professor Center for Drug Discovery, Northeastern University. Co-founder and research associate of spinoff biotechnology companies Bionature and ReNeuroCell. Venture Partner at BigPi Ventures. Chairman of the International Advisory Committee, Athens LifeTech Park. His research team is developing synthetic compounds, neurotrophin receptor agonists, with neuroprotective, anti-neuroinflammatory and neurogenic properties with potential applications in the treatment of neurodegenerative diseases. In addition, his team focuses on 3D scaffold technologies that host brain cells and neural stem cells for the development of neurobiosensors for the development of neuroprotective and neurogenic drugs, and neuroimplants for the treatment of spinal cord injury and brain trauma.

Evangelos Kiskinis

Assistant Professor of Neurology, Feinberg School of Medicine, Northwestern University, USA

Evangelos Kiskinis PhD is an Assistant Professor of Neurology and Neuroscience at Northwestern University Feinberg School of Medicine and a New York Stem Cell Foundation Robertson Investigator. Dr. Kiskinis earned a PhD from Imperial College London and carried out postdoctoral training at Harvard University where he pioneered the first models of ALS using personalized stem cell-based approaches. His laboratory was established at Northwestern in 2015 and seeks to harnesses the power of human pluripotent stem cells to study neuronal development as well as to understand how neuronal function is impaired as a result of injury or disease. The overarching goal of his research is to provide novel insights into neuronal development and identify points of targeted and effective therapeutic intervention for epilepsy and ALS/FTD. At Northwestern he also serves as the Scientific Director of the Stem Cell Core Facility.

Jari Eerik Koistinaho

Director of Helsinki Institute Of Life Science, Helsinski Institute of Life Science

Jari Koistinaho is the Director of Helsinki Institute of Life Science (HiLIFE) and Professor of Neuropharmacology at the University of Helsinki, and Professor of Regenerative Medicine in the A.I. Virtanen Institute, Faculty of Health Sciences, at the University of Eastern Finland. Koistinaho's research focuses on the neurobiology and genetics of neurodegenerative diseases using iPSC technology. Koistinaho was trained as a physician and completed doctoral studies at the University of Tampere. He has worked abroad at the US National Institute of Health, University of California San Francisco, Stanford University, Indiana and Purdue Universities, and the Karolinska Institute. His group was the first to discover the neuroprotective effects of minocycline, a semisynthetic antibiotic, a finding that led to over 30 clinical trials. Koistinaho is also the founder of three spin-out companies: Cerebricon Ltd acquired by Charles River Discovery Research Services 2009; Medeia Therapeutics Ltd 2006, and Aranda Pharma Ltd 2015.

Tilo Kunath

Chair Of Regenerative Neurobiology, Institute for Stem Cell Research, University of Edinburgh, UK

Tilo Kunath obtained his PhD from the University of Toronto in 2003 investigating novel stem cell systems in the laboratory of Professor Janet Rossant. His postdoctoral studies with Professor Austin Smith at the Institute for Stem Cell Research at the University of Edinburgh were focused on neural induction and differentiation of mouse and human embryonic stem (ES) cells. He started his laboratory in the Centre for Regenerative Medicine at the University of Edinburgh in 2007 with Parkinson's UK funding, where he has pioneered the use of patient-specific induced pluripotent stem (iPS) cell technologies to establish robust models of Parkinson's. He also has a major focus on driving forward technologies to advance the use of human ES and iPS cells for use in cell replacement therapies for Parkinson's. He hosted the 31st NECTAR conference in Edinburgh in November 2021. Tilo is currently funded by Cure Parkinson's and the MRC, and was recently promoted to Chair of Regenerative Neurobiology.

