MOSA

CONFERENCE

Program book

Content

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MAASTRICHT | HASSELT

1. Welcome to MOSA Conference 2024

We are delighted to welcome you to the 28th edition of the MOSA Conference, a remarkable event where (bio)medical innovation, student-driven excellence, and global opportunities converge. Hosted by Maastricht University (UM) in partnership with Hasselt University (UH) and MUMC+, this international conference stands as a beacon of innovation, knowledge sharing, and boundless potential.

MOSA is where student researchers meet discerning judges, healthcare leaders, and industry visionaries. We serve as a bridge between academia and industry, facilitating the exchange of cutting-edge ideas and research. Each year, we delve into a (bio)medical theme through captivating lectures and workshops, fostering fresh perspectives and connections.

The 2024 edition promises a global perspective as we continue to attract renowned speakers. We invite you to join us and become part of this thriving community. This year's theme, "Big Data & Artificial Intelligence (AI) in (Bio)medicine," highlights the integration of vast datasets and advanced AI analytics, enabling personalized medicine, improving clinical outcomes, and fostering innovation in biomedicine.

Welcome to MOSA Conference 2024 - Igniting the Future of (Bio)medical Research.

2. Short program

Tuesday, 25 June 2024	
Time	Program
08:00 – 09:00	Registration open
09:00 – 09:10	**Opening by Prof. Jan Theys**
09:10 – 09:20	**Intro from Prof. Annemie Schols (FHML Dean)**
09:20 – 09:30	**Intro from Prof. Dr. Veerle Somers (UH Biomed Dean)**
09:30 – 10:30	**Guest lecture by Dr. Pieter Kubben**
10:30	Coffee break
10:00 – 13:00	***CV check***
10:00 – 13:00	***Job market***
10:45 – 12:15	***Oral presentations I -*** *Embracing The Wide Range of Biomedical Research: Talks About Cardiovascular Disease, Enteric Nervous System, and Tissue Engineering* ***Oral presentations II -*** *Advances in Pediatric Care, Anti-Immuno-Aging, Acne Treatment, and HBV*
10:45 – 12:15	***Poster walks***
12:00 – 13:00	Lunch
13:00 – 14:30	**Interactive workshop part 1 (MANDATORY for UM and UH students)**
14:30 – 15:00	Coffee break
15:00 – 16:00	**Guest lecture by Prof. Dr. Liesbet Peeters**
15:00 – 16:00	**Guest lecture by Prof. Dr. Rianne Fijten**
16:15 – 17:45	**Workshops in parallel**
18:00	**End of the first day**

Wednesday, 26 June 2024	
Time	Program
08:30 – 09:00	Registration open
09:00 – 10:00	**Interactive workshop part 2 (MANDATORY for UM and UH students)**
10:00 – 14:30	***CV check***
10:00 – 16:00	***Job Market***
10:00	Coffee break
10:15 – 12:30	***Oral presentations III -*** *NeuroInsights: Advancements And Challenges In Neurological Health And Treatment.* ***Oral presentations IV -*** *Advancements in Genotoxicity, Cancer Research, and Machine-Assisted Innovations*
10:15 – 12:30	***Poster walks***
12:00 – 13:00	Lunch
13:10 – 14:10	**Guest lecture by Dr. Mehrdad Seirafi**
14:20 – 15:50	**Workshops in parallel**
15:50 – 16:00	Coffee break
16:00 – 17:00	**Guest lecture by Dr. William van Doorn and Dr. Paul van Dam**
17:10 – 18:10	**Panel discussion: "Ethical Implications of Big Data and AI in Biomedicine"**
18:20 – 19:20	**Awards and closing**
19:20 - 21:00	**Reception**

3. Complete program

Tuesday, 25 June 2024		
Time	Program	Location
08:00 – 09:00	Registration open	Onderwijsplein / UNS40 main entrance
09:00 – 09:10	**Opening by Prof. Jan Theys**	MSM conference Hall
09:10 – 09:20	**Intro from Prof. Annemie Schols (FHML Dean)**	MSM conference Hall
09:20 – 09:30	**Intro from Prof. Dr. Veerle Somers (UH Dean)**	MSM conference Hall
09:30 – 10:30	**Guest lecture by Dr. Pieter Kubben** Opening lecture on Big data and AI *Moderator: Jan Theys*	MSM conference Hall
10:30 - 10:45	Coffee break	Onderwijsplein / Drielandenpunt
10:00 – 13:00	***CV check***	*Tongeren Hall*
10:00 – 13:00	***Job market***	*Onderwijsplein*
10:45 – 12:15	***Oral presentations I -*** *Embracing The Wide Range of Biomedical Research: Talks About Cardiovascular Disease, Enteric Nervous System, and Tissue Engineering* *Moderators: Elke Smeets (UHasselt) & Niels Vanden Bosshe (Maastricht University)* I.1. Endothelial Cell Volume in Arteriovenous Malformations: Everolimus and Growth Factor Effects- *Mohammadhossein Izadloo, Universidade Católica Portuguesa* I.2. Serratus Anterior Plane Block: High vs. Low Dose for Post-Minimally Invasive Heart Valve Surgery Pain- *Laurien Veulemans, Hasselt University* I.3. Fabrication of strong and tough hydrogel networks processable for tissue engineering - *Anneleen Jacobs, Hasselt University* I.4. Sound-based assembly of a spatially organised neurovascular network - *Greta Cocchi, Maastricht University* I.5. The impact of microbiota on gut function and behaviour in a mouse model for psychiatric disorders - *Kobe Neven, Hasselt University* I.6. miR-146a-5p Regulation of Enteric Glial Cell Status in Gastrointestinal Health and Disease - *Jorunn Vranken, Hasselt University* ***Oral presentations II -*** *Advances in Pediatric Care, Anti-Immuno-Aging, Acne Treatment, and HBV* *Moderators: Iveta Dzivite (Maastricht University) & Tia Ackermans (Maastricht University)* II.1. Hyperleukocytosis in paediatric patients with acute lymphoblastic leukaemia - a single center experience- *Aleksandra Dembowska, Medical University of Lubin* II.2. Animal milk oligosaccharides inhibit the biofilm of Cutibacterium acnes - a preliminary study - *Wiktoria Stańska, Medical University of Warsaw* II.3. When you hear hoofbeats, think of horses, but also of zebras. Paraneoplastic syndromes in paediatric oncology - *Maciej Dubaj, Medical University of Lubin* II.4. Human in vitro models to study the role of pulmonary neuroendocrine cell hyperplasia in the	*Heerlen Hall* *Tongeren Hall*

	pathophysiology of congenital diaphragmatic hernia - *Isabel Bougie, Erasmus University Medical center* II.5. Exercise: a powerful tool to counteract immune ageing via IL-15-induced autophagy - *Mariken Lemmens, Hasselt University* II.6. Hepatitis B virus core protein as an antiviral target for functional cure of chronic hepatitis B - *Emerance Ishimwe, Hasselt University*	
10:45 – 12:15	***Poster walks:*** Group 1-6	
12:00 – 13:00	Lunch	Onderwijsplein / Drielandenpunt
13:00 – 13:10	**Interactive workshop - explanation (only for UM and UH students, MANDATORY)**	dsm-firmenich hall (Maastricht Hall)
13:10 - 14:30	**Interactive workshop - part 1 (only for UM and UH students, MANDATORY)**	Multiple halls
14:30 – 15:00	Coffee break	Onderwijsplein / Drielandenpunt
15:00 – 16:00	**Guest lecture by Prof. Dr. Liesbet Peeters** Real-world data *Moderator: Jan Theys*	dsm-firmenich hall (Maastricht Hall)
15:00 – 16:00	**Guest lecture by Prof. Dr. Rianne Fijten** Shared-decision making in cancer *Moderator: Niels Vanden Bossche*	Tongeren Hall
16:15 – 17:45	**Workshops in parallel**	Multiple halls
18:00	**End of the first day**	

