Universität Zürich[UZH]

iPSZÜRICH

Welcome to the

iPSZurich Symposium 2024

4-5th April 2024 • Zurich, Switzerland

160th
日本・スイス国交樹立記念
Anniversary of Diplomatic Relations
between Japan and Switzerland

Workshop: KOL-F-101

Public Lecture & Symposium: KOH-B-10

Rämistrasse 71

8006, Zurich

University of Zurich[UZH] Institute for Regenerative Medicine • IREM

CiRA

京都大学 KYOTO UNIVERSITY

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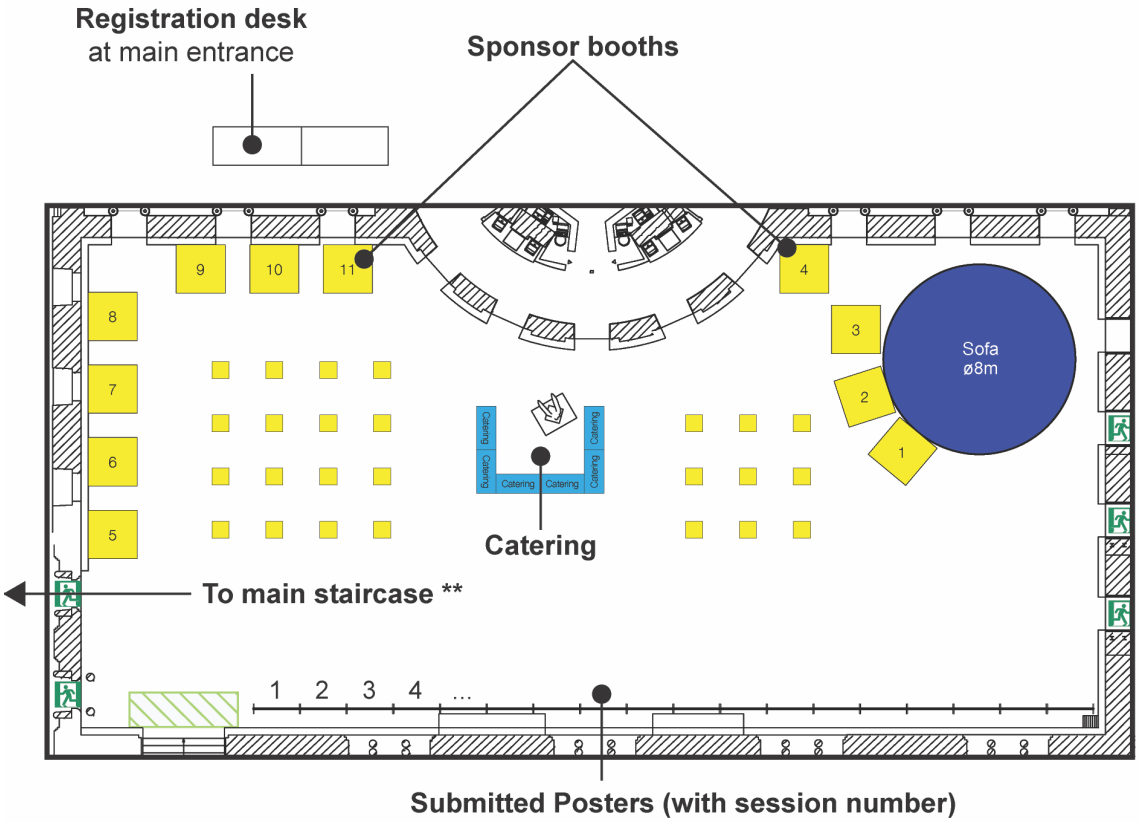
Orientation

UZH Lichthof, Floor D



3 **West Entry via Künstlergasse**
Station: Neumarkt (Line 3)
12' walk to main station

6 **9** **10** ** **Main Entry from staircase via Rämistrasse 71**
Station: ETH/Universitätsspital (Line 6+9+10)

** **Freight delivery access via Karl-Schmid-Strasse 4**

UZH map

KOL - Main building
Rämistrasse 71
8006 Zürich

1	STEMCELL Technologies
2	IotaSciences
3	Agilent Technologies
4	OMNI Life Science
5	MaxWell Biosystems
6	MaxWell Biosystems
7	3Brain
8	3Brain
9	Bucher Biotec / Nanocellect
10	SEED Biosciences
11	Miltenyi Biotec

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Voting QR codes

Don't forget to vote for the your favourite poster and presentation using the QR-codes below.

Favourite Poster

https://strawpoll.com/eJnvvakQ4nv

Favourite Oral Presentation

https://strawpoll.com/wAq3AoL7My8

Sponsors

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The Swiss National Science Foundation (SNSF) funds excellent research at universities and other institutions – from chemistry to medicine to sociology. Thousands of teams are generating knowledge for a better future for all. Together with our partners, we play a key role in shaping research in Switzerland.

The Kyoto University Foundation More info here

The Kyoto University Foundation for Education and Research provides grants for the promotion of high-level education and research at Kyoto University, the continuation and development of important academic fields that are not necessarily in great demand from a social and economic perspective, human resource development for young researchers, and contribution activities for the revitalization of local communities, thereby contributing to the promotion of science, technology, and other interests.

UZH Alumni More info here

Through alumni organizations, former students not only stay connected to each other and their alma mater, but also actively shape the latter's vitality. They thus remain an essential part of the UZH family even after graduation. The UZH alumni networks boasts 18'400 members, across 25 alumni organisations.

3Brain More info here

3Brain is the world's first company to connect cells with sophisticated silicon chips in cell culture plates. Stemming from their passion for technology and scientific progress, their co-founders have worked for more than 15 years on CMOS-powered cell-electronic interfaces with the aim of boosting research in major fields like neuroscience, ophthalmology and cardiology.

MaxWell Biosystems More info here

MaxWell Biosystems AG is an electronics and biotechnology company based in Zurich, Switzerland that provides instrumentation and solutions to advance neuroscience research and accelerate drug discovery. The company was incorporated in September 2016. Applications include a electrophysiology cell-imaging platform and High-Resolution MEA Technology based on micro-sensor fabricated in CMOS technology.

Bucher Biotec AG More info here

Bucher Biotec AG is a Swiss distributor representing leading manufacturers of highly innovative life science research instrumentation. We are extremely proud of our distinguished customer base in the pharmaceutical, biotechnology, agricultural, food and related industries, in all life science research oriented academic institutions, in numerous governmental, clinical and environmental labs and in all university hospitals. Our highly competent, well educated team is focused on understanding our customer's needs in order to be able to propose optimal solutions for the demanding research tasks.

NanoCellect Biomedical, Inc. More info here

NanoCellect is committed to empowering every scientist to make discoveries one cell at a time, with modern and simple technologies for to advance cell based assays that are affordable, compact, and easy-to-use. Our microfluidic flow cytometry platforms enable biomedical scientists to analyze and sort cells required for drug discovery, single cell-omics, cloning, and basic research. The company was founded in late 2009 as a spinout from UCSD and dedicated 6 years developing the foundation of the WOLF's technology before introducing the WOLF to early adopters in 2016. Initial funding of R&D was graciously provided by multiple NIH SBIR grants and

contracts. Additionally, we are backed by Illumina Ventures, FusionX Ventures, Anzu Partners, Agilent Technologies, Vertical Ventures and other private investors.

SEED Biosciences More info here

SEED Biosciences developed DISPENCELL with the vision of democratising the use of single cells in research. DISPENCELL enables scientists to isolate single cells three times faster and at a cost 10 times lower than existing solutions. Its ambition is to set new standards in single-cell biology in order to accelerate the translation of precision medicine from research to personalised therapies for the benefit of patients.

Miltenyi Biotec More info here

For more than 30 years, Miltenyi Biotec has played an important role in the design, development, manufacture, and integration of products that empower the advancement of biomedical research and enable cell and gene therapy. Miltenyi has 17,000 Products available worldwide and at the heart of their business lies MACS Technology, a magnetic cell separation method based on the use of MACS Columns and MACS MicroBeads.

OMNI Life Science More info here

OMNI Life Science is your Partner for Solutions in 3D Cell Culture, Cell Counting, Cell Assays, Cell Imaging and Microbiology. Innovative systems for research in Life Sciences: From cell culture to real-time cell assays, cell counting and microbiology. 3 Reasons to partner with OLS: (1) match your need with the broad range of excellent cell analysis instruments, (2) Gain best results in less time with user-friendly instruments and powerful software, (3) Benefit from the OLS experts´ know-how, instrument trainings and ongoing service.

Agilent More info here

Agilent is a global leader in life sciences, diagnostics and applied markets, recognized for uncompromising integrity in all we do. Our mission is to advance quality of life by focusing our expertise in six key markets. With a team of approximately 18,000 employees spread across the world, our global footprint includes sales offices, logistics centers, and business facilities in three major geographies.

iotaSciences More info here

We are a young and dynamically growing spin-out company from Oxford, UK. The company was founded in summer 2016 by scientists from the Department of Engineering Science and the Sir William Dunn School of Pathology, University of Oxford, UK. Since its inception, iotaSciences has been committed to offer cell researchers highly user-friendly, reliable and powerful technologies with unique capabilities that greatly simplify and facilitate breakthrough advances in cell biology.

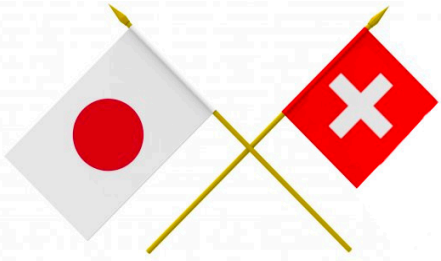
STEMCELL Technologies More info here

STEMCELL Technologies provides high-quality cell culture media, cell separation technologies, instruments, accessory products, and educational resources to scientists around the world working on stem cell, immunology, cancer, regenerative medicine, and cellular therapy research. At STEMCELL, we are dedicated to improving lives through advanced knowledge and scientific discovery, through our commitment to fostering diversity and inclusion in STEM and the life sciences industry, and through our investments in sustainability, community, and social responsibility.

4th April 2024 – CiRA-IREM Joint Workshop

07:30 – 08:00	**Registration**	**Registration desk**
08:00 – 08:30	**Welcome & Introduction KU and UZH**	**Room KOL-F-101**
	Welcome and Introduction of Kyoto University and University of Zurich as a hub of life science and innovations	
08:30 – 09:30	**Experience Reports**	**Room KOL-F-101**
	Experience Reports of Swiss living/studying in Japan and vice versa *Invited speakers: Thomas Maurissen (Industry), Reiko Akiyama (UZH), Sora Matsumoto, (EMBL), Debora Kehl (Industry)*	
09:30 – 10:30	**Meet & Greet**	**Lichthof, Floor D**
	Meet and greet with our invited workshop guests with Gipfeli and coffee	
10:30 – 11:00	**Funding opportunities**	**Room KOL-F-101**
	Leslie Reinhard, Global Affairs, UZH Dr. Kelvin Hui, CiRA	
18:00 – 19:30	**Public Lecture**	**Room KOH-B-10**
	Public lecture by Prof. Jun Takahashi, CiRA and Prof. Simon P. Hoerstrup, IREM	

Prof. Jun Takahashi
Kyoto University



160th 日本・スイス国交樹立記念 Anniversary of Diplomatic Relations between Japan and Switzerland

Prof. Simon P. Hoerstrup
University of Zurich

University of Zurich[UZH] Institute for Regenerative Medicine • IREM

CiRA

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5th April 2024 – Symposium

08:00 – 08:30	**Welcome & Registration**	**Registration desk**
08:30 – 10:00	**Session 1: Development and Aging**	**Room KOH-B-10**
	Guest speaker: Prof. Knut Woltjen (30 +10 min), CiRA Abstract Selected Speaker (10+3 min): Linh Nguyen, Kyoto University, Japan Ermanno Malagola, University of Zurich, Switzerland Ramon Pfaendler, ETH Zurich, Switzerland	
10:00 – 10:30	**Coffee Break**	**Lichthof, Floor D**
	Refreshments provided in the Lichthof	
10:30 – 12:00	**Session 2: Modelling in Health and Disease**	**Room KOH-B-10**
	Guest speaker: Dr. Lauriane Cabon (30 +10 min), Roche Abstract Selected Speaker (10+3 min): Edna Grünblatt, University of Zurich, Switzerland Sarah Guimbal, University of Bern, Switzerland Martina Nubie, University of Zurich, Switzerland Gold sponsor: MaxWell Biosystems (5 min)	
12:00 – 13:00	**Lunch**	**Lichthof, Floor D**
	Refreshments provided in the Lichthof	
13:00 – 14:00	**Poster Session I**	**Lichthof, Floor D**
	Selected posters session (Odd numbers must be by poster)	
14:00 – 15:30	**Session 3: Gene Regulation**	**Room KOH-B-10**
	Guest speaker: Prof. Hirohide Saito (30 +10 min), CiRA Abstract Selected Speaker (10+3 min): Alberto Catanese, Ulm University, Germany Tatsuya Yamakawa, Kyoto University, Japan Jiang-An Yin, University of Zurich, Switzerland	
15:30 – 16:30	**Poster Session II**	**Lichthof, Floor D**
	Selected posters session (Even numbers must be by poster)	