Sabah Mozafari

Researcher University of Paris, Paris, France

EDUCATION
-PhD in Neurosciences (2016), Sorbonne University, Paris, France
-Master in Neurosciences (2012), Sorbonne University, Paris, France
-Master in Medical Physiology (2008), Tarbiat Modares University, Tehran, Iran
-Bachelor in Nursing-Anesthesia (2004), Booshehr University of Medical Sciences, Iran

RESEARCH EXPERIENCES
-Researcher, Material and Complex Systems (MSC) Laboratory, CNRS, University of Paris, (2022 – current)
-Postdoc., Institute Mondor of Biomedical Research (IMRB); INSERM, France (2021 – 2022)
-R&D Scientist, MedDay Pharmaceuticals Paris, France (2019-2021)
-Postdoc., ICM, Paris, France (2016 – 2019)
-Graduate student, Sorbonne University, ICM, Paris, France (2011 – 2016)
-Researcher, Department of Stem Cell Biology, Royan Institute, Tehran, Iran (2010 – 2011)
-Research assistant, Tehran University of Medical Sciences, Tehran, Iran (2009-2010)
-Graduate student, Tarbiat Modares University, Tehran, Iran (2005 – 2008)
CLINICAL EXPERIENCES:
-Nurse Anesthetist and Intensivist, Iran (2001 –2009)
PUBLICATION:
20 publications, 32 proceedings, 705 citations, h-index = 12
1st author in 8, 2nd author in 3, Corr. author in 2
ACHIEVMENTS:
8 Individual Research Fundings, 1 Young Researcher Prize (FdT), 10 Travel Fellowships, 3 Best presentation prizes, 2 First Class Honours in BSc. & MSc., 2 Ranked 4th (~ 2000 candidates each) in National Entrance Competitions for master.

Malin Parmar

Lund Stem Cell Center, Sweden

Dr. Parmar is a Professor in the Cellular Neuroscience Department at Lund University, Sweden, where she is focusing on bringing new cell-based therapies for Parkinson's disease to the clinic by replacing lost dopamine neurons with new, healthy cells. Her work in cellular reprogramming opens up the possibilities of personalized treatments of patients with healthy versions of their own cells. She completed her postdoctoral studies at Lund University, Sweden and Edinburgh University, Scotland.

Luís Pereira de Almeida

Associate Professor | President, University of Coimbra, Center for Neuroscience and Cell Biology, Portugal

Luís Pereira de Almeida research is developed at CNC-Center for Neuroscience and Cell Biology, University of Coimbra Portugal where he is Principal Investigator and President. Luis also coordinates the Center for Innovative Biomedicine and Biotechnology (CIBB) and is Associate professor at Faculty of Pharmacy, University of Coimbra.

Luis did his PhD (1998-2003) in the Gene Therapy Center of Lausanne CHUV, Switzerland with Profs Patrick Aebisher and Nicole Déglon and spent short sabbatical leaves at CEA, France(2005), and at Massachussetts Institute of Technology (MIT-2010) and has been vice-president of the Portuguese Society for Stem Cells and Cell Therapy (2013-2015).

Luis leads a talented research group focused on gene and stem cell therapy approaches for brain disorders, particularly Machado-Joseph disease/spinocerebellar ataxia type 3, which has published over a hundred peer-reviewed papers (h-factor 46). Luis mentored 12 pos doctoral fellows and 17 PhD students and coordinated or participated in over 30 research projects, such as the transnational projects Synspread and ModelPolyQ within the European Joint Programme for Neurodegenerative Diseases (JPND), and presently including ViraVector - a Research Infrastructure for production and experimentation with viral vectors that integrates the national roadmap of scientific infrastructures, and the European iMi project ARDAT (Accelerating Research and Development in Advanced Therapies).

Stefano Pluchino

Professor of Regenerative Neuroimmunology, Cambridge University, UK

The Pluchino team studies whether the accumulation of neurological disability observed in patients with chronic inflammatory neurodegenerative diseases can be slowed down by reprogramming the host immune system with advanced therapeutics. He has been first or senior author in seminal papers that have established the potential of stem cell and exosome therapeutics for a broad range of neurological conditions. His research has recalibrated the classical view that cellular grafts only function through structural cell replacement, while opening a new therapeutic avenue by which to use cellular and acellular advanced therapeutics.

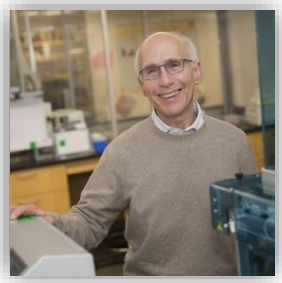
He is recipient of numerous national and international awards, among which the Italian Multiple Sclerosis Foundation Rita Levi-Montalcini prize for outstanding research in MS (2007) and the International Royan Award for outstanding research in Stem Cell Biology and Technology (2010). He is a 2009 Italian Ministry of Health Young Investigator Awardee and 2010 ERC Starting Independent Researcher.