Wednesday, 26 June 2024		
Time	Program	Location
08:30 – 09:00	Registration open	Onderwijsplein / UNS40 main entrance
09:00 – 10:00	**Interactive workshop part 2 (only for UM and UH students, MANDATORY)**	dsm-firmenich hall (Maastricht Hall)
10:00 – 14:30	***CV check***	*Tongeren Hall*
10:00 – 16:00	***Job Market***	*Onderwijsplein*
10:00 - 10:15	Coffee break	Onderwijsplein / Drielandenpunt
10:15 – 12:30	***Oral presentations III -*** *NeuroInsights: Advancements And Challenges In Neurological Health And Treatment.* *Moderators: Charlotte Peetersem (UHasselt) & Laurien Veulemans (UHasselt)* III.1. The Real-World Effectiveness and Safety of Cyclophosphamide in Patients with Progressive Multiple Sclerosis - *Ian Meyssen, Hasselt University* III.2. The good, the fat, the ugly: fatty acid elongation in control of lesion repair in multiple sclerosis - *Elke Smeets, Hasselt University* III.3. The isotype-dependent impact of ApoE on miR-146A-mediated autoimmunity in multiple sclerosis - *Dylan Kidjemet, Hasselt University* III.4. Temperature-Dependent Axonal Growth Inhibition via TRPV4 Activation - *Femke Cornelissen, Hasselt University*	*Heerlen Hall*

	III.5. The Haunted Treatment Against Stroke: Harnessing the Power of Stem Cell Nanoghosts - *Sarah Willems, Hasselt University* III.6. Turning back time: Can Targeting Cellular Senescence Enhance Recovery After Spinal Cord Injury? - *Yanne van Reusel, Hasselt University* ***Oral presentations IV -*** *Advancements in Genotoxicity, Cancer Research, and Machine-Assisted Innovations* *Moderators: Hanne Coenen (UHasselt) & Josephine-Elisabeth Pippi (Maastricht University)* IV.1. bioGWAS: a Simple and Flexible Tool for GWAS Datasets Generation - *Anton Changalidi, Maastricht University* IV.2. Machine Learning Approaches Using Complete Blood Count for Diagnosis Chronic Lymphocytic Leukemia - *Oğuzkan İlmaz, Giresun University* IV.3. MET activity prevents actin cap alignment: implications for cell migration and nuclear morphology - *Jasmijn Timmermans, Maastricht University* IV.4. A survey of protective effect of melatonin against trifluoperazine-induced genotoxicity in peripheral blood lymphocytes via micronucleus assay- *Sahar Bezhad, Zabol University* IV.5. Lost in Translation: Unravelling cancer's dependence on aberrant mRNA translation - *Ozgu Gumustekin, Maastricht University* IV.6. Detection of Low Diastolic Ocular Perfusion Pressure Using Transfer Learning from Fundus Photographs - *Betin Bilkan Karaman, Eskişehir Osmangazi University Medical School* IV.7. Cathepsins Beyond the Lysosome: Extracellular Cathepsin B as a Therapeutic Target for MASH-HCC - *Jorn Steeghs, Maastricht University*	*Tongeren Hall*
10:15 – 12:30	***Poster walks:*** Group 7-11	
12:00 – 13:00	Lunch	Onderwijsplein / Drielandenpunt
13:10 – 14:10	**Guest lecture by Mehrdad Seirafi** Application of AI in neurological research *Moderator: Jan Theys*	dsm-firmenich hall (Maastricht Hall)
14:20 – 15:50	**Workshops in parallel**	Multiple Halls
15:50 – 16:00	Coffee break	Onderwijsplein / Drielandenpunt
16:00 – 17:00	**Guest lecture by Dr. William van Doorn and Dr. Paul van Dam** Machine learning for risk stratification in the emergency department *Moderator: Jan Theys*	dsm-firmenich hall (Maastricht Hall)
17:10 – 18:10	**Panel discussion: "Ethical Implications of Big Data and AI in Biomedicine"** *Moderator: Jan Theys*	dsm-firmenich hall (Maastricht Hall)
18:20 – 19:20	**Awards and closing**	dsm-firmenich hall (Maastricht Hall)
19:20 - 21:00	**Reception**	Faculty bar

Workshops

Tuesday, 25 June 2024	
Workshop	**Location**
Science communication	Heerlen Hall
AlphaFold	Luik Hall
dsm-firmenich	Diepenbeek Hall
Drug discovery & AI	Keulen hall
Blender	dsm-firmenich hall (Maastricht Hall)
Clinical Data Harmonisation and Machine Modelling	Tongeren Hall

Wednesday, 26 June 2024	
Workshop	**Location**
Science communication	Heerlen Hall
dsm-firmenich	Diepenbeek Hall
Drug discovery & AI	Keulen hall
Blender	dsm-firmenich hall (Maastricht Hall)
Microscopy	Tongeren Hall

Interactive workshop (UM & UH)

Location: Maastricht Hall						
Group	**Name**					
1	Elisa Simone	Jens Gielen	Asier Martin Carzorla	Marina Porras Macua	Edmée Snijders	Aina Tudela Gonzalez
2	Mariken Lemmens	Lien Van Otterdijk	Sophia Wanner	Andrea Pääro	Karmen Aslankurt	Manon van der Pas
3	Dylan Kidjemet	Femke Cornelissen	Cas CTMA Swinkels	Alexandra Bosch	Iveta Dzivite	Julie Mounir Labib
4	Janne Vandyck	Dario Geebelen	Matteo Cesario	Jolijn Driessen	Ulas Babaygit	Elini Papadopoulou
5	Yanne Van Reusel	Anton Brosens	Cobi Bergsma	Nick Welten	Valentine Deremiens	Camilla Ferrari
6	Anessa Pijalovic	Ruben Knevels	Veerle Horsting	Christos Konstantaros	Jorn Steeghs	Caitlin Peeters
7	Renée Rita Moonen	Anouar Dlatat	Niels Vanden Bossche	Luisa Wensky	Sarah Veenstra	Olivia Smyth
8	Merel Hufkens	Lisa Steegen	Sem Peijnenborgh	Jasmijn Timmermans	Zuzanna Lech	Anne Oude Egberink
9	Homayoon Yazdanshenas	Freddy Leenders	Panagiotis (Panos) Barlampas	Anouk Aaldering	Mart Smidt	
10	Yana Heyvaert	Yara Lambrechts	Mehmet Koyuncuoğlu	Lisi Wagener	Daan Arts	
11	Hanne Coenen	Thomas Raps	Bram Brandt	Lara Stassen	Madalena Santos Neves Rêgo Cabrita	