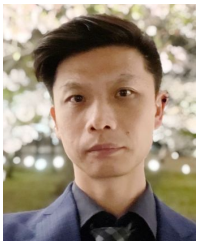
16:30 – 18:00	**Session 4: Translational Application**	**Room KOH-B-10**
	Guest speaker: PD Dr. Ute Modlich (30 +10 min), IREM, UZH Abstract Selected Speaker (10+3 min): Emiri Nakamura, Kyoto University, Japan Lewis Jones, ETH Zurich, Switzerland Blandine Clément, ETH Zurich, Switzerland Gold sponsor: 3Brain (5 min)	
18:00 – 18:30	**Closing remarks and Awards**	**Room KOH-B-10**
18:30 – 20:00	**Networking & Apéro**	**Lichthof, Floor D**

iPSZürich organizing team

Melanie Generali

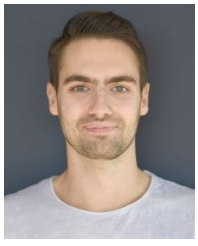
Vanessa Budny

Melanie Eschment



Kelvin Hui

Clara Duré



David Taborsky

Universität Zürich[UZH]

iPSZÜRICH

Public Lecture

Prof. Jun Takahashi
Kyoto University

CiRA

160th
日本・スイス国交樹立記念
Anniversary of Diplomatic Relations between Japan and Switzerland

Institute for Regenerative Medicine • IREM

Prof. Simon P. Hoerstrup
University of Zurich

The Current State of Regenerative Medicine in Japan and Switzerland

"CIRA'S MISSION IS NOW IN PROGRESS"

The Center for iPS Cell Research and Application was established in 2010 with a clear mission: the medical application of iPS cells and related technologies. We now have three research buildings with about 600 faculty members, researchers, and students in Kyoto, a beautiful historical city in Japan. As you may know, iPS cells have two characteristics: self-renewal and pluripotency, meaning they can proliferate indefinitely as stem cells, and under appropriate conditions, they can differentiate into the main cell types of our body. Taking advantage of these characteristics, we are trying to apply iPS cells for regenerative medicine, namely transplanting iPS cell-derived somatic cells to replace lost or damaged cells. Alternatively, we use iPS cells to generate disease models to investigate disease mechanisms and develop new drugs. In my talk, I will introduce our efforts and results in both directions, especially my work on an iPS cell-based therapy for Parkinson's disease.

"IREM – A BLUEPRINT FOR TRANSLATIONAL REGENERATIVE MEDICINE"

Based on bio-inspired principles and developmental engineering/re-engineering approaches, the Institute for Regenerative Medicine (IREM) aims to replace or regenerate dysfunctional human cells, tissues, or organs with the goal of restoring normal functions. By using its designated clinical translational facilities, ranging from Cleanroom Technologies (Center for Therapy Development), iPS Core Unit, and a Clinical Trial Unit (Center for Prevention and Dementia Therapy), IREM fulfills its mission to translate excellent basic research into innovative clinical application and advancing molecular life sciences into the future.
Furthermore, the interaction with Wyss Zurich, a unique center with the ETH Zurich and the University of Zurich, drives the fields of regenerative medicine and robotics, together with bionics technologies. Wyss Zurich brings together some of the world's leading experts to form multidisciplinary teams, pooling their knowledge and expertise.

Invited Workshop Speakers

Maurissen, Thomas

Thomas obtained his BSc/MSc in Bioengineering at EPFL, during which he participated in a summer exchange program between EPFL and the University of Tokyo. This experience in Japan sparked his interest to apply and later obtain his PhD from Kyoto University, Graduate School of Medicine. Thomas greatly enjoyed 3.5 years of iPS cell research at CiRA in the Woltjen lab. Now, he is a scientist and research project leader in a pharmaceutical company, working on retinal/vascular diseases.

Akiyama, Reiko

Reiko is a plant ecologist by training and is a lab manager at the Department of Evolutionary Biology and Environmental Studies at the University of Zurich. During her MSc study at Kyoto University, she stayed at Uppsala University in Sweden as an exchange student. With a PhD from Uppsala University, she came to Switzerland as a postdoc and worked with collaborative research projects between Switzerland and Japan. She embraces the culture and nature of both countries.

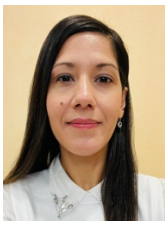
Matsumoto, Sora

Sora is currently pursuing a PhD in Dr. Sinem Saka's group at the European Molecular Biology Laboratory (EMBL) in Heidelberg. Prior to this, he earned his MSc and BSc at Kyoto University under the guidance of Dr. Hirohide Saito in CiRA. Through a collaborative project with researchers in France, he had the opportunity to familiarize himself with the European research environment, which eventually led him to apply for his PhD studies at EMBL. His research interests span across RNA biology, synthetic biology, liquid-liquid phase separation, and spatial omics. Outside the lab, Sora enjoys bouldering and cooking Japanese food.

Kehl, Debora

Debora Kehl holds a PhD in Integrative Molecular Medicine from the University of Zurich and currently works in clinical research in the pharmaceutical industry in Switzerland. As a postdoctoral fellow at the Institute for Regenerative Medicine (IREM), Debora supported the establishment of a scientific collaboration with the Center for iPS Cell Research and Application (CiRA) in Kyoto. As visiting researcher she stayed at CiRA and focused on iPSC-derived cardiomyocytes to regenerate ischemic heart tissue.

iPSZürich Symposium 2024 CiRA-IREM Travel Award Recipients



Denise Zujur

Linh Nguyen

Emiri Nakamura

Naoya Amimoto

Tatsuya Yamakawa

Invited Symposium Speakers

Woltjen, Knut More info here

GENOME AND EPIGENOME EDITING TOOLS FOR STEM CELL RESEARCH

Establishing control over the genome and epigenome is desirable for the study of human health and aging as well as the development of reliable cellular therapeutics. To achieve accurate disease models, we have focused on predicting DNA repair outcomes following gene editing in human induced pluripotent stem (iPS) cells with CRISPR-Cas9. In this way we have established an isogenic allelic series for single nucleotide variants, provided a genome-wide resource for creating deletion mutations, and are exploring the design requirements for cutting edge methods such as Prime Editing. In an alternative strategy, we are employing epigenome editing for cell engineering without permanent genetic modification. Examples of partial reprogramming of somatic cells for biological age reversal and targeted gene repression for the creation of 'universal' iPS cells will be presented. The current challenges of using epigenome editing for cellular rejuvenation and cell therapies will be discussed.

Saito, Hirohide More info here

RNA SYNTHETIC BIOLOGY TO PROGRAM CELLS

Research in synthetic biology, which aims to understand biological phenomena by creating biomolecules and life systems, is advancing and shows promise in fostering new technological breakthroughs. In this presentation, I will introduce studies based on the interactions between RNA and RNA-Protein (RNP). Our team has been progressing research on mRNA switches that can regulate translation based on the state of the cell. We have developed a switch that turns off translation in response to proteins that characterize cells and microRNA (miRNA) - referred to as the miRNA-responsive OFF switch. However, with just this OFF switch, purification efficiency was a challenge for certain cell types. Recently, we have developed an innovative switch that, contrary to the OFF switch, activates translation in response to miRNA, known as the miRNA-responsive ON switch. In the development of this ON switch, we discovered a unique RNA design technique where an artificial sequence is inserted downstream of the mRNA's poly-A tail. By combining both the ON and OFF switches, we successfully purified cardiomyocytes differentiated from iPS cells at high efficiency and eliminated unwanted cells without the need for equipment like cell sorters. Along with these

RNA switch technologies, this talk will also introduce the latest achievements in cell reprogramming studies based on RNA.

Modlich, Ute More info here

GENE THERAPY FOR INHERITED BLOOD DISORDERS

There are more than 7000 inherited disorders caused by monogenetic mutations, many of which affect the blood and immune system. Because all blood cells are produced by hematopoietic stem cells (HSC), transplantation of allogeneic HSC from healthy and compatible donors can cure these disorders. However, suitable donors are not always available (e.g. in 2/3 of Caucasian patients). In these cases, gene therapy using autologous patient HSC is a viable alternative, using an ex vivo gene modification, either adding a healthy gene copy or correcting the mutated gene, and transplantation back into the patients.

In our research group, we focus on gene therapy affecting myeloid cells, especially macrophages, or megakaryocytes and platelets. For targeted expression, we developed a lentiviral vector platform for expression in these cell types. Due to limited access to patient-derived primary cells, the use of iPSC-derived hematopoietic cells represents an important test platform for developing gene therapies. Using iPSC with disease-specific mutations, the specificity of transgene expression and correction of the phenotype can be studied. Differentiation of hematopoietic cells from iPSC is guided by specific transcription factors, typically induced by cytokines. By inducible overexpression of these transcription factors during hematopoietic commitment, blood cell differentiation can be supported. Inducible expression systems in iPSC for megakaryocytic development and the challenges of tight expression control will be discussed.

Cabon, Lauriane More info here

INSTITUTE OF HUMAN BIOLOGY AT ROCHE

In this talk, Lauriane will present how novel therapeutic modalities in the oncology space led to the establishment, improvement and adoption of adult stem cell derived organoids assays in drug development. T-cell bispecific (TCBs) antibodies are an innovative tool for harnessing the immune system to fight cancer whereby TCBs simultaneously bind to a T-cell and a tumor antigen resulting in tumor killing. TCBs efficacy relies in part on the ability of the T-cell to access the tumor tissue; while their safety is driven by levels of expression of the tumor antigen in normal tissues. Relying on human relevant preclinical assays to evaluate and better understand the benefit/risk ratio of such molecules became crucial over the past years. Lauriane will more specifically highlight the

advances her team and colleagues at Roche have made by introducing lymphocytes into organoids assays, from peripheral blood mononuclear cells to tissue resident lymphocytes. She will then outline a few other examples of stem cell derived organoids assays applied in the ophthalmology and immunology disease areas.

Selected Speaker Abstracts

Session 1: Development and Aging

Nguyen, Linh **Kyoto University, JP**

"ALL MEN ARE CREATED EQUAL" BUT ARE ALL IMSCS CREATED EQUAL?

Mesenchymal stem cells (MSCs) are a promising cell source in regenerative medicine for treating various diseases owing to their immuno-regulatory effects and multi-lineage differentiation capacity. The defining characteristics of MSCs are 1) adherence to plastic culture dishes, 2) expression of specific surface markers CD73, CD90, and C105, and 3) differentiation into osteoblasts, chondrocytes, and adipocytes. However, MSCs extracted from the human body often show inconsistent quality and heterogeneity. Although iPSC-derived MSCs are expected to overcome such problems, the nature of iPSC-derived MSCs from different lineages remains unknown, and comprehended research is required. In this study, we successfully generated MSCs from the same human induced pluripotent stem cells (iPSCs) through different lineage-specific routes, including cranial neural crest cells, trunk neural crest cells, paraxial mesoderm (somite), lateral mesoderm, and limb mesenchyme. We then compared these differentially originated iPSC-derived MSCs with tissue-derived MSCs from adipose tissue, bone marrow, muscle, and dental pulp. All five types of iPSC-derived mesenchymal stem cells fulfilled the criteria but showed different capacities for multi-lineage differentiation, depending on the differentiation route. Specifically, somite, cranial neural crest, and limb mesenchyme-derived MSCs showed higher osteogenic potency than trunk neural crest and lateral plate mesoderm-derived MSCs, implying the effect of developmental origin on different iMSCs' characteristics. PCA plot distinctly illustrates the variations in global gene expression patterns among the iMSCs. Furthermore, dental pulp-derived MSCs and neural crest-derived MSCs are shown not to cluster together, suggesting that factors beyond cellular origins, such as functional attributes or environmental influences, might play a role in determining the global gene expression of MSCs. Our research represents a thorough investigation into different iMSCs' characteristics, contributing to a greater understanding of the heterogeneity associated with iPSC-derived MSCs, which will be critical for their therapeutic applications.

Malagola, Ermanno **University of Zurich, CH**

ISTHMUS PROGENITOR CELLS CONTRIBUTE TO HOMEOSTATIC CELLULAR TURNOVER AND SUPPORT REGENERATION FOLLOWING INTESTINAL INJURY (MOUSE)

The currently accepted intestinal epithelial cell organization model proposes that crypt base columnar (CBC) cells marked by high levels of Lgr5 expression represent the sole intestinal stem cell (ISC) compartment. However, previous studies have indicated that Lgr5+ cells are dispensable for intestinal regeneration, leading to two major hypotheses: one favoring the presence of a quiescent reserve stem cell population, the other calling for differentiated cell plasticity. To investigate these possibilities, we studied crypt epithelial cell organization, during homeostasis and regeneration, in unbiased fashion, via high-resolution single-cell profiling. These studies, combined with in vivo lineage tracing, show that Lgr5 is not a specific ISC marker and that stemness potential exists beyond the crypt base in the isthmus region, whose cells, contrary to differentiated cells, participate in tissue homeostasis and support intestinal regeneration. Our results provide a novel model of organization for the intestinal crypt epithelium in which stemness potential is not restricted to CBC cells and suggesting that neither de-differentiation nor reserve stem cell populations are drivers of intestinal regeneration.