His laboratory research on Regenerative Neuroimmunology is documented in >230 publications in international journals, including articles in Nature, Cell, Cell Stem Cell, Nat Cell Biol, Nat Chem Biol, PLoS Med, to name a few. His publications have to date received >15.000 citations (ISI-WOK), having a Hirsch Factor of 55, according to Google Scholar.

Magdalini Polymenidou

Principal Investigator, Department of Quantitative Biomedicine University of Zurich, Switzerland

Magdalini Polymenidou is Associate Professor of Biomedicine at the Department of Quantitative Biomedicine of the University of Zurich (UZH). She is an expert on protein aggregation, prions and prion-like phenomena, as well as RNA-binding protein biology. She joined UZH as an Assistant Professor in 2013 and since then, she has been leading a research team focusing on the molecular mechanisms of ALS and FTD. The team focuses on the formation and properties of the physiological and pathological states of TDP-43, FUS and the dipeptide repeat proteins, combining structural and biochemical analyses with cellular assays and animal models. Originally trained as a pharmacist in Aristotle University of Thessaloniki (Greece), she did her PhD on prion diseases in the laboratory of Adriano Aguzzi at the University Hospital of Zurich (Switzerland). As a postdoctoral fellow in the group of Don Cleveland at the University of California in San Diego (USA), she used genome-wide approaches to understand the function of TDP-43 and FUS. Among other honors, she was awarded the ERC Consolidator Grant (2022), the Franco Regli Prize (2019), the EMBO Young Investigator Award (2018), the Georg-Friedrich Götz Prize (2015), the SNSF Professorship (2013), the HFSP Career Development Award (2013) and the NIH Pathway to Independence Award (2011).



Lee Rubin

Professor Dept of Stem Cell and Regerative Biology, Harvard University, USA

Dr. Rubin has a broad experience in translational neuroscience research in both academia and industry. His group discovered the first small molecule agonists and antagonists of the hedgehog-signaling pathway. One of the antagonists (Erivedge) was partnered with Genentech and is now approved as a treatment for metastatic basal cell carcinoma. One of the agonists is now widely used by stem cell investigators to produce motor neurons and other types of neurons from stem cells. Since coming to Harvard, Dr. Rubin has focused on producing differentiated patient-specific neurons from induced pluripotent stem cells to model disease and discover therapeutics for disorders including Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA). His group's work in SMA led to the observation that there is a cell-autonomous defect in muscle development and regeneration and stimulated us to work on muscle-directed therapeutics. Finally, his lab has initiated a series of experiments focused on identifying aging related factors, such as GDF11, that may stimulate functional improvement in patients suffering from neurodegenerative diseases.

Dr. Rubin received his PhD in Neuroscience from the Rockefeller University and had postdoctoral training, also in Neuroscience, at Harvard Medical School and Stanford University School of Medicine.

Frederic Saudou

Institut Neurosciences, Grenoble France

2014: **Director of the Grenoble Institute of Neurosciences** (GIN) (app. 250 persons) INSERM U836, University Grenoble Alpes, 38000 Grenoble, France
2014: **Full Medicine Professor**, Hospital Practitioner, Grenoble University Hospital
2014: **Group leader GIN**

Jens Christian Schwamborn

Prof. For Developmental And Cellular Biology, Luxembourg Centre for Systems Biomedicine, Luxembourg

In 2002 Jens obtained a diploma in Biochemistry from the University Witten/Herdecke in Germany and in 2005 a PhD in Biology from the University Muenster in Germany. He worked as a postdoctoral researcher at the Institute for Molecular Biotechnology in Vienna / Austria. Since 2013 Jens is head of the Developmental and Cellular Biology group at the Luxembourg Centre for Systems Biomedicine (LCSB) as well as Professor at the University of Luxembourg.
Jens Co-founded OrganoTherapeutics SARL-S, where he currently acts as CEO, in 2019. Before he was co-founder and CSO of Braingineering Technologies SARL (2016-2018).
The focus of his work over the last years was on Neurobiology, Stem Cell research and Parkinson's disease. In particular he is interested in using human induced pluripotent stem cells for the development of brain organoid and assembloid models, which are used for in vitro disease modeling.