Location: Tongeren Hall						
Group	**Name**					
12	Amber Theunissen	Anouk Bollen	Ana Neves Da Fonseca	Borja Zaragoza Gauchia	Greta Cocchi	
13	Birgit Vrancken	Jelena Geens	Paula Perez Climent	Linda Kehr	Nina Lier	
14	Willem Awouters	Miele Martens	Sanne Dekker	Anastasia Karatza	Josephine-Elisabeth Pippi	
15	Michèlle Hendriks	Charlotte Peetersem	Ines Santos	Silke van Reeuwijk	Jenny Surholt	
16	Robin Schellingen	Elien Mees	Louise Beuk	Demi Gerritsen	Martijn Simons	
17	Joke Aerts	Sofiia Vorobiova	Rachel Chin Kwie Joe	Luc Hooglugt	Isabel Bougie	
18	Sarah Willems	Anneleen Jacobs	Ivan Saify	Delphine lemaire	Alessandra Papitto	

Location: Heerlen Hall						
Group	**Name**					
19	Elif Korkmaz	Algoe Hemafelincia	Quinten Lok	Levi Iperenburg	Lynn Theunissen	
20	Semih Bayram	Elke Smeets	Denzel Hageman	Tia Ackermans	Romy Khan	
21	Quinn Croughs	Jule Richartz	Fleur Wiertz	Lotta Regtop	Diego Fadrique Valle	

Location: Keulen Hall						
Group	**Name**					
22	Zoë Donders	Kobe Neven	Laura Wubben	Elisa Santarsiero	River Hummel	
23	Jonas Schimmel	Rombout Moors	Liset Jacobs	Dario Strikwerda	Nienke Meskes	
24	Laurien Veulemans	Niki Sciarrino	Ioanna Tzavara	Jente Schmeetz	Loïs Reijnders	
25	Jorunn Vranken	Kirsten Poelmans	Pierre Cantagallo	Lisa Kalisvaart	Marie Poncelet	

Location: Diepenbeek Hall						
Group	**Name**					
26	Malou Reverendo	Jill Grondelaers	Fabiola Marques Trujillo	Anna Ouwerkerk	Maaike Bruurs	
27	Amber Delbroek	Melisa Erdal	Emmee Stevelmans	Niek Stassen	Tim Diebels	
28	Marie Miseur	Heike Blockx	Bruno Mendes Dinho Pinto Da Silva	Pablo Infante Villanueva	Delana Deijck	
29	Dario Cosemans	Anouk Delaet	Leela Chatterji	Sewan Abbas	Pierre Klemmer	

Location: Luik Hall						
Group	**Name**					
30	Hanne Eerdekens	Liam Coenen	Marta Sossai	Steffanie Hartjes	Faith-Nova Folkerts	
31	Emma Geerits	Imke Guery	Erik Meyer	Maryam Bouzidi	Eva Hensenne	
32	Emma Gesquire	Eline Simons	Lucia Malikova	Marie Vandormael	Vignesh Shaju	Ozgu Gumustekin
33	Ian Meyssen	Yousra Boutayniout	Emerance Ishimwe	Lourdes Matas Sancho	Jasper JJJA Ploos van Amstel	Senne Zon

4. Poster walks

Tuesday, 25 June 2024		
Poster walk 1, Area around Maastricht Hall		
1A	Jill Grondelaers	Fluoxetine's Anti-inflammatory Potential Hinges on Lipid Metabolism Integrity
1B	Michèlle Hendriks	Tackling Cancer Cachexia in Colorectal Cancer Patients by Unraveling Skeletal Muscle Characteristics
1C	Ana Neves da Fonseca	Exploring the roles of ARID4B and BMPR2 in the progression of KMT2A-rearranged Acute Lymphoblastic Leukemia
1D	Emmee Stevelmans	The glaucoma gambit: rescuing retinal ganglion cells from dysfunction
1E	Charlotte Peetersem	The triad between lipids, apolipoprotein E, and microglial activation in Alzheimer's disease explored
Poster walk 2, Onderwijsplein		
2A	Jonas Schimmel	The influence of extracellular matrix cues on aging retinal pigment epithelium biomechanics
2B	Inês Santos	Implementing up-front ctDNA liquid biopsy using ddPCR for the molecular genotyping of advanced NSCLC
2C	Amber Theunissen	Unraveling the role of reactive oxygen species during epithelial to mesenchymal transition
2D	Merel Hufkens	Uncovering the Differential Roles of Microglia and Macrophages in MS: Foamy or Functional?
2E	Eline Simons	The membrane-associated periodic scaffold regulates endocytosis along the proximal axon
Poster walk 3, Onderwijsplein		
3A	Anton Brosens	Exploring the role of DNA repair protein APEX1 on the development and longevity of the ENS.
3B	Renée Rita Moonen	EVEN SUPERHEROES NEED THEIR VITAMINS. Investigating Riboflavin's Influence on Planarian Regeneration
3C	Zoë Donders	Downstream PDE4D Signaling: Illuminating the Path to Myelin Regeneration in Multiple Sclerosis
3D	Josephine-Elisabeth Pippi	Investigating the therapeutic potential of antibody Candidate 14 in amyloid-β induced neurotoxicity
3E	Eva Hensenne	The glutamate/GABA balance as a novel therapeutic target in 22q11.2 deletion syndrome: a clinical trial
Poster walk 4, Drielandenpunt		
4A	Lisa Steegen	Echocardiography evaluation to allow early detection of doxorubicin-induced cardiotoxicity in rats
4B	Niki Sciarrino	Acyl-CoA-binding protein (ACBP) deletion in brown adipose tissue impairs high-fat diet-induced cardiac remodeling
4C	Dario Geebelen	The impact of polystyrene micro- and nanoplastic on neuroregeneration in Schmidtea mediterranea
4D	Cobi Bergsma	Structure-activity relationship between per- and polyfluoralkyl substances (PFAS) and their effects on the NF-κB pathway in innate immune toxicity: a matter of headgroups?
4E	Anouar Dlalat	DNA damage and repair in the spinal cord after traumatic injury
Poster walk 5, Drielandenpunt		
5A	Hanne Coenen	The MIF/CD74 Axis as a Driver of Pro-inflammatory B Cell Responses Following Spinal Cord Injury
5B	Elisa Simone	Interrogating the essentiality of RBBP7 chromatin modifiers in IDH1-mutant glioma.
5C	Quinn Croughs	The fight against head and neck cancer on a nano-scale: ROS-responsive carriers for targeted therapy
5D	Amber Delbroek	Investigating Microglial Saltatory Migration and Efferocytosis in Disc1 LI Mouse Models