Pfaendler, Ramon **ETH Zurich, CH**

A SYSTEMS VIEW OF CELLULAR STATE HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELLS

Early human embryogenesis relies on cellular fate transitions of pluripotent stem cells orchestrated by various signalling pathways and spatio-temporal dynamics. While recent progress deepened our understanding of the gene regulatory programs underlying these processes, the molecular determinants governing cellular state heterogeneity of pluripotent stem cells at the single-cell level remain incompletely quantified and understood. Here, we investigate the functional and phenotypic variability of induced pluripotent stem cells (iPSCs) upon drug perturbations using a combination of high-content microscopy, computer vision, and high-throughput molecular profiling. Quantification of the high-content imaging data using self-supervised deep learning unveiled diverse single-cell morphological states of iPSCs across drug mode-of-actions. In addition, multiplexed transcriptomics and high-throughput proteomics revealed condition-specific expression dynamics of key proteins and transcripts involved in early differentiation. Intriguingly, integrating single-cell morphological states with this multimodal molecular data enables us to link the underlying molecular processes that govern iPSC cellular states. Globally, this suggests that iPSCs navigate an intricate path between simultaneously changing cellular morphology and fate. Overall, our study provides valuable insights into the molecular landscape underlying phenotypic heterogeneity in a cellular system characterised by maximal developmental plasticity across scales.

Session 2: Disease Model

Grünblatt, Edna **University of Zurich, CH**

ADHD NEURODEVELOPMENTAL ALTERATION ASSOCIATED TO WNT PATHWAYS, OXIDATIVE STRESS AND INFLAMMATION: PERSONALIZED TREATMENT PROSPECTS

Due to its polygenic and phenotypic spectrum, attention-deficit hyperactivity disorder (ADHD) is a complex neurodevelopmental disorder. Over 5% of children and adolescents worldwide are affected, with 60% persisting into adulthood. While the cause of ADHD is unknown, neurodevelopmental delays associated with Wnt-pathways and subsequent oxidative stress (OS) and inflammation suggest plausible causes. Response to Methylphenidate (MPH), a psychostimulant, is one of the highest in mental disorders, yet some do not benefit due to either partial or no response. Nevertheless, the mechanism of action remains unclear. Some patients also benefit from polyunsaturated fatty acids (PUFAs), particularly omega-3, the effect that may be attributed to its anti-inflammatory and anti-OS properties. We used patient-derived induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), and forebrain cortical neurons (FCNs) to model ADHD to identify the critical time point for alterations and whether we could identify treatment-specific response. Intriguingly, modifications began only at the NSC phase, where cells establish neural fate. Compared to controls, ADHD cells exhibited decreased growth-rates, linked to alterations in Wnt-activity. These alterations associated with personalized genetic predisposition and clinical scores, enhancing the model's clinical translation ability. Additionally, ADHD FCNs showed elevated OS and inflammatory markers, supporting clinical findings. MPH and omega-3 reduced cellular alterations, which was encouraging. Notably the response to MPH was only detected in clinically responsive patients, suggesting specific mechanisms. Overall, our findings contribute to explaining ADHD's etiology and treatment mechanisms in patient-specific neural cell models, paving the path to novel approaches, preventive measures, and discoveries of new therapeutic targets.

Nubie, Martina **University of Zurich, CH**

DEVELOPMENT OF HUMAN IPSC-BASED MODELS TO TEST MICROGLIA-DIRECTED GENE THERAPY FOR NEURODEGENERATION

The deficiency of progranulin (PGRN), caused by mutations in the granulin (GRN) gene, is responsible for two fatal neurodegenerative diseases: neuronal ceroid lipofuscinosis-11 (CLN11) in children and young adults, and frontotemporal dementia (FTD) with typical onset before 65 years of age. Currently, no disease-modifying treatment is available. We develop an ex vivo hematopoietic stem and progenitor cell (HSPC) gene therapy that targets microglia, the brain-resident immune cells critically involved in the pathology. In an autologous transplantation setting, the gene-modified patient HSPCs generate new microglia-like progeny that secrete higher levels of PGRN. For this gene therapy, we designed a lentiviral vector (LVV) that drives phagocyte-specific PGRN expression. Prior to in vivo application, we tested the LVV in a model of microglia differentiated from induced pluripotent stem cells (iPSCs) carrying mutations in the GRN gene. We successfully generated

microglia expressing typical markers such as IBA1, TMEM119 and SALL1. Upon iPSC transduction with the gene therapy LVV, we could increase PGRN expression in the microglia to physiological levels. We also investigated the dynamics of transgene expression driven by our myelospecific promoter by monitoring the emergence of lineage-restricted myeloid markers during differentiation. We confirmed that our LVV is inactive in early differentiation and becomes activated during myeloid specification. To mimic the integration of PGRN-enhanced microglia in the patient's brain, other brain cell types need to be included in this model. In a pilot experiment, we defined the culture conditions that support the differentiation of iPSC-derived neurons, astrocytes and microglia. The development of this co-culture model will allow not only to test candidate LVVs for microglia gene therapy, but also to investigate the underlying human-specific pathogenic mechanisms of GRN-related neurodegeneration.

Guimbal, Sarah **University of Bern, CH**

NEW MOLECULAR UNDERPINNINGS OF BBB DYSFUNCTION IN MULTIPLE SCLEROSIS

Introduction: Blood-brain barrier (BBB) breakdown is amongst the earliest pathological hallmarks in multiple sclerosis (MS). The mechanisms leading to BBB dysfunction are incompletely understood and are generally thought to be a consequence of the autoimmune neuroinflammatory process in MS. Objective: We challenge this view and ask if intrinsic alterations in BBB endothelial cells manifested at the genetic, epigenetic, transcriptional, and phenotypic level contribute to altered BBB function. Methods: We made use of human induced pluripotent stem cells (hiPSCs) derived from 6 healthy controls (HC) and 10 MS patients and differentiated them into brain microvascular endothelial cell (BMEC)-like cells as in vitro model of the BBB. We performed a transcriptomic analysis on the donors available (3 HC and 4 MS patients) via RNA sequencing on HC and MS-derived BMEC-like cells stimulated with TNF-α and IFN-γ and unstimulated. Results: The RNA sequencing analysis showed an increase of regulated genes in unstimulated BMEC-like cells compared to the stimulated condition, strengthening that BBB may contribute directly to MS pathology. Moreover, it also revealed a strong modulation of the Semaphorin-4D (SEMA4D) signalling pathway in unstimulated MS-derived BMEC-like cells compared to the controls. We confirmed, via western blot and immuno-staining, the expression of SEMA4D and its downstream effectors, RHOB and ROCK2. EECM-BMEC-like cells were treated with a recombinant protein for SEMA4D and showed decreased mRNA expression of SEMA4D and a cytoskeletal reorganization. Conclusion: Our study suggests that SEMA4D and its downstream effectors could play a role in BBB dysfunction in the context of MS.

Session 3: Gene Regulation

Catanese, Alberto **Ulm University, GER**

HETEROZYGOUS KNOCKOUT OF SYNAPTOTAGMIN13 PHENOCOPIES ALS FEATURES IN HUMAN MOTOR NEURONS

Synaptic alterations represent a common feature shared across the heterogenous pathogenetic spectrum of Amyotrophic Lateral Sclerosis (ALS). In particular, we have recently shown that the presynaptic SNARE machinery is detrimentally impaired in ALS motoneurons (MNs), which indeed display reduced firing properties. In this work, we aimed at identifying specific presynaptic targets actively contributing to neuronal vulnerability and disease progression in ALS. We combined multi-omics and deep learning strategies with human iPSC-derived MNs to highlight Synaptotagmin 13 (Syt13) as a candidate synaptic protein contributing to neuronal vulnerability in ALS. By using CRISPR-Cas9 technology, we then investigated whether the heterozygous loss of Syt13 is sufficient to resemble an ALS phenotype. Syt13+/- hiPSC-derived MNs display a progressive manifestation of typical ALS hallmarks such as loss of synaptic contacts and accumulation of aberrant aggregates. By comparing the transcriptome of Syt13-deicificent cells to Syt13+/+ ones, we found a significant impairment in biological mechanisms involved in motoneuron specification and spinal cord differentiation. In addition, we identified an astonishing correlation of the Syt13+/- transcriptome with an ALS fingerprint generated using RNAseq data from human MNs and post mortem spinal cord samples. This significant overlap converged toward a detrimental upregulation of neuronal death and pro-inflammatory response, which was linked to a dysfunctional Akt-GSK axis. Our data show for the first time that the heterozygous loss of a single member of the synaptotagmin family is sufficient to trigger a typical ALS phenotype leading to the death of human MNs, thus revealing novel insights into the selective vulnerability of this cell population.

Yamakawa, Tatsuya **Kyoto University, JP**

NOVEL PATHOGENESIS OF DIAMOND-BLACKFAN ANEMIA EXPLORED THROUGH REGULATION OF PROTEOSTASIS

Diamond-Blackfan anemia (DBA) is a congenital bone marrow failure syndrome characterized by a significant decrease in red blood cells and physical abnormalities, including craniofacial malformations. DBA is known to result from heterozygous mutations in ribosomal protein (RP) genes, with over 20 causative genetic mutations identified to date. However, approximately 40% of patients do not exhibit these mutations, suggesting the presence of other causative genes or mechanisms. Furthermore, many aspects of DBA, including treatment, genetic diagnosis, and the mechanisms of onset, remain unclear. We have previously developed a comprehensive protein identification and quantification method by Mass spectrometry. We conducted large-scale trans-omics analysis of human induced pluripotent stem cells (hiPSCs) and somatic cells. While mRNA remained constant between these two cell-types, the analysis revealed 236 genes with significantly higher protein in hiPSCs including nearly all reported DBA genes. We generated hiPSCs derived from the patients with high-frequency mutations in DBA genes such as RPS19, RPL11, and RPL5.

These DBA-hiPSCs showed defective differentiation potential to early mesoderm. Furthermore, while the protein levels of RPs in DBA-hiPSCs were comparable to those from healthy hiPSCs, they decreased during mesodermal differentiation. These findings suggest that the DBA genes undergo different quantity control between hiPSCs and differentiated cells. Importantly, this regulation occurs not during transcription but post-transcriptionally. The data also imply abnormalities in the regulation of protein amount (proteostasis) during differentiation and development in DBA patients. This study could propose a novel mechanism to the onset of DBA through the regulation of proteostasis, and lead to a better understanding of common principles underlying mutation-independent DBA pathogenesis.

Yin, Jiang-An **University of Zurich, CH**

QUADRUPLE-GUIDE HUMAN GENOME-WIDE ARRAYED CRISPR LIBRARIES AND IPSC-BASED RESEARCHES

Unbiased genetic screening is a foundational technique greatly advanced by CRISPR technology. While current CRISPR screens utilize pooled guide RNA libraries with limited applicability to selectable phenotypes, arrayed CRISPR libraries significantly expand the scope of CRISPR screens. However, generating arrayed CRISPR libraries is challenging and their availability is limited. Utilizing a newly developed massively parallel plasmid cloning methodology, we have constructed genome-wide arrayed libraries for human gene ablation (19,936 plasmids), activation, and epigenetic silencing (22,442 plasmids). Each plasmid encodes an array of four non-overlapping sgRNAs designed to accommodate most human DNA polymorphisms. We achieve perturbation efficacies of 75–99%, 76–92%, and ~10,000-fold in deletion, silencing, and activation experiments, respectively. Our studies have demonstrated that our new libraries surpass existing resources in both superiority and versatility across various systems, particularly induced pluripotent stem cells (iPSCs) and organoids. Given that our libraries unlock vast biological frontiers previously inaccessible and the widespread utility of iPSC technology, we anticipate that the synergistic integration of these tools will significantly advance both fundamental and translational research in biomedicine.

Session 4: Translational Applications

Nakamura, Emiri **Kyoto University, CH**

TURBULENCE ENHANCES MITOCHONDRIA DELIVERY IN MEGAKARYOCYTE MATURATION, LEADING TO BIOGENESIS OF HIGH QUALITY OF IPSC-PLATELETS

Platelet (PLT) products, limited by blood donations, have a short shelf life of 4-5 days due to its efficacy limitation and risk of bacterial growth at room temperature storage. To address these issues, we proposed two breakthrough technologies to supply artificial PLTs: 1.) self-renewing megakaryocyte progenitors established from human induced pluripotent stem cells (iPSCs) as a master cell bank, which we named immortalized megakaryocyte progenitor cell lines (imMKCLs) (Nakamura, Cell Stem Cell, 2014). 2. turbulent-flow bioreactors that enabled high yield production of competent iPSC-PLTs from imMKCLs (Ito, Cell, 2018). We herein found turbulence increases CD42b (GP1bα) expression, a marker for PLT function, in iPSC-PLTs, which inversely correlated with phosphatidylserine exposure levels and subsequent CD42b shedding. We identified two populations of iPSC-PLTs: CD42b positive (+) and negative (-), where mitochondria activity was detected only in CD42b+ iPSC-PLTs. We also demonstrated using GPIX knockout imMKCLs or applying a mitochondrial oxidative phosphorylation uncoupler, that mitochondria activity is an upstream regulator of extracellular CD42b expression on iPSC-PLTs. Moreover, inhibiting mitochondrial fission also decreased CD42b+ iPSC-PLTs, strongly suggesting that CD42b- iPSC-PLTs result from impaired mitochondria delivery. Interestingly, we observed turbulence exposure only during the latter 4 days of the total 6 days of maturation is crucial for mitochondria delivery. Meanwhile, turbulence diminished F-actin levels by 20-30%, as detected via flow cytometry, whereas complete inhibition of F-actin lowered mitochondria inclusion in resulting iPSC-PLTs, implying that lower F-actin within an optimal range may be involved in mitochondria delivery in imMKCLs to iPSC-PLTs. From these findings we propose that turbulence induces the fine-tuning of mitochondria distribution via an actin mediated mechanism, to yield high-quality iPSC-PLTs.