Benjamin Stecher

Chair of the Patient Advisory Board (PAB), Rune Labs

Benjamin Stecher was born in Nairobi, Kenya but grew up just outside Toronto, Canada. He studied history and philosophy at the University of Guelph, but as soon as he graduated he took off and spent most of the next decade living and working in East Asia as an education consultant. All that changed when, at 29 years old, Benjamin was diagnosed with Parkinson's disease. The promising future he had been building towards suddenly faded and was replaced by a gloomy fog of uncertainty and despair. As his disease progressed, he realized that he needed to come back home to learn how to better manage his disease.

But, as he started visiting labs and attending conferences, he soon found himself enthralled by the rampant pace at which biomedical science has progressed in the 21st century. He spent the next couple of years traveling the world visiting research centers and pharmaceutical companies to learn from the top minds in the field all that he could about this disease and what therapies were on the horizon.

He now speaks regularly at academic centers and biotech companies on issues related to neurodegenerative diseases, research advocacy and healthcare. He is also the founder of Tomorrow Edition, and a patient advisor to several organizations including the Toronto Western Hospital Movement Disorder Clinic and Rune Labs, a San Francisco based brain-tech company, where he chairs their patient advisory board. He has also published a book along with Prof. Alberto Espay by Cambridge University Press titled Brain Fables.

Clive Svendsen

Chair/Executive Director, Cedars-Sinai Medical Center

Dr. Clive Svendsen did his predoctoral training at Harvard University. He received his PhD from the University of Cambridge in England, where he subsequently became a Welcome Fellow and established a laboratory focusing on stem cell research. He then moved to the University of Wisconsin in 2000 as Professor of Neurology and Anatomy and founded their Stem Cell and Regenerative Medicine Center. In 2010, he moved to Los Angeles and founded the Cedars-Sinai Board of Governors Regenerative Medicine Institute (RMI), which currently has 23 faculty members and over 120 staff. A main focus of the Institute is to both model and treat various human diseases with the use of induced pluripotent stem cells (or iPSCs), which are adult human cells taken back in time to a young stem cell state capable of making any tissue of the human body. At the heart of the RMI is the Cedars-Sinai Biomanufacturing Center which manufactures iPSCs and other cell types for research purposes and clinical trials. Additionally, Dr. Svendsen maintains a large lab that focuses on modeling neurodegenerative diseases including Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis (ALS), as well as Huntington's, Parkinson's and Alzheimer's Disease. Using "organ on chip" technology that combines stem cells and engineering, Dr. Svendsen is creating multicellular human systems for research and drug development. He has received many recognitions for this work, including a recent feature on the front cover of National Geographic as "The Future of Medicine". The other focus of his lab involves cutting-edge clinical trials using combinations of stem cells and growth factors. He spearheaded one of the first clinical trials to deliver a growth factor (GDNF) to patients with Parkinson's Disease, and is currently working closely with neurosurgeons, neurologists and other scientists to develop novel ways of using stem cells modified to release GDNF for Parkinson's patients. He is also finding ways to use stem cells for other neurological diseases such as Huntington's and Alzheimer's Disease, and is leading a new clinical trial using stem cells to treat an incurable eye disease Retinitis Pigmentosa. Finally, Dr. Svendsen has a long-standing interest in ALS and he was the Sponsor of the first-ever clinical trial delivering stem cells and GDNF to patients that was recently completed at Cedars-Sinai.