5E	Lien Van Otterdijk	S-palmitoylation hinders brain repair by triggering foam cell formation
Poster walk 6, Drielandenpunt		
6A	Bayram Semih	Human dental pulp stem cells as a patient-in-a-dish model for Charcot Marie-Tooth disease type 1A
6B	Jenny Surholt	A Two-Edged Sword: Impact of Anticoagulation Treatment on Atherosclerosis
6C	Luisa Wensky	Assessing genome instability markers in relation to obesity across different tissues and age groups in the ZSF1 hypertensive rat model
6D	Kirsten Poelmans	Promoting Phospholipid Synthesis via the TGF-β axis as a Potential Strategy to Rescue Remyelination in Demyelinating Disorders
6E	Jule Richartz	PDE4D enzymes as possible therapeutic target to alleviate dementia symptoms in Alzheimer's disease

Wednesday, 26 June 2024		
Poster walk 7, Area around Maastricht Hall		
7A	Willem Awouters	Light-induced degradation of N719 in dye-sensitized solar cell-based photovoltaic photographs
7B	Alessandra Papitto	Investigating Immune Responses in Severe Asthma and the Influence of Mepolizumab on Immune Cell's Profiles and Phenotypes
7C	Birgit Vrancken	Assessing nutritional interventions' impact on cognitive function in older at-risk adults
7D	Yara Lambrechts	Exploring lipid metabolism dynamics during human Schwann cell differentiation for CMT1A therapy
7E	Marie Poncelet	Exploring Transcranial Alternating Current Stimulation Modulation of Neural Oscillations
Poster walk 8, Onderwijsplein		
8A	Jens Gielen	Duration and Quality of Analgesia after Ambulatory Forefoot Surgery under Ankle Block
8B	Vana Stojić	Necrotizing fasciitis secondary to retroperitoneal abscess
8C	Hema Felincia Algoe	The effect of a potato- versus a pasta or rice-based food pattern on cardiometabolic health
8D	Anessa Pijalovic	Generation of Cortical Neurons from Mouse ES Cells to Study Val66Met Polymorphism in the BDNF Gene
8E	Lucia Malikova	Uncovering the uptake and cytotoxicity of micro- and nanoplastics in human intestinal cells
Poster walk 9, Drielandenpunt		
9A	Yana Heyvaert	Conductive MXene-based bioink for integrating biomimetic electronics into new 3D skin model
9B	Homayoon Yazdanshenas	Potential Mechanisms of PM2.5 Particulate Transfer via Extracellular Vesicles
9C	Rombout Moors	Identification of cellular processes elicited by the defects in ribosome synthesis that cause Diamond-Blackfan anemia
9D	Emma Geerits	Decoding tumor heterogeneity: cyclic staining to visualize the tumor microenvironment.
9E	Selma Mtoor	Roles of apical ectodermal ridge and zone of polarizing activity in chicken embryo limb development
Poster walk 10, Drielandenpunt		
10A	Elien Mees	Phenotypic and functional characterization of IgD-CD27- double negative B cells in multiple sclerosis pathology
10B	Thomas Raps	Evaluating suicide gene therapy with human dental pulp stem cells for oral squamous cell carcinoma

10C	Delphine Lemaire	Deciphering D-Amino Acid Dynamics in Cancer Cachexia: From Plasma Profiling to Muscle Cell Responses
10D	Joke Aerts	The role of inflammasome activation for T cell migration in Multiple Sclerosis
10E	Iveta Dzivite	In vitro differentiation profile of human periosteum-derived cell aggregates
Poster walk 11, Drielandenpunt		
11A	Emma Gesquiere	The neuroregenerative potential of IGF-II as a therapeutic strategy in ischemic stroke
11B	Robin Schellingen	Unraveling the Brain's Cleanup Crew: TRPV4 in Mitochondrial Movement during Microglial Phagocytosis.
11C	Freddy Leenders	Forever Young – The Epigenetic Clock of Oligodendrocyte Precursor Cells
11D	Hanne Eerdekens	NANO-ARD: Utilizing NANOvesicles for accelerating angiogenesis in Acute Radiation Dermatitis
11E	Miele Martens	Synergistic sensing: combining thermal and impedance sensors into the microwell format.

5. Lectures by keynote speakers

Pieter Kubben

Opening lecture on big data & AI

As a leading neurosurgeon at Maastricht UMC+, Dr. Kubben brings a wealth of expertise in cutting-edge research areas such as adaptive deep brain stimulation and brain-computer interfacing. His pivotal role in establishing the eHealth program at MUMC+ underscores his commitment to leveraging technology for healthcare advancement. As the opening keynote speaker, Dr. Kubben will illuminate critical concepts of big data and AI, equipping the audience with essential background knowledge as they explore the subject further in the following presentations.

Liesbet Peeters

Real-world data

Liesbet M. Peeters is an assistant professor, leading the research group of biomedical data sciences at Hasselt University (Belgium). This research group is affiliated with both the Biomedical Research Institute as well as the Data Science Institute. They are a multidisciplinary team that consists of researchers with a (bio)medical background as well as with a data science background (and everything in between). Together, they make the connections between the two very different worlds of biomedical sciences and data sciences. In her lecture, Liesbet will inspire the audience about how #DataSavesLives and how biomedical researchers can contribute to the wicked problem of health data sharing and use.

Rianne Fijten

Shared-decision making in cancer

Rianne Fijten, Assistant Professor at Maastricht University and Maastro, is at the forefront of Clinical Data Science, leveraging AI to enhance patient outcomes in medical decision-making. With a background rooted in biomedical sciences, Dr. Fijten's journey into data science began during her Master's and PhD studies. Here, she performed research on the diagnosis of diseases (including cancer) using the exhaled breath of patients. During her presentation, Dr. Fijten will highlight a variety of clinical cases in which AI can make a difference. In particular, she will focus on AI that supports decision-making by predicting future events such as cancer survival and recurrence or side effects of treatment.

Mehrdad Seirafi

Application of AI in neurological research

Mehrdad Seirafi, a neurotech entrepreneur based in Maastricht, holds a PhD in cognitive neuroscience from Maastricht University, where he originally studied physics. Alongside being the co-founder, he serves as the tech lead at Alpha Brain Technologies, a deep-tech medical device company. During his lecture, Dr. Seirafi will shed light on NEVOA-AI, an embedded-AI for continuous monitoring of the brain in real time developed by Alpha Brain Technologies. This AI is able to predict and potentially prevent brain-related catastrophes, such as epileptic seizures, before happening.

William Van Doorn

Paul van Dam

Machine learning for risk stratification in the emergency department

William Van Doorn, a resident in clinical chemistry, and Paul van Dam, an internist in acute medicine, are both working at Maastricht UMC+ and leading researchers of the MARS-ED trial. During their talk, they will introduce the audience to the MARS-ED trial and the use of AI tools to help decision-making for patient care.