Clément, Blandine **ETH Zurich, CH**

NERVE MODEL TO STUDY THE DIVERSE ELECTROPHYSIOLOGICAL PROPERTIES OF HUMAN IPSC-DERIVED SENSORY NEURON SUBTYPES

Neuropathic pain results from various types of nerve damage and is often inadequately treated. Most available in vitro tools to search for new therapeutic approaches fail to generate human translatable results. Nociceptors are a specialized subpopulation of sensory neurons conveying the perception of pain and are morphologically and functionally distinct from other neurons. The individual sensation of pain can be triggered by different subpopulations and may vary between patients. However, currently available painkillers against neuropathic pain have no cell specificity resulting in unwanted side effects. Thus, the development of tools that can differentiate nociceptive fibres would enable a more targeted compound screening. In this work, we present a multi-compartment nerve model platform combining a CMOS-based high-density microelectrode array with a polydimethylsiloxane (PDMS) guiding microstructure that aims to capture the electrophysiological responses of

individual dorsal root ganglion (DRG) subtypes. Human iPSC-derived sensory neurons were cultured at low density inside the microstructure and only a few axons grew in multiple parallel 4 x 10 µm microchannels before converging to a bigger bundle-forming channel. This configuration allowed the measurement of propagation speeds and stimulation-induced responses of close-to-individual axons in microchannels after stimulating electrically the fiber bundle. We observed different electrophysiological profiles highlighting the diversity of nociceptive fibres within a single network, suggesting that such a platform might be suitable to evaluate nociceptor-specific drug response for a given subtype of sensory neurons.

Jones, Lewis **ETH Zurich, CH**

USING METAMATERIALS AND CARDIAC TISSUE ENGINEERING TO ENGINEER ROBUST AND CONTRACTILE CARDIAC TISSUE PATCHES

Ventricular Septal Rupture (VSR) is a challenge in cardiac medicine with a high mortality rate of 45-60%. Current treatment methods use bovine pericardial patches (BPPs), which are non-contractile, tend to calcify over time, and fail to integrate effectively with the myocardium. Therefore, patients do not tend to fully recover cardiac function. To address these limitations, we are engineering a cardiac tissue patch that uses human stem cell-derived cardiomyocytes in hydrogel, reinforced with a metamaterial lattice. This approach allows us to tune the patch mechanical properties and contractility, while enabling stable implantation within the intraventricular space. Here, we will showcase our current results on metamaterial design and manufacturing, mechanical characterization (tunable stiffness), and biological characterization (biocompatibility, cell maturation, and tissue contractility). In summary, we will show how metamaterials can be combined with engineered cardiac tissues to fabricate centimeter scale, three dimensional, and implantable cardiac tissues.

Core Facilities

Abidi, Affef More info here

THE IPSCORE ZURICH

The iPSC Core Facility (iPSCore) is a technology platform of the University of Zurich established to provide a wide range of services from induced pluripotent stem cell (iPSCs) including reprogramming to differentiation, hands-on training, and project planning to the life science community in Zurich and beyond. Currently, following protocols have been established and are available for iPSC-derived cells: cardiomyocytes, smooth muscle cells, endothelial cells neural progenitor cells, multiple types of neurons, astrocytes and macrophages. Additional differentiation protocols are being established upon demand, such as sensory, motor neurons and brain organoids.

We have recently completed our portfolio, and extend the possibilities of disease modelling using iPSC, by offering CRISPR editing as a service, we can generate knock-out lines, mutation correction/insertion in control/disease lines and transgenic/reporter lines. The mission of the iPSCore is to empower its users with access to the state-of-the-art technologies in the fields of gene editing and stem cells, thus, enable the creation of research models best fit for the unique need of each lab. We provide the full range of service, from the idea to the final product, including the highest standard of quality control.

Jirkov, Paulin More info here

UZH 3R AWARD 2024: CALL FOR NOMINATIONS IS OPEN!

The UZH 3R Award is intended to highlight the efforts and successes of UZH staff in the field of 3Rs (replacement, reduction, refinement of animal use in science), both within and outside UZH, and thus promote the 3R principles at UZH.

- The prize is awarded to a member of the university (individual or team) who makes a significant contribution in the field of the 3Rs.
- Candidates must be employed or enrolled at UZH.
- Candidates can be nominated by any UZH member.
- The contribution may take the form of proposals to modify existing research techniques or innovative approaches that improve animal welfare in animal experimentation or reduce or replace the use of animals.
- Published scientific papers or publicly available preprints by UZH researchers as well as education and training projects and initiatives for the implementation of 3R methods by UZH members at UZH can be nominated. The publication, project or initiative should have been published or taken place in the year prior to the year of the award.
- The award is endowed with 5000 CHF and is presented annually.
- Deadline for nomination is 15.06.24, 23:59.

Othman, Alaa More info here

FUNCTIONAL GENOMICS CENTER ZURICH (FGCZ): 20 YEARS AT THE FRONTIER OF MULTI-OMICS SUPPORT FOR BASIC AND APPLIED BIOMEDICAL RESEARCH

The Functional Genomics Center Zurich (FGCZ) is a research and training platform of the ETH Zurich and the University of Zurich, offering the latest technologies and key expertise for multi-omics research.

The FGCZ is leveraging Next Generation Sequencing (NGS) technology to support research projects from population-scale profiling to measuring individual molecules in single cells. Bringing together the latest sequencing technologies, automation solutions, and expertise in handling ultra-low input material allows the scalable profiling of mutations, epigenetic states, transcriptomic responses, etc., in virtually any tissue. We further exploit our powerful NGS platforms and know-how by branching out into the field of genome editing and supporting CRISPR screening and Off-Target analysis for next-generation genome editing tools.

Penton Ribas, David More info here

THE ELECTROPHYSIOLOGY CORE FACILITY (E-PHAC) AT UZH

The Electrophysiology Facility (e-phac) offers expertise, instrumentation, and support in the area of in-vitro electrophysiology to researchers from UZH and associated institutions. We combine in-vitro electrophysiology with the advantages of image-based techniques to study bioelectrical signaling in cells and tissues. We utilize manual and automated patch clamp, as well as High-Density Microelectrode Arrays and other electrophysiological techniques, to assess the bioelectrical properties of ion channels, transporters, cells, and tissues. We provide training for researchers in the use of these techniques and make a user Lab available, or offer these techniques as a service. Please, get in touch with us to set up an experimental plan for your project.

Stefanic, Sasa More info here

NANOBODIES, HIGHLY SPECIFIC ANTIGEN-BINDING DOMAINS AS AN ALTERNATIVE TO CLASSICAL ANTIBODIES FOR USE IN RESEARCH, DIAGNOSTICS, AND THERAPY

Nanobodies are recombinantly produced antigen binding domains derived from antibodies that that lack light chains, termed heavy chain-only antibodies, which naturally occur in all camelid species and some cartilaginous fish. The main advantages over conventional antibodies is in much smaller size and solubility that reflects in superior tissue penetration, excellent thermal stability, and ease of expression and selection in vitro, whilst maintaining the same binding affinities as conventional antibodies. In addition, nanobodies are often able to recognise epitopes which are not accessible to conventional antibodies. Nanobodies can be easily genetically modified and recombinantly produced in unlimited amounts using any expression system (bacteria, yeast, insect cells) and further characterized in analytical and functional assay of choice. The Nanobody Service Facility of the University of Zurich has specialised in the production of nanobodies by immunization of alpacas with the target

protein, followed by the generation of a phage display library and in vitro selection of target-specific binders. Through our service we make the nanobody technology available to the Life Science Community (https://www.nsf.uzh.ch/en.html).

Applications in research range from immunohistochemistry, live imaging, pull-down assays, functional analysis of protein-protein interactions, modulation of protein functions in the cell, or as mediators of specific protein knock-out. To date many nanobodies were identified with potential to inhibit tumour growth and shown a great potential in non-invasive cancer imaging or immunotherapy, but also against viral and bacterial infections, and neutralizing toxins. The first nanobody was EMA and FDA approved for human therapy, and many more are currently in the late phase of clinical trials. Highly diverse nanobody libraries also offer a powerful high-throughput tool for development of diagnostic tests.

Poster Abstracts

1 . Achón Buil, Beatriz **University of Zurich, CH**

CRISPR ACTIVATION SCREEN IN HUMAN IPSC-DERIVED NEURAL PROGENITOR CELLS TO ELUCIDATE MOLECULES ENHANCING THEIR MIGRATION TOWARD STROKE TISSUE

Stem cell-based therapy has emerged as a promising strategy for treating stroke, but it still presents several challenges, such as the route of administration. In a clinical setting, endovascular delivery of cells is preferred to intracerebral engraftment. However, circulating stem cells must cross brain barriers and might get trapped in peripheric organs, resulting in an insufficient number of cells in the ischemic lesion. To optimize stem cell migration across brain barriers, we set out to mimic immune cells as they extravasate into the brain parenchyma following stroke. For instance, since the chemokine CXCL12 is released after stroke, we transduced human xeno-free iPSC-derived neural progenitor cells (NPCs) to overexpress its receptor (CXCR4) which can be found on the surface of leukocytes. Transduced NPCs migrated more efficiently than wild-type NPCs towards 10nM CXCL12 in a Boyden chamber assay. Although this is a promising result, it is a biased approach that neglects the complexity of the brain barrier extravasation. Therefore, we will develop a CRISPR activation (CRISPRa) screen to determine the optimal targets for crossing brain barriers after stroke. NPCs containing the CRISPRa system will be first tested in vitro by adding brain lysates to the Boyden chamber assay, or by performing adhesion assays. Top candidates will be then injected endovascularly in mice following stroke. NPCs that arrive at the ischemic lesion will be further analyzed to elucidate which molecules enhance their migration across brain barriers. The generation and preclinical validation of engineered NPCs with improved brain homing capacity will improve the efficacy of stem cell-based therapies for treating stroke via endovascular injection.

2 . Amimoto, Naoya **Kyoto University, JP**

RECONSTRUCTION OF CORTICOSPINAL TRACTS BY TRANSPLANTATION OF HIPS CELL-DERIVED CEREBRAL ORGANOIDS

Cerebrovascular disease is the second most common cause of long-term care needs. In addition, the annual medical cost of cerebrovascular diseases is 1.8 trillion yen, and the development of treatment methods is urgently needed from a medical and economic perspective. Cerebral infarction accounts for 80-85% of all strokes, which cause motor dysfunction due to damage to the corticospinal tracts (CST) that connect the cerebral motor cortex and the spinal cord. Various treatments for cerebral infarction, such as drug therapy and rehabilitation, are available, but the fact that many patients remain suffering from aftereffects makes it clear that the current therapies are insufficient. Therefore, as a novel therapeutic strategy, there are high expectations for reconstruction of corticospinal tracts by transplantation of pluripotent stem cell-derived neural cells such as iPS cells. The CST is responsible for the transmission of motor information, including fine motor skills, one of the most important motor movements in humans, but there are no reports demonstrating the reconstruction of the CST. Therefore, our laboratory aims to reconstruct CST by cell transplantation. We have already confirmed that transplantation of human iPS cell-derived

cortical neurons into the cerebral cortex of mice results in the elongation of neuro-axons along the CST to the spinal cord and improvement of motor function1,2). However, previous studies have not confirmed "graft-host connections" and "direct contribution to motor function." Therefore, in this study, I would like to demonstrate "graft-host connections" and "direct contribution to motor function" by cell transplantation using the rabies virus and Designer Receptor Exclusively Activated by Designer Drugs (DREADD). In fact, the study of input from the host to the graft revealed that it received input from the same locations as the actual input source, such as the cerebral cortex and thalamus. I believe that these techniques will demonstrate the integration of transplanted cells with the host's neural network of the brain and prove "reconstruction of the corticospinal tract by cell transplantation.

3 . Amos, Giulia **ETH Zurich, CH**

STUDYING LOCAL PLASTICITY IN LOW-DENSITY IPSC-DERIVED NEURONAL NETWORKS

To this day, we lack a basic understanding of how the human brain retrieves, processes and stores information. One particular challenge is unraveling how local plasticity mechanisms shape information transfer between neurons. In literature, we can find various plasticity-based learning paradigms, such as the Spike-Timing Dependent Plasticity or the Bienenstock-Cooper-Muncro synaptic learning rule. However, many of these paradigms face experimental and functional limitations as they exclusively encode spike timing or firing rate, and it is still unclear how they relate to each other. This might be attributed to the lack of well-defined model networks of neurons that can be reproducibly and quantitatively studied over an extended period of time. We introduce a bottom-up platform that combines engineered in vitro neuronal network models with microelectrode arrays (MEAs) to overcome this challenge. In this platform, human induced pluripotent stem cell (hiPSC)-derived neurons are cultured within custom-designed polydimethylsiloxane (PDMS) microstructures placed on top of the MEAs to engineer neuronal networks with constrained connectivity and compartmentalisation. These topologically constrained networks display complex spatiotemporal dynamics, making them valuable experimental tools to represent neuronal in vivo networks. Consequently, the platform enables to study plasticity rules in isolated neuronal networks with reduced complexity, while providing high reproducibility and throughput. We show that we can modulate the network activity using stimulation protocols that could potentially evoke spike-time dependent plasticity effects. Looking forward, we aim to test the learning paradigms further by simplifying the networks to contain 1-3 neurons. These investigations may provide insight into the levels of complexity required for learning within an in vitro laboratory setting and could provide a valuable complementary approach to in vivo methods.