Jun Takahashi

Director and Professor, Center For Ips Cell Research And Application, Kyoto University

Jun Takahashi is a director and professor of the Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan. He graduated from the Kyoto University Faculty of Medicine in 1986 and started his career as a neurosurgeon at Kyoto University Hospital after that. After earning his Ph.D. from the Kyoto University Graduate School of Medicine, he worked as a postdoctoral research fellow at the Salk Institute (Dr. Fred Gage), CA, U.S.A., where he started research on neural stem cells. After returning to Kyoto University Hospital, he conducted functional neurosurgery, including deep brain stimulation and research on stem cell therapy for Parkinson's disease. In 2012, he became a full professor at CiRA, pursuing stem cell therapies for Parkinson's disease patients. As a physician-scientist, he has laid the groundwork for the clinical application of iPS cells by developing effective differentiation protocols to dopaminergic neurons, selective sorting of the differentiated dopaminergic progenitor cells, and optimization of the host brain environment to maximize the survival and function of the transplanted cells in rodent and monkey brains. Based on these achievements, he started the clinical trial for Parkinson's disease using iPS cells in 2018.

Malú Gámez Tansey

Norman and Susan Fixel Professor of Neuroscience and Neurology, Co-Director Center for Translational Research in Neurodegenerative Disease and the Parkinson's Foundation Research Center, University of Florida, USA

Maria de Lourdes (Malú) was born in El Paso, Texas and attended Loretto Academy high school. Dr. Tansey obtained her B.S/M.S in Biological Sciences from Stanford University and her Ph.D. in Cell Regulation from UT Southwestern in Dallas, TX followed by post-doctoral work in neurorscience at Washington University Medical School. Prior to setting up her academic research lab in 2002 at UT Southwestern Medical Center in the Department of Physiology, she was head of the Chemical Genetics group at Xencor, a biotechnology company in Monrovia, working on novel TNF inhibitors that she used as tools in academia to investigate the role of neuroinflammation in neurodegenerative disease and which have now advanced to clinical trials in Alzheimer's disease and COVID19 for cytokine storm.

After becoming a tenured Associate Professor of Physiology at UT Southwestern in 2008, Dr. Tansey was recruited to Emory University in Atlanta, GA by the Department of Physiology where she became a member of the Center for Neurodegenerative Disease (CND) and also served as Senior Director of Graduate Studies (DGS) for the Emory Neuroscience Graduate Program and a member of the Executive Committee of the Emory Immunology and Molecular Pathogenesis (IMP) Graduate Program. While at Emory, she catalyzed multiple initiatives to expand and coalesce neuroinflammation efforts across the medical school and helped establish the Center for Neurodysfunction and Inflammation (CNI) at Emory in 2018. In 2019, she was recruited to the University of Florida to be Director of the Center for Translational Research in Neurodegenerative Disease (CTRND) and the first endowed Norman and Susan Fixel Chair in Neuroscience and Neurology at the University of Florida.

Today, Tansey's lab employs multi-disciplinary approaches to investigate the role of inflammation and immune system responses in brain health and the development of neurodegenerative diseases with particular focus on the gut-brain axis. Her long-term goal is to train the next generation of scientists who can/and to develop better therapies to prevent and/or delay these disorders.

As a Hispanic American, Dr. Tansey has served as a role model to numerous undergraduate, graduate and post-graduate trainees, many of them women from under-represented groups in STEM. She served as Co-Director of Emory's R25 Initiative for Maximizing Student Development (IMSD) whose mission is to strengthen institutional efforts to enhance recruitment and retention of diverse student and faculty bodies at Emory, by providing research training and mentoring opportunities to both. Dr. Tansey is a fierce advocate for women and other under-represented groups in STEM and has earned several mentoring awards from students and faculty for her efforts in this area.

Era Taoufik

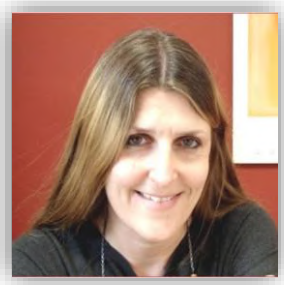
Hellenic Pasteur Institute, Greece

I am a researcher at the Laboratory of Cellular-Molecular Neurobiology and Stem Cells working in the Neuroimmunology and Stem Cell fields. At the beginning of my scientific career I focused on the interactions between the immune system and the central nervous system trying to understand how they co-operate at the molecular and cellular level to help maintain brain homeostasis under physiological as well as pathological conditions such as Multiple Sclerosis (MS) and Brain Ischemic Injury. For the last three years I have been shifting my interests to another neurological disease, Parkinson's, studying disease mechanisms by using Pluripotent Stem Cells (iPS) derived from patients with an inherited and familial form of the disease, both in vitro and in vivo, applying cutting edge technologies such as RNA-Sequencing, artificial synapse formation assays and cell transplantation protocols. I have also initiated a new collaborative project where I use iPS technology to investigate the cellular component, the neuron-glial interactions and the molecular mechanisms of another devastating neurological disease, Primary Progressive MS.