Determining the risk levels of patients after they are admitted to the emergency department could aid in making better patient care decisions. A recent retrospective study conducted at Maastricht UMC+ presented a new clinical risk score called the RISKINDEX. This score employs an AI model to predict the 31-day mortality of patients who visit the emergency department. The RISKINDEX surpassed the performance of internal medicine specialists; however, the extent of its beneficial value when applied in clinical practice was still uncertain. This led to the MARS-ED clinical trial, where the diagnostic accuracy, policy changes, and clinical impact of the RISKINDEX are determined on a large scale. The outcomes of this trial could revolutionize how we prioritize care in critical settings and even made the list of Nature Medicine's Top Clinical Trials for 2024.

6. Workshops

Jordi Morwani

From lab to likes:

Science communication on social media

Have you ever dreamed of sharing your love for science with the world through social media? Learn how to transform complex scientific concepts into engaging content that's perfect for various social media platforms! From crafting catchy posts to building your online presence, we'll equip you with the skills you need to stand out in the digital crowd. Plus, we will dive into group activities where you'll brainstorm social media strategies for hypothetical projects and get feedback from your peers. Get ready to unleash your inner science communicator and make a splash online! Don't miss out on this opportunity to turn your passion for science into social media gold.

Timo Rademakers

Unlocking the power of advanced microscopy

Are you curious about the possibilities of state-of-the-art microscopy in modern research and eager to explore the depths of cellular imaging and analysis? Join us for an interactive workshop where we'll unravel the mysteries of advanced microscopy. During this workshop, we will start with a short introduction to higher content microscopy, ranging from histology scanners to complex automated acquisition protocols. This will be followed by a hands-on experience using QuPath, in which we will cover topics such as tissue classification and cell detection in 2D (histological) data sets. Finally, we will end with a short showcase of more complex analysis pipelines for 3D/4D imaging.

Shervin Mehryar

Clinical data harmonization and machine modeling

In today's healthcare landscape, AI tools are becoming indispensable, influencing the decisions made by healthcare providers in clinical settings.This workshop allows participants to explore the tools used by data scientists to investigate real-life clinical data sets together in small groups, in addition to offering a unique outlook on the AI tools used for clinical modeling and harmonized clinical decision-making. Moreover, the interactivity of the session allows the students to be part of the optimization and personalization process for real-life clinical decisions, ensuring a hands-on learning experience that bridges the gap between theory and practice.

Caitlin Mellor

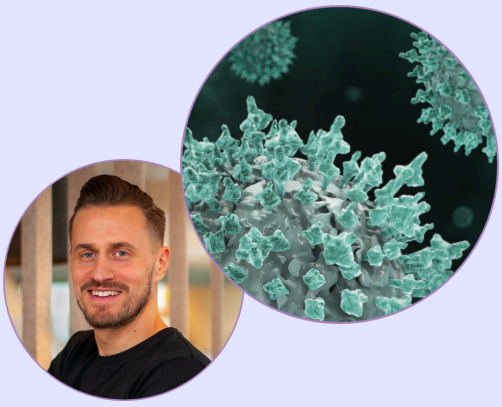
Artificial intelligence for drug discovery

Ever wondered how artificial intelligence (AI) is reshaping drug discovery? Curious about its real-world applications and potential future implications? Join us for an engaging workshop that delves into the fascinating intersection of AI and drug discovery. This workshop offers a holistic overview of the current uses of AI in drug discovery, drawing on real-world data and examples to demonstrate the power AI yields in this field. Moreover, in this workshop, we will explore the future of the field, considering the pitfalls and potential limitations of AI in drug discovery and how these ultimately translate to patients.

Paulyna Magaña

Redefining biomedicine through AI structural biology

Navigating the complex world of structural biology has taken an exciting turn with AlphaFold. This AI breakthrough by Google DeepMind isn't just about predicting protein structures with incredible accuracy, it's about opening doors to new discoveries in biomedicine. It's transforming the way we approach medical research, from speeding up drug development to getting a better grasp on protein evolution. In the workshop, we'll dive into how AlphaFold is reshaping biomedicine. We'll look at real-world examples where it's making a difference, highlighting its role in the fast-paced world of medical breakthroughs. The workshop will showcase AlphaFold as a technical achievement, but also as a beacon for future medical research, highlighting its potential to redefine the boundaries of biomedicine.



Stefan Giselbrecht

Blender: next-level visualization of science

Creating good quality figures for your written work and presentations is important: they make your complex concepts and data easier to understand for everyone. Plus, they make your work look solid and can boost the representation of your results. Blender is an open-source 3D creation program ideal for generating scientific figures and animations. During this workshop, participants will gain proficiency in Blender's interface, learn to utilize its key functions for 3D visualization, and explore advanced tools for textures and animations, empowering them to craft compelling scientific representations effectively.

Daniel Garcia

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7. Panel discussion

Dr. Stijn Denissen

Post-doc researcher at the AI-supported modelling in clinical sciences (AIMS) lab, VUB

Stijn Denissen studied rehabilitation sciences at the KU Leuven after which he pursued a PhD in medical sciences at the VUB focusing on AI applications to study the link between structural brain MRI and cognitive impairment in multiple sclerosis. He is currently a post-doc at the AI-supported modelling in clinical sciences (AIMS) lab at the VUB continuing the federated learning project he started during his PhD. The concept enables training AI models on decentralised data sets, mitigating the need for data sharing between clinical centres.

Prof. Dr. Seppe Segers

Professor moral science and theoretical and practical ethics at the Department of Philosophy and Moral Science of Ghent University

Prof. Dr. Seppe Segers' main research domains are theoretical and substantive ethics, with a focus on normative and meta-ethics, and, respectively, bioethics, medical ethics and engineering ethics. He has published in academic journals and popular media about the use of AI in medicine, its ethical relevance, and questions using large language models in (medical) ethics. He is a member and former secretary of the Bioethics Institute Ghent and deputy of the Ethics and Law special interest group of the European Society of Human Reproduction and Embryology.

Dr. Dorothee Horstkötter

Head of the Department of Health, Ethics and Society, Maastricht University, the Netherlands.

Dorothee Horstkötter (PhD) is trained as a philosopher and ethicist and has specialized in Neuroethics. Her ethics research targets traditional modes of prevention and intervention, innovative (neuro)technologies and increasingly the implementation of digital care technologies and the usage of ai-supported tools. Her research focuses on ethical values like autonomy, responsibility, consent, justice, and equity, while she is also interested in the wider implications of emerging technologies on the meaning and scope of human agency and the normativity of human behavior and decision-making.