4 . Budny, Vanessa **University of Zurich, CH**

THE ROLE OF APOLIPOPROTEIN E (APOE) ISOFORMS IN NEURAL CELL PHYSIOLOGY

Alzheimer's Disease (AD), characterized by the accumulation of beta-amyloid plaques and neurofibrillary tangles, is the most common age-related neurodegenerative disorder. The APOE4 allele is the major genetic risk factor for AD while APOE3 is defined as average risk and APOE2 is protective. Despite recent advances, the fundamental role of different APOE

alleles in brain homeostasis is still poorly understood. Here, we aim to uncover the functional role of APOE2, E3 and E4 in human neural cells and how this relates to AD pathophysiology. We differentiated APOE-isogenic iPSCs (APOE4, E3, E2 and APOE-knockout (KO)) to functional astrocytes ("iAstrocytes") and neurons ("iN cells"). iAstrocytes were analyzed for proteomic profiles using unlabeled mass spectrometry. GO enrichment analysis showed genotype differences in various cellular pathways including inflammatory signaling and energy metabolism. Accordingly, APOE4 iAstrocytes displayed the highest release of cytokines, while APOE2 and APOE-KO iAstrocytes secreted the lowest amounts. Furthermore, APOE4 iAstrocytes showed lower capacity for uptake of beta-amyloid and glutamate, while uptake was highest in APOE2. To investigate the role of APOE in energy metabolism of both, iAstrocytes and iN cells, we used the seahorse assay. Interestingly, total as well as mitochondrial ATP production were highest in APOE4 iAstrocytes and iN cells compared to the other genotypes, while no difference was observed in iPSCs suggesting a cell-type-dependent effect. Novel, single cell-based functional assays will be applied to characterize the APOE-dependent regulation of neural energy metabolism in more detail. We show that APOE plays a major role in several physiological and metabolic processes in human neural cells with APOE4-expressing iAstrocytes being pro-inflammatory and deficient in beta-amyloid and APOE4 iN cells and iAstrocytes being affected in their bioenergetic state.

5 . Carestiato, Silvia **University of Turin, IT**

HUMAN IPSCS-DERIVED NEURONAL MODEL TO STUDY THE PATHOGENIC MECHANISMS BEHIND TANGO2-DISEASE

The TANGO2 gene encodes for the homonymous protein mainly involved in vesicular trafficking, mitochondrial oxidation, and cellular homeostasis. While its subcellular localization is unclear, TANGO2-biallelic LoF variants are associated with an autosomal recessive disorder characterized by cardiac arrhythmia, metabolic crises, rhabdomyolysis, and kidney damage. A neurological component is also observed, including progressive neurodegeneration, developmental delay, and cerebral atrophy. Although a possible role in neuronal migration has been suggested, the effect of TANGO2-loss in neurons has never been investigated, constituting a major gap in understanding the disease. Human induced Pluripotent Stem Cells (hiPSCs) were generated from two clinically discordant TANGO2-affected siblings to derive neural rosettes (NRs), neural progenitor cells (NPCs) and mature neurons. Analysis of NRs showed a significative area reduction (~45%) and an impaired morphological organization in the severely affected patient. Furthermore, in the same patient, the wound-healing assay demonstrated altered migration in NPCs, supporting the previously reported role of TANGO2 in migration. Additionally, during the maturation of cortical neurons, an increase in cell death was also observed. In this regard, the Reactive Oxygen Species (ROS) assay showed an oxidative stress condition supporting the involvement of TANGO2 in mitochondrial functions and cellular homeostasis. In contrast, no significant differences were found in NRs, NPCs, and mature neurons in the asymptomatic patient compared to controls. In conclusion, we demonstrated the role of TANGO2 in early-stages of neurodevelopment, particularly in neural tube formation, cell migration, and neuronal maturation. Considering the peculiar siblings' case, this study suggests potential protective gene/genetic modifiers influencing patient phenotypes, highlighting the need for further transcriptomic and proteomic analyses for future therapies.

6 . Carreira, Olga **NOVA Medical School Research, PORT**

MODELLING RETINAL DEGENERATIVE MECHANISMS USING 3D RETINAL ORGANOIDS

The major causes of human vision loss and blindness are due to degenerative retinal diseases that involve function loss or death of specific retinal cell types. Recent studies have found retinal abnormalities in Parkinson's disease (PD) patients and retinal deposition of α synuclein (α syn). The development of reliable models to study the human retina has improved and 3D models like organoids derived from hiPSCs have been described to recapitulate the cellular organization and light sensitivity of the human retina. Here, we aim to study the effects of α syn overexpression in retinal degeneration, by generating retinal organoids derived from a PD-patient iPSC line with a triplication of the α syn gene (SNCA) and from a control cell line. Preliminary results indicate that PD-patient retinal organoids are efficiently generated and self-organized compared to control organoids. At different differentiation stages (days 80 and 120) PD-patient organoids revealed higher α syn protein levels and its phosphorylated form at serine 129. In parallel, we observed increased protein levels of antioxidant enzymes, such as catalase and superoxide dismutases (SODs) 1 and 2, together with increased mRNA levels of pro-inflammatory cytokines like interleucine 6 (IL6). Additionally, members of the B cell lymphoma 2 (BCL2) family like BCL2, Apoptosis Regulator (BAX) and BCL2 antagonist/killer 1 (BAK1) also displayed higher mRNA levels in PD-patient organoids, as well as higher levels of cleaved Caspase-3 (c-Casp3), indicating disfunction related with the mitochondrial pathway of apoptosis. Overall, this work supports 3D retinal organoids as a reliable model to study the molecular mechanisms involved in retinal degenerative diseases and further validate them as a tool for developing targeted and innovative therapeutic strategies against oxidative stress and degeneration.

7 . Collo, Linda **University of Genova, IT**

CHEMICAL STIMULATION OF NEURAL NETWORKS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

In vitro models combined with human induced pluripotent stem cells (hiPSCs) are a widely used tool to investigate the complex mechanism of the human brain. Indeed, the use of hiPSCs-derived neuronal networks allow to create in vitro patient-specific models that most closely resembles the processes of the human system, providing a reliable model to test neuroactive properties of different compounds. This study investigates how different drugs affected the electrophysiological activity of in vitro systems composed by excitatory (E) and inhibitory (I) neurons derived from hiPSCs. Our experimental model included three different culture configurations: 100% excitatory neurons (100E), 75% excitatory and 25% inhibitory neurons (75E25I), and 100% inhibitory neurons (100I). To these neuronal networks, 2-amino-5-phosphonovaleric (APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and bicuculline (BIC) were administrated. The recordings of the spontaneous and chemically stimulated electrophysiological activity were performed by exploiting Micro-Electrodes Arrays (MEAs). The administration of APV and CNQX resulted in an unaffected firing activity, while led to a decrease of the bursting and network bursting activity of both 100E and 75E25I configurations. On the other hand, BIC led to a significant increase of the firing activity of the 100I and 75E25I, and of the bursting and network bursting activity of the 75E25I configuration. This comprehensive investigation sheds light on the different effects of APV,

CNQX and BIC on the electrophysiological dynamics of neuronal networks with different excitatory-inhibitory compositions. These findings contribute to our understanding of drug-induced modulation in neuronal circuits and provide valuable insights for potential therapeutic investigations in neurological disorders.

8 . Dodi, Lorenzo Davide **ETH Zurich, CH**

INVESTIGATING ADHD BIOMARKERS USING HIPSC DERIVED NEURONS ON MEAS

Attention-deficit hyperactivity disorder (ADHD) is among the most prevalent neurodevelopmental disorders worldwide, with up to 7% of children and 4% of adults being affected. Psychostimulants are currently the first-line treatment in childhood and adult ADHD. Despite their high efficacy, approximately 30% of children and over 50% of adults do not respond sufficiently to psychostimulants, and a substantial proportion of patients stop treatment due to side effects. Predictive markers for an early identification of potential non-responders currently do not exist, leading to unnecessary drug exposure for many patients. Current state-of-the-art approaches to study ADHD have significant limitations: animal models fall short in representing complex human mental disorders, and neuroimaging does not offer access to single-cell data at high temporal resolution, which is a key prerequisite for in-depth network characterization. The main goal of this project is to perform electrophysiological characterization of disease-induced effects in patient-derived cells at high spatiotemporal resolution, combining human stem cell biology with cutting-edge high-density microelectrode array (HD-MEA) technology. Human iPSC-derived neurons retain the unique genetic signatures of their donors and enable the study of complex polygenic disorders, such as ADHD. HD-MEA technology provides access to network activity at high spatiotemporal resolution, ranging from subcellular compartments through individual neurons to entire networks. This combination potentially enables data-driven phenotypic screening to facilitate medication assessment and support the development of personalized treatment strategies. It may contribute to improving treatment outcomes of individual ADHD patients, reducing individual suffering, and developing novel treatment options.

9 . Duré, Clara **University of Zurich, CH**

IN VIVO SINGLE-CELL RIBOSOME PROFILING TO INVESTIGATE TRANSLATIONAL REGULATION IN AGED EPIDERMAL STEM CELLS

Adult stem cells are characterised by a tightly regulated and globally suppressed translation. With advanced age, stem cell function declines and the capability to self-renew and regenerate a tissue decreases. There is direct evidence that translation plays a major role in ageing since low levels of protein synthesis are associated with longevity. However, how the translational landscape of stem cells is altered during ageing remains largely unknown. Here, we employ in vivo single-cell ribosome profiling of the mouse epidermis to unravel the role of translational regulation in epidermal stem cells and the consequences of translational deregulation on skin regeneration. By optimizing the recently published single-cell ribosome profiling protocol specifically for in vivo epidermal stem cells, we comprehensively explore the translational landscape of young and aged epidermis at single-cell resolution, elucidating cell-type-specific translational programs during ageing. Furthermore, by coupling single-cell ribosome profiling with single-cell RNA sequencing, we uncover strongly deregulated

translation specifically in aged epidermal stem cells, which contrasts with the prevalent transcriptomic changes observed in aged differentiated epidermal cells. Additionally, aged epidermal stem cells show elevated expression of inflammatory response genes, that are translationally regulated. Systemic inflammation is a hallmark of ageing and together with our findings, it could be under translational control in ageing stem cells. Together, our findings advance our understanding of how deregulated translation contributes to age-related systemic inflammation, stem cell exhaustion and might pave the way for improving stem cell fitness, which is particularly relevant for wound healing of aged skin.

10 . Elsafadi, Sara **ETH Zurich, CH**

GENERATION OF INDUCED PLURIPOTENT STEM CELLS (iPSCS) FROM ROE DEER (CAPREOLUS CAPREOLUS) EMBRYONIC FIBROBLASTS VIA EPISOMAL VECTORS

Embryonic diapause, a period of developmental delay, remains an enigmatic process despite being widely observed among various mammalian species. This phenomenon was first documented in the European roe deer (Capreolus capreolus) in 1854. It features a distinctive extended phase lasting about four months, by which slow but continuous embryonic growth occurs. The collection and in vitro production of roe deer embryos for research purposes are fraught with field work difficulties, positioning induced pluripotent stem cells (iPSCs) as a promising research tool. The iPSC provides an innovative means to delve into the complexities of cellular potency and embryonic mechanisms of developmental delay. In this study, we strived to generate iPSC lines from roe deer embryonic fibroblasts utilizing episomal vectors encoding crucial pluripotency factors - Oct3/4, SOX2, KLF4, L-MYC, and LIN28 - in a feeder- and serum-free milieu. Although our initial analyses reveal that OCT4 expression in the reprogrammed cells likely originates from the introduced vectors rather than endogenous activity, early results are encouraging, indicating the potential for reprogramming roe deer fetal fibroblasts with human factors under these conditions. Comprehensive cell characterization is pending to ascertain their true stem cell capabilities and reliance on these vectors. Developing an iPSCs model for the roe deer promises to unravel the cellular and molecular intricacies of embryonic diapause. This advancement has the potential to significantly advance our comprehension of mammalian reproductive and developmental mechanisms, enriching the field of stem cell research by providing insight into species-specific pluripotency and cell cycle regulation.