Anne Marion Taylor

Chief Scientific Officer, Xona Microfluidics, USA

Dr. Taylor received her Ph.D. in Biomedical Engineering from the University of California at Irvine, and postdoctoral training at Caltech in Neurobiology under Dr. Erin Schuman (now a Director of the Max Planck for Brain Research in Frankfurt, Germany). Dr. Taylor was previously faculty at the University of North Carolina at Chapel Hill for 9 years before moving to Xona Microfluidics full-time in 2019. Dr. Taylor invented the microfluidic technology used to compartmentalize neurons, together with the scientific co-founders of Xona. As an independent investigator at both UNC-Chapel Hill and Xona Microfluidics, Inc., she continues to study of how cellular mechanisms of axon damage, an early event in acute neural injuries and neurodegenerative disease, cause synaptic remodeling. Her research has been published in many high-profile publications including Nature Methods, Neuron, and Nature Communications.



Dimitra Thomaidou

Research Director, Neurobiology Deprtment, Hellenic Pasteur Institute, Greece

Dimitra Thomaidou is Research Director in Hellenic Pasteur Institute (HPI) Neurobiology Department and Scientific Responsible of HPI's Light Microscopy Unit. DT has 25 years of experience in Neuroscience with her research interests focusing on adult neurogenesis and Neural Stem Cell biology. She has received her BSc degree in Biology from the University of Athens and her PhD in Neuroscience from the University of Patras. She continued to post-doctoral studies initially in University College London and then in Rockefeller University New York, with scientific focus on cell cycle regulation and neurogenesis during nervous system development.

She returned back to Greece in 1999 awarded with "Career Award" Program as a senior post-doc in HPI and elected in Researcher position in 2002. The current research interests of her group focus on the exploration of the mechanism of action of novel genes/ miRNAs in instructing direct neurogenic reprogramming of astrocytes and the assessment of their properties in vitro and in vivo in animal models of neurodegeneration / neuroinflammation. To this end, she has introduced long time live cell imaging to follow the route of reprogrammed cells towards induced-neurons in vitro and 2-photon intravital imaging to in vivo explore astrocytes-microglia dynamic interactions following brain injury and neuroinflammation. As Head of Light Microscopy Unit, DT has played a key role in the establishment of the Unit, acquisition of latest technology imaging systems and development of state-of-the-art imaging approaches. Her publication record consists of 71 articles, 41 of them in peer-reviewed journals, among which J. Neuroscience, JBC, Cell Reports, Stem Cell Reports, PNAS and Brain.

PubMed publication list: https://pubmed.ncbi.nlm.nih.go

Mark Tuszynski

Director, Ucsd Translational Neuroscience Institute, University of California San Diego, USA

Dr. Tuszynski received his undergraduate and M.D. degrees from the University of Minnesota in Minneapolis. He received clinical training in neurology at Cornell University Medical Center in NY, NY from 1984-1987, and became board-certified in neurology in 1989. He attended graduate school at the University of California-San Diego from 1988-1991, earning a Ph.D. in neuroscience. Dr. Tuszynski joined the faculty of the Department of Neurosciences at the University of California-San Diego in 1991. He is currently Professor and Director of the UCSD Center for Neural Repair, and Founding Director of the UCSD Translational Neuroscience Institute.

Dr. Tuszynski's research focuses on the role of growth factors in influencing cell survival, plasticity and regeneration in the adult central nervous system. He actively researches the topics of Alzheimer's disease, aging, cellular mechanisms of normal memory, and spinal cord injury. In 2001 he began the first human clinical trial of gene therapy to treat an adult human neurodegenerative disease, testing the effects of nerve growth factor gene delivery in patients with early Alzheimer's disease. He has won 10 national awards for neurodegeneration research, and is the author of over 200 scientific and medical publications.