Prof. Dr. Leonard Wee

Assistant professor of Clinical Data Science at Maastricht University

Prof. Dr. Leonard Wee specializes in using real-world clinical data generated from routine encounters of patients with the healthcare system, in order to generate new insights and testable clinical hypotheses. To do this, he uses the Personal Health TRAIN (PHT) which is an open-source, demonstrably safe and proven ethical framework of performing federated learning, which means that mathematical models and statistical descriptions are shared but NOT any of the individual level patient data. Leonard is presently lead investigator or work leader across a number of multi-institutional and EU-funded federated learning studies.

Prof. Dr. Rik Wehrens

Associate professor in the sociology of digital health at Erasmus School of Health Policy & Management

Prof. Dr. Rik Wehrens has a background in the interdisciplinary field of Science & Technology Studies, combining perspectives from sociology (of science), anthropology and philosophy of technology. Working from a socio-technical approach that takes the intertwinement of technological and social developments as an analytical focus, his research work focuses on the various implications of the transformation to digital health for healthcare practitioners and patients. He utilizes ethnographic and discursive methods to analyze how data-driven technologies reconfigure knowledge practices, professional roles, and ethical decision-making in healthcare. Recent publications include papers in Social Science & Medicine, Big Data & Society, and Science, Technology, & Human Values.

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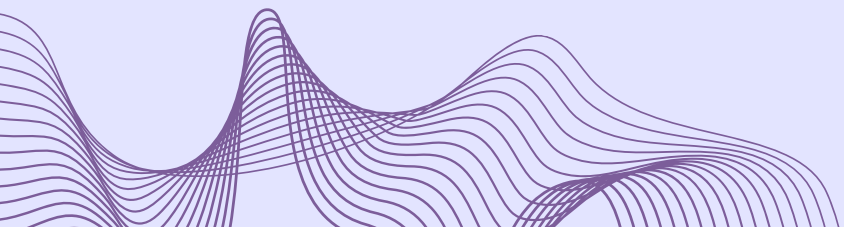

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Cardiovascular Research Institute Maastricht

CARIM is one of the six schools of the Faculty of Health, Medicine and Life Sciences of Maastricht University and is embedded within the Maastricht University Medical Center+ . Moreover, they are known for their close collaboration with the Heart+Vascular Center of Maastricht UMC+.

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9. Location

This year's MOSA conferences takes place at the Faculty of Health, Medicine & Life Sciences of Maastricht University.

Universiteitssingel 40, 6229 ER Maastricht, The Netherlands

How to reach the conference?

By car

From the A2/E25 North

Take the exit *MECC, Randwyck, Academic Hospital (no. 55)*. Continue towards *Universiteitssingel 40*.

From the A2/E25 South

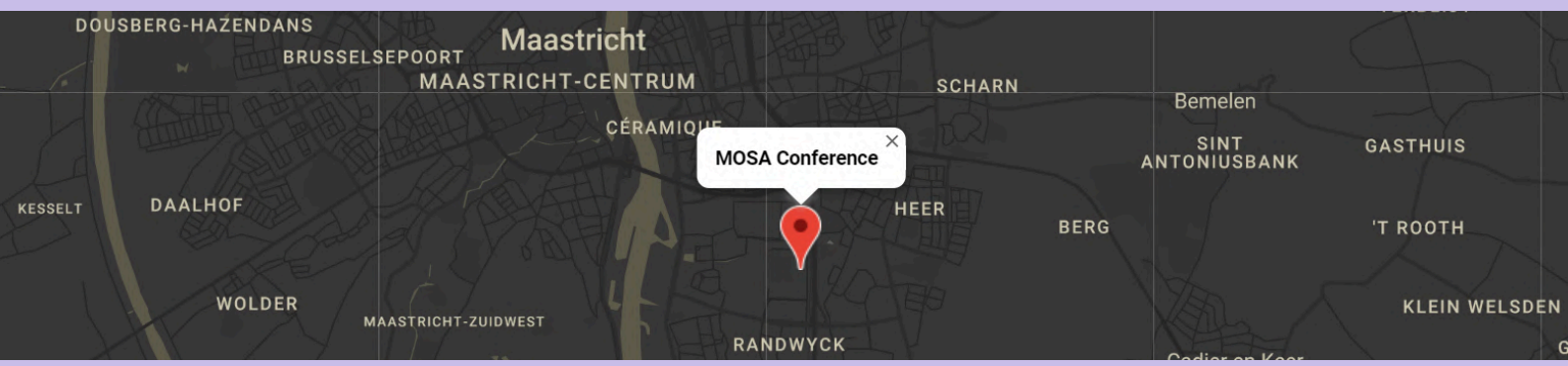
Take the exit *Gronsveld, MECC, Academic Hospital (no. 55)*. Continue towards the academic hospital until you cross the motorway, then follow *Universiteitssingel 40*.

By train

From Maastricht Central Station, a train departs four times an hour (travel time: 3 minutes) to *Maastricht-Randwyck station*. Once arrived, leave the platform to the right and follow towards *Universiteitssingle 40*.

By bus

Take the bus towards *Randwyck station*, *Forum MECC*, or *Endopolsdomein* when coming from Maastricht center or Maastricht station. When coming from Belgium, there is a direct connection between Hasselt and Maastricht center with bus no. 20a.



Free parking space is provided for participants

Follow the directions towards the P. Debyelaan 50 parking
Parkingspace, P. Debyelaan 50, 6229 HG Maastricht, The Netherlands

At the end of the *Sorbonnelaan*, you will find an intercom. Please ring the bell and kindly inform them that you are a participant of the MOSA Conference. Parking will be provided free of charge. Space is limited, so please arrive early to secure a spot. From the parking lot, walk towards Universiteitssingel 40. This is the entrance to the conference (approximately a 10-minute walk). See the additional instructions below for a detailed map or directions.

Registration

Please note that we kindly ask Hasselt University and external participants to report to the registration desk at the main entrance of UNS40, and Maastricht University participants at Onderwijsplein. Registration is mandatory.

10. Organizing team

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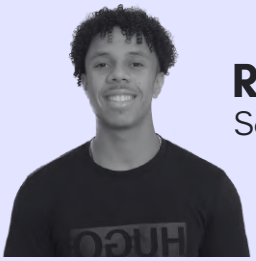

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11. Abstracts
Oral Presentation Session 1

Embracing The Wide Range of Biomedical Research: Talks About Cardiovascular Disease, Enteric Nervous System, and Tissue Engineering

1. Mohammadhossein Izadloo, Universidade Católica Portuguesa
2. Laurien Veulemans, Hasselt University
3. Anneleen Jacobs, Hasselt University
4. Greta Cocchi, Maastricht University
5. Kobe Neven, Hasselt University
6. Jorunn Vranken, Hasselt University

Endothelial Cell Volume in Arteriovenous Malformations: Everolimus and Growth Factor Effects

Mohammadhossein Izadloo1, Cláudio Franco[2], Ana Figueiredo[2]

1Universidade Católica Portuguesa, Católica Medical School, Lisbon, Portugal
[2]Universidade Católica Portuguesa, Católica Medical School, Católica Biomedical Research Centre, Lisbon, Portugal

Background: Arteriovenous Malformations (AVMs) are abnormal shunts between artery and veins without an intervening capillary bed. Among different promoting factors hypertrophic venous cells have been identified as an essential initiating factor for shunt formation. Pharmacological interventions such as mTOR inhibitors and changes in Growth factor have shown to have a significant effect on AV shunts formation through inhibition of pathological endothelial cells (EC) volume expansion. This study aims at demonstrating the effect of Everolimus as an mTOR inhibitor and the comparison between different growth factor concentrations on Human umbilical vein endothelial cells (HUVEC) volume.