11 . Eschment, Melanie **University of Zurich, CH**

AN IPSC-DERIVED CEREBRAL ORGANOID MODEL FOR CEP290-ASSOCIATED NEURODEVELOPMENTAL CILIOPATHIES IDENTIFIES ANOMALIES IN CILIARY MORPHOLOGY

Ciliopathies are a group of human Mendelian disorders caused by dysfunction of primary cilia, small ubiquitous sensory organelles protruding from the surface of most cells, required for signal transduction. These disorders are associated with central nervous system (CNS) anomalies, particularly exemplified by the neurodevelopmental disease Joubert syndrome (JBTS). JBTS is characterized by a highly specific mid-hindbrain malformation whose underlying pathomechanism remains unclear. Moreover, the presence of non-structural CNS defects such as seizures or intellectual disability implies a role for cilia in neuronal function beyond transmission of developmental signaling pathways. To understand the role of cilia

and of JBTS genes in the CNS, we are generating iPSC-derived in vitro models for JBTS. We established a biobank for JBTS through generation of CRISPR-engineered isogenic human induced pluripotent stem cell (hiPSC) lines that carry mutations in selected JBTS-associated cilia- related genes such as the ciliary gene CEP290. To elucidate the consequences of mutations in CEP290 on brain development, we differentiated control, CRISPR-edited and patient-derived hiPSCs into 3D cerebral organoids. We find that CEP290-mutated hiPSCs can differentiate into cerebral organoids and single-cell RNA sequencing confirmed that the overall generation of a variety of neuronal cell populations does not appear to be significantly impacted up to day 80. We do, however, observe an increased propensity to generate choroid plexus in mutant organoids compared to controls and a disorganization of cortical plate units. Furthermore, we observe striking morphological anomalies of a subset of primary cilia in the lumen of cortical plate units generated, which is a consistent finding throughout our genetically engineered and patient-derived cerebral organoids. Altogether, this study represents an advancement in understanding JBTS in the context of human brain development.

12 . Fujisawa, Miwako **University of Bern, CH**

INTRINSIC BLOOD-BRAIN BARRIER DYSFUNCTION CONTRIBUTING TO MULTIPLE SCLEROSIS PATHOGENESIS

Blood-brain barrier (BBB) breakdown is amongst the earliest pathological hallmarks observed in multiple sclerosis (MS). The mechanisms leading to BBB dysfunction are incompletely understood and are generally thought to be a consequence of the autoimmune neuroinflammatory process in MS. Investigating BBB dysfunction in MS is hampered by the limitation of MS tissue samples. Therefore, we made use of human induced pluripotent stem cells (hiPSCs) from MS patients and healthy controls (HC) and established in vitro models of the BBB by differentiating hiPSCs into brain microvascular endothelial cell (BMEC)-like cells using the extended endothelial cell culture method (EECM) recently developed by us. EECM-BMEC-like cells from MS patients showed impaired barrier properties and an inflammatory phenotype when compared to those derived from HCs. Comparing the transcriptional profile of the first EECM-BMEC lines we found 438 significantly differentially expressed genes in non-stimulated MS- versus HC-derived EECM-BMEC-like cells and 343 differentially expressed genes in cytokine-stimulated MS- versus HC-derived EECM-BMEC-like cells. These findings underscore that intrinsic dysfunction of the BBB contributes to MS. To further substantiate these findings and to explore the underlying mechanisms we have now established hiPSCs from 3 additional HC and 8 additional MS patients and differentiated them into EECM-BMEC-like cells. EECM-BMEC-like cells from two additional MS patients prior to diagnosis (radiologically isolated syndrome: RIS) and at the time of diagnosis with MS are included to determine if BBB dysfunction establishes prior to clinical signs of MS. Characterization of these data will be presented. The differences in transcriptional profile and phenotype of these MS- versus HC-derived EECM-BMEC like cells will be presented.

13 . Gatta, Beatrice **University of Zurich, CH**

INVESTIGATING THE ROLE OF DEFINED TDP-43 STATES IN HEALTHY AGING AND ALS PATIENT-DERIVED NEURAL NETWORKS

TAR DNA-binding protein 43 (TDP-43) is the main aggregating protein in several neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD). Yet the mechanisms that trigger the transition of TDP-43 from a highly regulated protein to a non-functional and pathological form that in turn causes toxicity and neuronal death remain unknown. An interesting hypothesis is that TDP-43 can exist in different functional states that allow the protein to facilitate its many functions in cells. Some of these states could be less functional then others and therefore may initiate the shift from a physiological to a pathological state of TDP-43 in disease. In order to decipher the functionality of distinct TDP-43 states and to elucidate their possible role in driving pathology, we use aging human neural networks established in our lab. Notably, this human neural model consists of self-organized and multi-layer glia and neural cultures with synaptically connected and electrophysiologically active neurons, which matures into a long-lived functional neural network over time. An important advantage of this system is its high reproducibility and significant longevity that makes it ideal to study neurodegenerative diseases such as ALS. Taking advantage of engineered iPSCs harboring ALS-linked mutations as well as ALS patient-derived iPSCs, we want first to generate aging neural cultures and then perform state-of-the art single-cell transcriptomic analysis as well as molecular and biochemical assays on these long-lived cultures to investigate the emergence of disease-relevant TDP-43 states and their impact on human neural cultures over time. Most importantly, we aim at evaluating the long-term impact of TDP-43 states in human neurons so as to understand how relevant pathological features develop in the course of time with the attempt of recapitulating the late-onset events associated to TDP-43-causing-neurodegenerative diseases. This research will allow us to confirm the possible role of relevant TDP-43 states in ALS disease and to shed light on new possible pathways of toxicity underlying TDP-43 pathology.

14 . Haenseler, Walther **University of Zurich, CH**

HUMAN IPSC DERIVED MODELS TO EXAMINE ALPHA-SYNUCLEIN PATHOLOGY

A Hallmark of Parkinson's Disease is α-synuclein (αS) accumulation in aggregates called Lewy bodies. To examine the pathogenic role of αS, we have generated induced Pluripotent Stem Cells (iPSC) from early onset Parkinson's Disease patients with SNCA A53T and SNCA Triplication mutations as well as gene edited isogenic sets. We characterized iPSC derived macrophages and found significantly reduced phagocytosis capability in SNCA Triplication macrophages, but not A53T macrophages. This phagocytosis defect can be phenocopied by adding αS to the cell culture medium of control macrophages. We characterized iPSC derived dopaminergic neurons and found increased αS aggregation and mitochondrial defects. In ongoing projects, we use limited proteolysis followed by mass spectrometry (LiP-MS) to identify structural changes in the proteome. We used SNCA KO iPSC derived cortical neuron lysates and spiked them with monomeric and fibrillar αS to identify αS structure specific αS interaction partners with LiP-MS. Furthermore, we are now characterizing the differences in the structural proteome between healthy controls and the SNCA triplication patient and the consequences of spiking αS fibrils into live neurons derived from control, SNCA KO and SNCA Triplication iPSC. With these human iPSC models we have confirmed finding from animal models and patient samples, but also got novel insight into αS pathology. They are an unlimited source of live patient material normally not available for disease modeling.

15 . Helfenstein, Carmen **University of Zurich, CH**

SAFETY SWITCH SYSTEMS IN HUMAN IPSC-DERIVED NEURAL PROGENITOR CELLS TO ENSURE SAFE APPLICATION IN STROKE

Ischemic stroke is a leading cause of severe long-term disability and death due to the limited regenerative capacity of the brain. Stem-cell-based therapy holds promise for stroke treatment, yet the proliferation potential poses a risk of tumorigenesis. Safety switch systems, based on suicide gene therapy from tumor research, offer a potential solution by inducing cell death specifically in modified cells. They consist of a cell-suicide-inducing transgene and a specific molecule that, when combined in the cell, cause cell death. Most studied safety switch transgenes are herpes simplex virus thymidine kinase (HSV-TK) and inducible Caspase9 (iC9) genes that induce cell death upon the administration of ganciclovir (GCV) or AP20187 respectively. Human iPSC-derived neural progenitor cells (NPCs) were transduced separately with two lentiviral constructs containing HSV-TK or iC9, together with firefly luciferase and a fluorophore reporter. We further plan to carry out a double transduction to remove all cells (iC9) or only dividing cells (HSV-TK). We determined in vitro the optimal drug concentration to trigger cell death via bioluminescence and immunostaining. In future experiments, modified NPCs will be intracerebrally injected into a photothrombotic stroke mouse model followed by drug administration. NPCs will be tracked in vivo via bioluminescence imaging, supplemented by post-mortem immunostaining for comprehensive analysis. These findings represent a significant stride toward the safer application of stem-cell-based therapy in treating stroke patients.

16 . Lloyd-Davies Sánchez, Daniel **University of Cambridge, UK**

OF MICE AND MEN - CEREBRAL ORGANOIDS FOR THE STUDY OF BRAIN DEVELOPMENT

Cerebral organoids grown with minimally guided protocols are able to recapitulate aspects of species-specific differences in timings of brain development which exist in-vivo. Furthermore, these stem-cell derived models can be used to make inter-species comparisons which would otherwise be technically or ethically inaccessible to experimentation. Being able to properly understand human-specific aspects of brain development will require us to be able to understand the phylogenetic context and how we and our developmental trajectories differ from close relatives such as apes, and also more distantly related mammals such as rodents. Indeed, there is much variation in development between species; and as a large literature exists for mouse as an in-vivo model, understanding the implications of this for human brain development requires new models which can bridge this knowledge gap across species. We adapted the cerebral organoid protocol, previously used on human-derived stem cells, for other species including mouse and observed intrinsically faster developmental trajectories and differentiation of neural fates compared to human cerebral organoids. Additionally, prolonged culture at the air-liquid interface reveals establishment of early neuronal layering and further cell-type differentiation, as well as neuron and axonal connections and dynamics.

17 . Kasap, Pelin **University of Bern, CH**

A HUMAN IPSC-DERIVED ISOGENIC MODEL OF THE NEUROVASCULAR UNIT TO EXPLORE BLOOD-BRAIN BARRIER DYSFUNCTION IN NEUROINFLAMMATION

The blood-brain barrier (BBB) is established by brain microvascular endothelial cells (BMECs) that maintain their characteristics by continuous crosstalk with pericytes and astrocytes. BBB breakdown and immune cell infiltration into the CNS are the earliest pathological hallmarks of multiple sclerosis(MS). The mechanisms of BBB breakdown in MS are largely unexplored due to the lack of patient-derived BBB models. To address this, we employed human induced pluripotent stem cell(hiPSC) technology to build an isogenic neurovascular unit (NVU) on-chip that models the anatomical structure of the postcapillary venules, where the immune cell infiltration into the CNS parenchyma takes place. We built the µSiM platform (microphysiological system enabled by a silicon nitride nanomembrane) that features ultrathin, transparent, and permeable membranes forming a “blood compartment” lined with hiPSC-derived BMECs and a “brain compartment” harboring hiPSC-derived pericytes and astrocytes. The µSiM offers the simplicity of the TranswellTM system while enabling live imaging, the use of different membranes with variable pore sizes, and the addition of modules converting it to a microfluidic or a tricellular culturing device. We produced hiPSC-derived EECM-BMECs that are characterized by an endothelial transcriptome. EECM-BMECs cultured in µSiM formed a uniform monolayer with mature tight junctions, developed barrier properties, and showed cytokine-induced expression of adhesion molecules. We successfully added hiPSC-derived pericytes and astrocytes to the µSiM, forming a fully isogenic NVU. We demonstrated successful T-cell migration from the blood compartment to the brain compartment in the µSiM under static conditions. Overall, our data demonstrate the suitability of the µSiM device to study the function of the human NVU in health and neuroinflammation. The addition of a flow unit will finally allow the investigation of immune cell trafficking across the NVU in an autologous manner.

18 . Kido, Jun **Kumamoto University Hospital, JP**

PATIENT DERIVED IPS CELLS AS CELLULAR MODEL FOR BASIC RESEARCH INTO CITRIN DEFICIENCY AND FOR DEVELOPMENT OF NOVEL THERAPIES

Citrin deficiency (CD) is an autosomal recessive disorder caused by a defect of the inner mitochondrial membrane transporter citrin resulting from mutations in SLC25A13. A defect of citrin primarily affects the function of the malate-aspartate shuttle but has several downstream metabolic effects impairing energy production due to hampered glycolysis, gluconeogenesis, tricarboxylic acid cycle function and beta-oxidation, and impairs nitrogen detoxification in the urea cycle. The condition is prevalent in East Asia, but also present in the Western world and hence considered a global disorder. Patients require as high-fat and low-carbohydrate diet with supplementation of medium-chain triglycerides (MCT) although this treatment has been introduced empirically without sound scientific evaluation. Especially the mechanism of action of MCT in this condition has never been systematically studied. We made use of patient derived skin fibroblasts, which were reprogrammed into iPS cells using standard protocols prior to differentiation into hepatocytes. These cells were used for studies of energy metabolism yielding decreased ATP levels and increased NADH/NAD+ ratios confirming their usefulness as CD model cells. For control, we used cultured patient fibroblasts and the hepatoma cell line HepaRG. Finally, we evaluated the effect of medium-chain fatty acids (MCFAs) on the ATP level and NADH/NAD+ ratios. Hereby, we could show in preliminary experiments that MCFAs lead to an increase of ATP levels indicating the improvement of energy production in disease iPSCs. In conclusion, this study

shows the usefulness of patient derived iPSCs both as disease model for CD and as a tool for studying novel treatments for this condition.