Maria Xilouri

Investigator C', Assistant Professor Level, Biomedical Research Foundation of the Academy of Athens, Greece

My research is mostly focused on alpha-synuclein neurobiology, a protein strongly implicated in Parkinson's disease (PD) and multiple system atrophy (MSA) pathogenesis. In contrast to PD, where alpha-synuclein inclusions are formed within neurons, MSA is characterized by abnormal accumulation of alpha-synuclein within oligodendrocytes that physiologically are considered to express low to non-detectable levels of the protein. Up to date, this unique ectopic alpha-synuclein expression remains an enigma in the field. The cellular pathways investigated relate to protein misfolding and aggregation, which may lead to impairment of protein degradation systems and eventually to neuronal demise. Beyond clearance, we are also targeting the release (occurring partly via small nanovesicles called exosomes), uptake and seeding of alpha-synuclein in neurons and oligodendrocytes, in an attempt to scrutinize whether similar or different protein strains cause PD- and MSA-like pathology, respectively. Our approach encompasses a variety of cellular and animal models of PD and MSA, as well as human patient-derived material (brain, iPSCs and biological fluids) coupled with state-of-the art technologies. Collectively, our research aims to may provide further insights into the mechanisms underlying disease initiation and progression. Once such mechanisms are understood, they can be harnessed in order to provide meaningful disease-modifying therapies.

Chiara Zurzolo

Institut Pasteur, Paris, France

Chiara Zurzolo, MD, PhD, received her doctorate degrees from the University of Naples "Federico II" before spending 4 years as a postdoctoral fellow at Cornell Medical School in New York. She returned to Naples University Medical School to establish her own research group as an Assistant and then Associate Professor. In 2003, she moved to Paris as a Director of Research. In 2015 she was nominated Professor at the Pasteur Institute and elected as an EMBO Member.

Zurzolo is an internationally well-known researcher, very often invited to speak at meetings around the globe. She is also highly involved in teaching at Pasteur and internationally, organizing and participating in high-level courses for Master and PhD students. She has also been active in other organizations such as ELSO, demonstrating her energy and engagement for promoting science.

Chiara Zurzolo has made several seminal discoveries in the fields of cell biology and neurodegeneration. During her postdoc, she focused on apical sorting mechanisms of GPI-anchored proteins and showed (against the prevailing hypothesis) that the GPI-anchor is NOT an apical signal. Her recent findings suggest that the sorting mechanism of GPI-APs in the Golgi regulates their apical organization and function (Nat Chem Biol 2014). This regulatory sorting mechanism is a novel concept that opens new avenues to elucidate conditions in which polarity is lost, such as in cancer, the current focus of several projects in her lab.

She started working on prion trafficking at the Pasteur Institute in 2003. She uncovered the intracellular site of pathological conversion of the prion protein (PlosPath 2009) and the mechanism of prion dissemination to the brain. She showed that prion dissemination between neurons and from dendritic cells to neurons occurs by Tunneling Nanotubes (TNTs), a new mechanism of direct intercellular communication (Nat Cell Biol 2009). Importantly, she has found that this mechanism is relevant for the intercellular spreading of other amyloid aggregates involved in neurodegenerative diseases (JCS 2013, BJ review 2013, Sci Rep 2015, EMBO J 2016, Biol Open 2017, ActaNeu 2017). She proposes that TNTs are involved in the spreading of different amyloidogenic proteins in the brain (JCB 2017) and therefore represent a suitable target to halt incurable neurodegenerative diseases. This represents a new paradigm in neurodegenerative diseases.

Her laboratory is now working on further assessing the role of TNTs in neurodegenerative diseases as well as in different cancer models, and to understand the molecular mechanisms involved in TNT formation. Her group discovered that TNTs and filopodia are molecularly and structurally different (Sci Rep 2016, JCS 2018, Nat Comm, under revision), representing novel organelles. Currently, she and her group are concentrating on identifying these structures *in vivo*, normal and diseased conditions. Her fundamental cell biology work has excellent translational potential in many fields where TNTs might be involved (e.g. cancer, development, immunity, etc.).

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