Methods: The methods involved in studying endothelial cell volume under varied conditions began with culturing human umbilical vein endothelial cells (HUVECs) until confluence in flasks. This protocol ensured accurate examination of endothelial cell volume under different conditions, including normal growth condition, reduced FBS 0.1%, FBS starvation, and a control group containing EtOH. On Day 3, cells were treated with Everolimus diluted in EtOH at concentration of 0.5μM and fixed with paraformaldehyde (PFA). Immunostaining followed, involving primary antibody incubation with VE-Cadherin Goat antibody and secondary antibody incubation with Alexa Fluor 555 Donkey anti-Goat. The samples were then analyzed under confocal laser scanning microscopy and the images were quantified via Imaris 9.8 program.

Results: The ECs among the three control and treatment groups with the given conditions: normal growth medium, 0.1% FBS and 0% FBS had significant differences in their volume and area ($P<0.05$). However, the treatment group with Everolimus 0.5μM and control group within the conditions of 0.1% FBS and 0% FBS show no significant difference in T-test analysis of variances. (P:0.3, P:0.4). Therefore, the effect of Everolimus treatment on ECs volume at the given concentration was statistically insignificant.

Conclusion: While differences in endothelial cell (EC) volume were noted across conditions, Everolimus treatment at 0.5μM concentration yielded no significant effects. Future research should focus on optimizing experimental parameters, including reducing cell numbers for easier quantification, comparing ethanol-treated and untreated groups, and conducting dose-response experiments to determine the optimal Everolimus concentration range. These refinements will enhance the precision and clinical relevance of pharmacological interventions for arteriovenous malformations (AVMs).

Serratus Anterior Plane Block: High vs. Low Dose for Post-Minimally Invasive Heart Valve Surgery Pain

Laurien Veulemans1,[2], Ina Callebaut1,[2], Laurien Geebelen[2], Alaadin Yilmaz[3], Jeroen Vandenbrande[2] and Björn Stessel1,[2]

1Faculty of Medicine and Life Sciences, Hasselt University, Campus Diepenbeek, Agoralaan Gebouw D - 3590 Diepenbeek
[2]Department of Anesthesia and Intensive Care Medicine, Jessa Hospital, Stadsomvaart 11, 3500 Hasselt, Belgium
[3]Department of Cardiothoracic Surgery, Jessa Hospital, Stadsomvaart 11, 3500 Hasselt, Belgium

Background. A prior study demonstrated a 40% decrease in opioid consumption and pain following heart valve surgery with the administration of a local anaesthetic via serratus anterior plane block. Yet, evidence on the optimal dosage to align with enhanced recovery is scarce. The current study evaluated the efficacy of a higher dose of the local anaesthetic administered via the serratus anterior plane block in managing postoperative pain following minimally invasive heart valve surgery.

Methods. This double-blinded randomized controlled superiority trial enrolled patients undergoing total endoscopic aortic or mitral valve surgery via the right anterolateral thoracotomy. Participants were randomly assigned in a 1:1 ratio to either a high-dose bupivacaine (local anaesthetic) + epinephrine serratus anterior plane block intervention group or a low-dose bupivacaine serratus anterior plane block control group. Both groups received a patient-controlled intravenous analgesia system with morphine and standard care. The primary outcome measure was cumulative morphine consumption during the first 24 postoperative hours. Secondary outcomes comprised pain scores at 4, 8, 12, and 24 hours post-block placement, as well as on the 7th day, along with opioid-related side effects and length of hospital stay. We hypothesized a 25% reduction in opioid consumption among participants randomized to the high-dose group compared to those in the low-dose group.

Results. Seven patients were analyzed (n=4 in group A, n=3 in group B). Morphine consumption in group A was higher (24.36±22.49) compared to group B (8.00±9.17). Nevertheless, no significant difference (p=0.30)between the groups was observed. Still, no significant differences were seen in opioid consumption between the two groups when only considering aortic (p=0.58) or mitral (p=1.00) valve surgery. In addition, pain scores in rest were lower in group B at 7 days after block placement, while pain scores on deep respiration were never lower in group B compared to group A. Nonetheless, none of the differences were significant. Next, extubation time was significantly higher in group B (234.67±120.02 min) compared to group A (71.50±32.03 min) (p=0.04). Other outcomes, such as length of stay and opioid-related side effects, were similar between both groups.

Conclusion. Given the double-blinded design of this ongoing study, the group assignments remain undisclosed. However, no significant differences were observed in cumulative morphine consumption or pain scores. These findings suggest comparable efficacy between higher and lower serratus anterior plane block doses. Notably, the ongoing nature of the study, and therefore the limited sample size, preclude definitive conclusions.

Fabrication of strong and tough hydrogel networks processable for tissue engineering.

Anneleen Jacobs1, Marianna Arreguin Campos1, Louis Pitet1

1Advanced functional polymer group, Institute for Materials Research, Universiteit Hasselt, Campus Diepenbeek, Agoralaan Gebouw D - B-3590 Diepenbeek

Background. Hydrogels are polymeric materials that have been studied extensively due to their hydrophilic nature. Conventionally, they comprise a single network, resulting in a soft and elastic material ideal for application in wound patches, contact lenses, and drug delivery systems. However, due to their brittleness and lack of strength, they are not ideal for mechanically demanding applications, such as bone and cartilage scaffolds. Hence, other methods have been studied, like combining multiple networks to improve the mechanical characteristics. However, these hydrogels are made via a multi-step process, which does not allow the processing of the materials and, in turn, limits patient-tailored applications.

Methods. Hydrogels of poly(ethylene glycol) dimethacrylate combined with gelatine methacrylate, alginate, and alginate methacrylate were fabricated to improve the intrinsic properties of hydrogels. Their characteristics were determined by mechanical, swelling, and biocompatibility testing using chondrocytes. Based on these results, one composition was selected for embedded 3D printing to process the hydrogels to facilitate patient-tailored applications.

Results. We developed three systems of hydrogels using two networks that employ different types of chemistry to facilitate a one-step synthesis. The first system was based on poly(ethylene glycol) dimethacrylate, which uses covalent bonding, and alginate, which uses ionic bonding. They showed no difference under compression but seemed tougher under tensile conditions, which could be related to the two different crosslinking mechanisms causing a lower crosslink density. The second system of poly(ethylene glycol) dimethacrylate and gelatine methacrylate utilized covalent bonding for both networks, connecting them together. Due to their higher crosslink density, they showed improved properties under compression but less prominent improvements under tensile conditions. Poly(ethylene glycol) dimethacrylate and alginate methacrylate formed the third system, and the second network combined covalent and ionic bonding for the second network. Due to this, this system showed a much stronger network under compression. Embedded 3D printing of the first system with poly(ethylene glycol) dimethacrylate compositions was used to prove the processability of the systems.