19 . Küchler, Joël **ETH Zürich, CH**

BIOLOGICAL NEURONAL NETWORKS AS COMPUTATIONAL UNITS: ACHIEVING FUNDAMENTAL LOGICAL OPERATIONS

Artificial intelligence (AI) has proven to be effective in solving complex problems in fields such as computer vision, natural language processing and medicine. However, in terms of energy efficiency, deep learning approaches are still outmatched by their biological counterparts. Yet, the underlying fundamental mechanisms of information storage, processing and computation of biological neuronal networks (BNNs) remain poorly understood. In bottom-up neuroscience, we tackle this challenge by analyzing low-density, engineered BNNs in vitro. Primary hippocampal rat neurons or human induced pluripotent stem cell (hiPSC) derived ngn2 neurons are seeded on top of high-density microelectrode arrays (HD-MEAs), where their growth is confined by polydimethylsiloxane (PDMS) microstructures. This approach allows us to record and stimulate BNNs to study the propagation of information on a subcellular level. It further has been demonstrated that the mean firing rate of a response induced by periodic continuous stimulation does not change significantly over periods of multiple hours. This gives a basis for using BNNs as a computational unit. In this work, we use PDMS microstructures with nanochannels which can generate isolated feed-forward circuits consisting of two input and one output node. Artificial inputs are encoded by stimulation patterns and the response of the middle node is captured with rate or temporal coding. By implementing different stimulation protocols, we successfully achieve three fundamental logical operations: NOT, AND, and OR. Furthermore, these operations are robust to stochasticity in the input and reproducible across different circuits. In addition, we compare stimulation-induced and spontaneous responses. Initial results show similarities not only in terms of spike timing but also in the principal components of the recorded signal shape. Looking ahead, this observation would allow interconnecting small logic gates to do more complex operations.

20 . Maurer, Benedikt **ETH Zurich, CH**

QUANTIFYING ENVIRONMENTAL EFFECTS ON SPIKING ACTIVITY IN ENGINEERED NEURAL NETWORKS

In vitro networks of human induced pluripotent stem cell (hiPSC) derived neurons have the potential to provide a more physiologically relevant, high-throughput platform to study electrical and chemical modulation of brain function under healthy and diseased conditions. However, variability between cultures due to their complexity and instable environmental parameters can have detrimental effects on readout robustness and reproducibility. In our bottom-up approach, small neural networks of reduced complexity are engineered by seeding hiPSC ngn2 neurons into polydimethylsiloxane (PDMS) microstructures on top of microelectrode arrays (MEAs) to spatially confine the location of their soma and guide neurite growth. Repetitive stimulation of these networks yields stable and reproducible spiking patterns in the evoked responses over days, which can be modulated by adding drugs. We present a custom readout and incubation system, Inkube, with recording and

closed-loop stimulation capability for 4 standard 60-electrode MEAs in parallel as well as fully integrated temperature and CO2 control. The medium temperature is controlled individually with an immersed resistive temperature sensor. Additionally, a syringe pump based perfusion system with a feedback sensor for the fluid volume in the wells is added. We demonstrate the temperature dependence of spike transmission latency in patterned networks of reduced complexity. It is crucial to isolate drug induced effects from variations caused by fluctuating environmental parameters. With our approach we aim to increase reproducibility in network studies and provide a robust drug screening tool that serves the 3Rs principle (replacement, reduction, refinement).

21 . Nunes, Carolina **ETH Zurich, CH**

HUMAN ESC-DERIVED RETT SYNDROME ELECTROPHYSIOLOGY AND MOLECULAR PHENOTYPIC SCREENING PLATFORM FOR THE DEVELOPMENT OF NEW THERAPIES

Rett syndrome (RETT) is a rare neurodevelopmental disorder characterized by an early period of normal development, followed by a sudden loss of acquired psychomotor skills. Most cases of RETT are caused by sporadic mutations in the Methyl-CpG-binding protein 2 (MECP2) gene on the X chromosome, which is essential for normal development, maturation, and function of neurons in the brain. Consequently, many new treatment strategies for RETT try to target MECP2 expression levels and their downstream pathways – but as of today – there is no cure for RETT. For a deeper understanding of how mutations in MECP2 relate to neuronal function and the observed range of symptoms in patients, we set out to develop an integrated multimodal phenotypic screening approach. Here, we present preliminary data obtained from 2 and 3D human ESC-derived control/ mutant neuronal cultures using a combination of functional and genomics assays, including high-throughput high-density microelectrode array (HD-MEA) measurements, patch clamp recordings, and gene expression analyses. Preliminary data indicate that MeCP2-deficient neuronal cultures show a higher division rate and lower levels of mature neuron markers (e.g., markers of microtubule-associated protein 2 and 200 kD neurofilament), as well as higher neuronal firing rates during early development. Generally, neuronal networks developed more robustly in 3D-spheroid cultures. When fully functional, this platform will be adapted to hiPSC-derived cultures and used to probe phenotypic rescue approaches including pharmacological and gene-therapeutic interventions aimed at restoring MECP2 expression levels.

22 . Oryshchuk, Anastasiia **MaxWell Biosystems**

LABEL-FREE FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED NEURONS AT SUBCELLULAR RESOLUTION

In recent years, brain models derived from pluripotent stem cells have become a fundamental tool for studying common neurological disorders, such as epilepsy, Alzheimer's disease, and Parkinson's disease. The ability to measure the electrical activity of human iPSC derived neurons in real time and label free can provide much needed insights into the complexity of the neuronal networks. Nowadays, combining single cell resolution with high throughput physiological assays, which can potentially deepen our understanding of subtype specific neuronal activity, is especially valuable and yet difficult to achieve. In this study, the

MaxTwo System (MaxWell Biosystems, Switzerland), a multi well high density (HD) MEA platform was used. MaxTwo HD MEA System allows in vitro extracellular recordings of action potentials at different scales, ranging from network through single neuron to subcellular features. Moreover, we showed the advantages of having an HD-MEA system featuring 26,400 electrodes per well, which are key to increase the statistical power of the data collected from iPSC derived neurons over multiple days, weeks and to capture the smallest neuronal signals. Finally, we present the Axon Tracking Assay, a tool for automated recording and analysis of individual axonal arbors of many neurons in parallel. The Axon Tracking Assay enables to measure action potential conduction velocity, axonal length, and number of axonal branches. With this unique method, we characterized the function and axonal structure of different iPSC derived neuronal cell lines. Our HD MEA platforms and the extracted metrics, such as firing rate, spike amplitude, and network burst profile among several others, provide an extremely powerful and user friendly approach for in vitro drug screening and disease modelling.

23 . Parodi, Giulia **University of Genova, IT**

INVESTINGATING THE EFFECT OF CHEMICAL AND ELECTRICAL MODULATION IN HUMAN-IPSCS-DERIVED NEURONAL NETWORKS COUPLED TO HIGH DENSITY ARRAYS

In the brain, the neuronal activity is kept and controlled by a precise and well-defined “Excitatory/Inhibitory balance” among neurons, which holds importance in neurodegenerative and neurodevelopmental disorders. With the final aim of generating a reliable in vitro system of the human brain, in this work, we focused on the interplay between excitatory glutamatergic (E) and inhibitory GABAergic (I) neurons in neural networks derived from human induced pluripotent stem cells (hiPSCs), deepening the critical role of heterogeneity. We exploited high-density Micro-Electrode Arrays (MEAs) characterized by 2304 electrodes to explore two neuronal culture configurations: 100% excitatory (100E) and 75% excitatory / 25% inhibitory (75E25I) neurons. This allowed us to broadly characterize the spontaneous electrophysiological activity of mature neuronal cultures at 56 Days In Vitro, a time point in which the GABA shift has already occurred. Moreover, we explored the impact of heterogeneity through chemical stimulation. In particular, the administration of BIC led to an increase in terms of firing and bursting activity only in the 75E25I configuration, while APV and CNQX caused substantial alterations on both dynamics and functional connectivity. On the other hand, the electrical stimulation revealed that the 100E configuration responded reliably, while the 75E25I required additional parameter tuning for improved responses. Our findings advance the understanding of different neuronal interactions and their role in the network activity, providing insights for potential therapeutic interventions in neurological conditions. Overall, our work contributes to the development of a valuable human in vitro system for investigating physiological and pathological conditions, highlighting the critical role of neuron diversity in neural network dynamics.

24 . Röck, Ruth **University of Zurich, CH**

CELL FATE DETERMINATION OF DIRECTLY REPROGRAMMED MOUSE AND HUMAN KIDNEY CELLS

Despite the increasing demand to understand and treat kidney diseases, options for modeling these conditions in vitro remain limited. We are interested in deciphering the molecular programs guiding kidney development and disease and have pioneered the direct reprogramming of renal tubular cells. By using only four transcription factors we achieve cell type conversion of fibroblasts to induced renal epithelial cells (iRECs) that are morphologically and functionally highly similar to primary kidney cells but can be stably cultivated and bioprinted into tubular structures at near physiological scales. However, the process of direct reprogramming isn't fully understood. In-depth analysis, utilizing bulk and single-cell RNA sequencing, further characterizes the molecular nature of iRECs concerning assay stability and cellular heterogeneity. These endeavors aim to refine direct reprogramming methods, enhancing their effectiveness and reliability for potential applications in drug testing and disease modeling in the future.

25 . Scherbakov, Dimitri **University of Zurich, CH**

ERROR-FREE TRANSLATION AS A NOVEL THERAPEUTIC APPROACH AGAINST AGE-RELATED NEURODEGENERATIVE DISEASES SUCH AS PARKINSON'S DISEASE

The prevalence of age-related neurodegenerative diseases (NDDs) is increasing worldwide as the human population ages. Currently, Parkinson's disease (PD) is the second most common NDD, affecting an estimated 7-10 million people worldwide. The increasing frequency of PD poses a significant challenge to healthcare systems, as PD is typically chronic, progressive, and associated with high levels of disability and caregiver burden. The accumulation of protein aggregates, particularly alpha-synuclein, linked with degradation of proteostatic machinery is thought to play a central role in the development of PD. In turn, protein misfolding/malfunction/aggregation is connected to the accuracy of gene expression where protein translation is the most error-prone step. Translation errors lead to misfolding and aggregation of proteins, thus contributing to progression of age-related NDDs such as PD. We propose to reduce the amount of erroneously translated misfolding-prone proteins by modulating the work of translation machinery and increasing protein translation fidelity. Error-free translation reduces the level of intracellular protein misfolding and aggregation thus representing a promising strategy for treating/preventing the development of NDDs such as PD. We have identified some genetic and pharmacological interventions decreasing translation errors and reducing protein aggregation. A further step of preclinical validation requires an appropriate experimental model such as PD patient-derived iPSC lines. A large number of PD patient-derived iPSC lines generated from sporadic patients with different risk profiles (see the Foundational Data Initiative for PD; www.foundinpd.org) offers a realistic possibility to analyze potential of increased translation fidelity as therapeutic approach for PD treatment.

26 . Taborsky, David **University of Zurich, CH**

THE ROLE OF TRANSLATIONAL REGULATION IN EARLY DEVELOPMENT AND GASTRULATION

Developmental processes such as gastrulation involve rapid and profound changes in tissue architecture and cell state. These changes in cell state are the result of alterations in gene expression of individual cells. Gene expression can be regulated at the level of transcription and translation, the latter being known as translational control. While transcriptional

changes have been extensively studied in developmental processes, the potential role of translational control in driving the proteome changes required for cellular transformations are far from understood. We aim to leverage an in vitro organoid model of mammalian gastrulation, the gastruloids, together with ribosome profiling to monitor altered translational efficiencies throughout gastruloid development. Using this approach, we provide evidence for translational control of various pathways essential for early development, including components of the Wnt signalling pathway, as well as pervasive translational regulation of the translational machinery itself. We then follow up on this translational landscape of gastrulation with a single-cell CRISPR screen targeting RNA-binding proteins to uncover the impact of translational control on shaping cell fate and germ layer differentiation in gastruloids.

27 . Tringides, Christina **ETH Zurich, CH**

DESIGNING PHYSIOLOGICALLY-MIMICKING PLATFORMS FOR IMPROVED NEURAL INTERFACES

Biomaterial scaffolds have emerged as a tool to build 3D cultures of cells which better resemble biological systems, while advancements in bioelectronics have enabled the modulation of cell proliferation, differentiation, and migration [1]. Here, we first describe a porous conductive hydrogel with the same mechanical modulus and viscoelasticity as neural tissue [2]. Electrical conductivity is achieved by incorporating low amounts (<0.3% weight) of carbon nanomaterials in an alginate hydrogel matrix, and then freeze-drying to introduce a highly porous network. The mechanical and electrical properties of the material can be tuned to modulate the growth and differentiation of human iPSC neural progenitor cells (NPCs). In addition to forming neurite networks that span the material in 3D, the NPCs can differentiate into astrocytes and oligodendrocytes. With increasing hydrogel viscoelasticity and conductivity, we observe the formation of denser neurite networks and a higher degree of myelination. Application of exogenous electrical stimulation can then be applied to the scaffolds to further promote NPC differentiation. To investigate the functionality of neurite networks in 3D, we begin by placing a polydimethylsiloxane (PDMS) microstructure on an underlying multielectrode array (MEA), as previously described [3], to confine the network growth. We then explore different materials and techniques to integrate hydrogels into the PDMS microstructures, such that the hydrogel can facilitate human iPSC-derived sensory neurons to form 3D networks while still confined by the PDMS. This platform is compatible with various methods to assess neuronal functionality, and can be used to understand the effect(s) of hydrogel properties on the resulting neuronal networks. Both described biomaterial platforms can support the growth of neuronal cells for over 6 weeks, and could facilitate the development of biohybrid electronic devices to understand neuronal development and disease.

28 . Vehring, Leah **University of Bern, CH**

INVESTIGATING PARACRINE INTERACTIONS IN HIPSC DERIVED IN VITRO MODEL MODEL OF NEUROVASCULAR UNIT

Pericytes are mural cells supporting brain microvascular endothelial cells (BMECs) at the level of brain capillaries and their dysfunction has been implicated in various neurovascular

disorders. However, only little is known on their direct influence on BMECs in Multiple Sclerosis (MS) pathogenesis. We therefore want to elucidate the paracrine contribution of pericytes to the previously observed intrinsic blood brain barrier (BBB) dysfunction and increased immune cell interaction. To do so, conditioned medium from human iPSC derived brain pericyte like cells (BPLC) will be analyzed with mass spectrometry to identify possible candidates that are capable of inducing impaired BBB characteristics in human iPSC-derived BMECs. This will be assessed applying various assays, such as permeability assay and adhesion assays, as well as junctional protein staining of BMECs. Lastly, these results will be complemented with the investigation of adhesion molecule expression, namely VCAM-1 and ICAM-1, for immune cell interaction. Interestingly, we showed that treatment of BMECs with BPLC conditioned medium and pro-inflammatory cytokines (TNF-a/IFN-y) increased the expression of relevant adhesion molecules. Taken together, this study will provide a better understanding of the role of pericytes in BBB dysfunction in the context of MS.

29 . Vogt, Sarah **ETH Zurich, CH**

EXPLORING MORPHOLOGICAL HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELLS WITH DEEP LEARNING

Induced pluripotent stem cells (iPSCs) exhibit maximal developmental plasticity, enabling differentiation into various cell types in response to environmental cues, resulting in alterations in their cellular states, including morphology. Investigating these diverse iPSC morphologies at a high-throughput scale requires efficient characterisation and quantification of morphological variations within high-content imaging data. Deep learning methods are highly suited for this task, given their efficient pattern recognition capabilities. In our study, we demonstrate the potential of scDINO (single-cell distillation with no labels), a self-supervised deep learning approach, to comprehensively characterise the complete range of cellular morphologies of iPSCs subjected to diverse small molecule perturbations in a fully unbiased fashion. Beyond mere classification, our investigation illustrates how reliably characterising and quantifying single-cell morphologies opens avenues for mapping bulk molecular data back to the level of individual cells. This approach holds promise for uncovering intricate relationships between molecular changes and alterations in cellular morphology, providing deeper insights into iPSC behaviour and differentiation pathways.

30 . Walter, Natalie **University of Zurich, CH**

ELUCIDATING THE FUNCTIONAL EFFECTS OF OMEGA-3 FATTY ACIDS AS A TREATMENT IN ADHD AGAINST INFLAMMATION AND OXIDATIVE STRESS

Attention-deficit hyperactivity disorder (ADHD) is the most frequently reported neurodevelopmental disorder, with a worldwide prevalence of ca. 5%, affecting children and adolescents. Inflammation and oxidative stress may play a crucial role in ADHD, indicated by altered serum levels of IL-6 and TNFα cytokines as well as increased reactive oxygen species discovered in ADHD children. Therefore, the non-pharmacological treatment of omega-3 (ω-3) polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), display potential candidates, as they take part in several biological mechanisms, including regulations of inflammatory processes and attenuating oxidative stress. However, the underlying molecular mechanisms of ω-3 PUFAs involved in ADHD remains unknown.

Thus, we investigated IL-6 as well as TNFα release and oxidative stress in human induced pluripotent stem cell (iPSC)- derived forebrain cortical neurons (FCNs) from ADHD patients and healthy individuals as control after DHA (10µM) and EPA (10µM) treatments. Investigations of pro-inflammatory cytokines released in FCN supernatants showed tendencies of decreased TNFα secretion in ADHD. These alterations could be increased to control levels after DHA treatment, which may suggest TNFα being crucial for healthy brain development. Moreover, as mitochondria demonstrate the main source of reactive oxygen species production we investigated superoxide accumulation derived from mitochondria in FCNs by conducting MitoSox live imaging. ADHD cell lines were discovered to produce significantly higher levels of superoxide's compared to controls (Mann-Whitney, *p=0.0159), which could be decreased after DHA and EPA treatments. These findings may help understand the importance of inflammation and oxidative stress more evidently and furthermore may reveal the functional effects of ω-3 PUFA to be considered as an additional treatment approach in ADHD in a personalized manner.

31 . Weber, Rebecca Z. **University of Zurich, CH**

HUMAN IPSC-DERIVED CELL GRAFTS PROMOTE FUNCTIONAL RECOVERY BY MOLECULAR INTERACTION WITH STROKE-INJURED BRAIN

Stroke is a leading cause of disability and death due to the brain's limited ability to regenerate damaged circuits. To date, stroke patients have only limited therapeutic options and are often left with considerable disabilities. Induced pluripotent stem cell (iPSC)-based therapies are emerging as a promising therapeutic approach for stroke recovery. In this study, we demonstrate that local transplantation of GMP-compatible iPSC-derived neural progenitor cells (NPC) improve long-term recovery-associated brain tissue responses and reduced neurological deficits after cerebral ischemia in mice. We observe long-term graft survival over the course of five weeks and preferential graft differentiation into mature neurons without signs of pluripotent residuals. Transplantation of NPCs led to a set of recovery associated tissue responses including increased vascular sprouting and repair, reduced blood-brain barrier disruption, reduced microglial activation and increased neurogenesis compared to littermate control animals receiving sham transplantation with phosphate-buffered saline (PBS). Employing deep learning assisted behavior analysis, we revealed improved gait performance and reduced fine-motor deficits. To dissect the molecular graft composition and identify graft-host interactions, we performed single nucleus profiling of graft and host tissue that identifies graft differentiation towards preferentially GABAergic-like cells with remaining cells acquiring glutamatergic neuron, astrocyte, and NPC-like phenotypes. Interactions between graft and host transcriptome indicate that GABAergic cell grafts are primarily involved in graft-host communication through regeneration-associated NRXN, NRG, NCAM and SLIT signalling pathways. In conclusion, our study indicates that transplanted iPSC-derived NPCs differentiate mainly into GABAergic-like cells contributing to long-term anatomical and functional recovery and identifies potential mechanisms involved in this process. These results provide a basis for a therapeutic strategy to improve brain regeneration after stroke.

32 . Yde Ohki, Cristine Marie **University of Zurich, CH**

INVOLVEMENT OF THE WNT SIGNALING IN METHYLPHENIDATE (RITALIN) TREATMENT OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER

Attention-deficit hyperactivity disorder (ADHD) is a multifactorial neurodevelopmental disorder that affects over 5% of children and adolescents around the world. However, molecular mechanisms involved in ADHD etiology are still under investigation. Methylphenidate (MPH), the first-line treatment for ADHD, ameliorates brain maturational delays in patients. The Wnt signaling pathway might be associated with the ADHD pathophysiology. We aim to investigate whether MPH modulates Wnt signaling, which might contribute to clinical improvements observed in patients. ADHD patients who respond to MPH treatment and matching controls (6-18 years old) were recruited by the Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich. Induced pluripotent stem cells (iPSCs), iPSC-derived neural stem cells (NSCs) and forebrain cortical neurons (FCNs) were generated. Proteomic analysis via Western Blot was conducted in these cells and Wnt reporter assays were performed in NSCs to analyze Wnt activity after treatment with the agonist Wnt3a, the antagonist DKK1, and MPH. Growth rates of iPSCs and NSCs were also measured via xCELLigence/WST-1. Synaptogenesis in FCNs was assessed via immunocytochemistry. ADHD NSCs proliferated significantly less than controls for both xCELLigence (Mann Whitney, *p=0.014) and WST-1 assays (Welch's t test, *p=0.012). This was slightly improved by MPH 10 nM in a Wnt-dependent manner. Increased active β-catenin and decreased inactive GSK3β (Mann Whitney, *p=0.043 and *p=0.018, respectively) indicated Wnt signaling alterations in ADHD NSCs, which was confirmed by lower EC50 values for Wnt3a (Welch's t test, *p=0.033) and increased IC50 values for DKK1 in reporter assays. Additionally, Wnt activity seems to be modulated by MPH 10 nM. Synaptic connectivity is higher in ADHD FCNs (Mann Whitney, *p=0.032). These results might be associated with the clinical outcomes observed in patients and with MPH-induced improvements in this context.

33 . Zanini, Giorgia **University of Genova, IT**

EFFECTIVE PARAMETERS FOR ELECTRICAL STIMULATION ON HUMAN IPSCS-DERIVED NEURONAL NETWORKS COUPLED TO MICRO ELECTRODE ARRAYS

In vitro models of neuronal networks have proven to be a valuable tool for gaining deeper insights into the intricate mechanisms governing the human brain. Notably, the integration of human induced pluripotent stem cells (hiPSCs) with micro-electrode arrays (MEAs) provides a controlled in vitro environment to replicate and analyse both the structural and functional elements of the human brain. As neuronal communication relies on the emission of electrical (and chemical) stimuli, the employment of electrical stimulation becomes crucial for a comprehensive exploration of neuronal assemblies, to better understand their inherent electrophysiological dynamics. However, the establishment of standardized stimulation protocols for cultures derived from hiPSCs is still lacking, thereby hindering the precise delineation of efficacious parameters to elicit responses. To fill this gap, this study aims to investigate the stimulation parameters aiming to induce independent and reliable evoked responses in human-derived neurons. We utilized biphasic voltage pulses with variable amplitudes (1.5, 2, 2.5, and 3V) delivered at different frequencies (0.1 and 0.2Hz). As an experimental model, we exploited glutamatergic (excitatory, E) and GABAergic neurons

(inhibitory, I) to construct fully excitatory (E:I 100:0) and mixed (E:I 75:25) cortical networks derived from hiPSCs coupled to MEAs. This approach allowed us not only to optimize stimulation parameters but also to understand their specific impact on the functional dynamics of both excitatory and inhibitory elements within the neuronal networks. This study represents a stepping-stone in the exploration of efficacious stimulation parameters, thus broadening the electrophysiological activity profiling of neural networks sourced from hiPSCs.

34 . Ziak, Nicole **University Hospital Zurich, CH**

UNDERSTANDING THE SELECTIVE NEUROTOXICITY OF SERINE-PALMITOYLTRANSFERASE MUTATIONS IN MOTOR AND SENSORY NEURONS

Serine–palmitoyltransferase (SPT) is the key enzyme for the de novo synthesis of sphingolipids (SLs). Mutations in SPT were previously linked to the Hereditary Sensory and Autonomous Neuropathy Type 1 (HSAN1) - a predominantly sensory neuropathy that is caused by the pathological formation and accumulation of an atypical class of neurotoxic 1-deoxySL. Recently, mutations in SPT have also been associated with Amyotrophic Lateral Sclerosis (ALS), where disease manifests differently. ALS is a progressive, neurodegenerative disease of the lower and upper motor neurons (MN), characterized by severe muscle wasting, eventually leading to paralysis and death. An altered homeostatic control leads to an overshooting formation of canonical SL. MN seem to be particularly susceptible to these changes whereas sensory neurons (SN) appear to be specifically sensitive to 1-deoxySLs accumulation, that occurs in HSAN1. In this PhD project, we want to understand how mutations in the same enzyme –SPT- can lead to such opposing clinical phenotypes. This will hopefully provide insights into the underlying pathomechanisms of both diseases, but also in the metabolic differences between SN and MN, opening up the possibility for novel therapeutic targets. Therefore, we will analyze the lipid profiles in three in vitro models, increasing in complexity. The first two are based on genetic engineering, where neuronal differentiation is induced by transcription factor overexpression. These models are called inducible Neurons (iNeurons). The first experiment uses wild type iNeurons, whereas in the second one, ALS and HSAN1 specific mutations are introduced by CRIPSR-Cas9. The third model, which is the most physiological one, uses patient derived iPSCs. These iPSCs will be differentiated according a small-molecule approach into MN and SN. The lipid profile of mature neurons and how it changes during the differentiation and the effect of perturbing the SL pathway will be assessed in all three models.

35 . Zujur, Denise **Kyoto University, JP**

GENERATION OF IPSC-DERIVED CHONDROGENIC SPHEROIDS VIA NEURAL CREST CELL INDUCTION FOR CARTILAGE REPAIR AND BIOFABRICATION

Articular cartilage is a highly specialized connective tissue providing a lubricated surface to bone joints. Unlike most tissues, articular cartilage does not have blood vessels, nerves, or lymphatics, resulting in limited self-healing capacity. To date, there is no effective long-lasting treatment to repair articular cartilage. In terms of regenerative medicine approach, primary chondrocytes and mesenchymal stem cells (MSCs) are the most common cell sources used in clinical settings for repairing articular defects. However, both cell types

possess limitations such as dedifferentiation, donor morbidity and limited expansion. We developed a stepwise differentiation method to generate cartilage spheroids from iPSCs via neural crest cell (iNCC) induction. iNCCs are further differentiated into iMSCs and chondrocytes. The cell strategy uses a combination of growth factors and small molecule inducers to produce controlled-size spheroids and enhanced cartilage extracellular matrix production with no sign of dedifferentiation, fibrotic cartilage formation or hypertrophy. In particular, we demonstrate that the use of a thienoindazole derivative, TD-198946, synergistically enhances chondrogenesis of iMSCs derived from iNCCs. These findings may provide a novel cell source for stem cell-based cartilage therapy. In addition, as the chondrogenic spheroids also have the potential to fuse within few hours, we are using them as building blocks for biofabrication of larger cartilage tissue using technologies such as the bioprinting Kenzan method.

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