Conclusion. This study aimed to combine poly(ethylene glycol) dimethacrylate with alginate, alginate methacrylate, and gelatine methacrylate to establish higher mechanical properties and facilitate the processing of hydrogels. We showed that the different systems had good mechanical properties and proved the possibility of processing the mixtures with embedded 3D printing.

Sound-based assembly of a spatially organised neurovascular network

Greta Cocchi[1,2], Riccardo Tognato[1], Tiziano Serra[1,2]

[1]AO Research Institute Davos, Davos, Switzerland
[2]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands

Background. Chronic low back pain imposes a significant economic and social burden, both for patients and EU. With intervertebral disc disruption contributing to pain in nearly half of low back pain cases, understanding the pathophysiology of discogenic pain is crucial. The intervertebral disc is a cartilaginous structure located between two vertebrae. Normally, it doesn't have nerves and vessels in its deeper region, whereas deep ingrowth of nociceptive nerve fibres and vessels are observed in painful intervertebral discs. The ingrowth of nociceptive nerve fibres and vessels in painful intervertebral discs remains poorly understood. To address this, our research combines the sound-based bioassembly approach with the innovative synthesis of cell-loaded gelatin beads to spatially organise endothelial and mesenchymal stem cells, prior to random seeding of dorsal root ganglion cells, in fibrin hydrogel. This concept promotes the creation of tube-like structures and thus a 3D in vitro neurovascular model.

Methods. Gelatin microbeads (150 mg/mL) for cell encapsulation were obtained by emulsion process in dextran solution (200 mg/mL) and consequently tested for biocompatibility using the Live/dead assay. For the fibrin gel, fibrinogen and thrombin solutions were prepared and mixed at different concentrations. The obtained hydrogels were tested using the rheometer to define the define the gelation kinetics and find the optimal concentration for the sound-based assembly approach. Cell-loaded gelatin beads were consequently dispersed in the fibrin solution and spatially organized in concentric circles through sound-based bioassembly. Vascular network formation was assessed via VE-cadherin staining and neuronal ingrowth of the randomly seeded neuronal cells was observed through CGRP/NF staining and calcium imaging.

Results. Gelatin microparticles demonstrated a high rate of encapsulation efficiency (90%) and promising biocompatibility results immediately after the encapsulation process and over 5 days of cell culture. Concentrations of fibrinogen and thrombin, respectively of 5 mg/mL and 0.5 IU/mL was proved to create a fibrin solution with suitable gelation kinetics for the sound-based bioassembly approach. Additionally, after bioassembly and 5 days of culture of the model, tight junctions were present between the patterned endothelial and mesenchymal stem cells, and it nerve growth was observed to follow the pattern of the newly obtained vascular network.

Conclusion. Overall, this could be a suitable method to generate spatially orchestrated porous constructs, opening the way to create reproducible, shape-defined multicellular systems for biological modelling. In this specific case, the model holds great promise for researchers and institutes studying discogenic pain and other pathophysiological conditions involving neurovascular growth.

The impact of microbiota on gut function and behavior in a mouse model for psychiatric disorders.

Kobe Neven1, Kinga Réka Tasnády1,2, Alessio Cardilli3, Ibrahim Hamad3, Amy Marie Holland1,2, Reindert Jehoul1, Marion Gijbels2, Markus Kleinewietfeld3, Akira Sawa4, Bert Brône1, Veerle Melotte2,5, Werend Boesmans1,2

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2Department of Pathology, GROW-School for Oncology and Reproduction, Maastricht University Medical Centre, Maastricht, The Netherlands
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4Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, US
5Department of Clinical Genetics, Erasmus University Medical Centre, Rotterdam, The Netherlands

Background: Neurodevelopmental disorders such as schizophrenia, major depressive, bipolar, and autism spectrum disorders are often accompanied by gastrointestinal (GI) comorbidities. However, the precise mechanisms underlying GI symptomatology in psychiatric disorders remain elusive. Aberrant expression of Disrupted in Schizophrenia 1 (DISC1), a hub and scaffold protein which plays a key role in neural maturation and connectivity, serves as a critical risk factor for several psychiatric conditions. We aim to elucidate whether DISC1 perturbation affects gut function, microbiome composition, and how these relate to behavior.

Methods: DISC1 expression and gut function were compared between adult DISC1 locus impaired (LI) mice and wild type (WT) littermates. Intestinal microbiota composition was profiled using 16S ribosomal RNA gene amplicon sequencing. Fecal microbiota transplantation (FMT) was carried out to investigate the role of microbiota in GI function and behavior (marble burying, nest building and shredding, Y-maze alternation, and object-location tests (OLT)).

Results: Knock-down of DISC1 in the enteric nervous system of DISC1 LI mice was confirmed by RNAscope. While small intestinal transit and colonic bead propulsion was not affected, naïve DISC1 LI mice displayed faster whole gut transit. In addition, DISC1 LI mice exhibited diminished wet stool weight. Intestinal microbiome analysis revealed enrichment of Bacteroides, and reduced levels of Muribaculaceae and Clostridia in DISC1 LI mice. Interestingly, FMT reduced whole gut transit time in DISC1 LI mice. Instinctive and repetitive behavior, as assessed by the marble burying, nest building, and shredding tests was not affected. Nevertheless, and although, a general spatial working memory test (Y-maze) did not reveal differences between the LI-FMT group and their controls, significant improvement of cognitive capacity was found with a hippocampus-dependent spatial working memory test (OLT).

Conclusion: Our data indicate that DISC1 disruption alters gut function and induces intestinal dysbiosis in naïve animals. The impact of FMT on GI transit and hippocampus-dependent spatial working memory emphasizes the crucial role of host-microbiome interactions and the gut-brain axis in psychiatric disorders, offering promising avenues for future treatment modalities and research.

miR-146a-5p Regulation of Enteric Glial Cell Status in Gastrointestinal Health and Disease

Jorunn Vranken1*, Amy Marie Holland1,2, Fränze Progatzky3,4, Reindert Jehoul1, Stefan Boeing3,5, Vassilis Pachnis3, Veerle Melotte2,6, Werend Boesmans1,2

1Biomedical Research Institute (BIOMED), Hasselt University, Diepenbeek, Belgium
2GROW-School for Oncology and Reproduction, Maastricht University Medical Center, Maastricht University, Maastricht, The Netherlands
3Development and Homeostasis of the Nervous System Laboratory, Francis Crick Institute, London, UK
4Kennedy Institute of Rheumatology, Oxford, UK
5Bioinformatics & Biostatistics STP Francis Crick Institute, London, UK
6Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

Background. Enteric glia, the glial cells of the enteric nervous system (ENS), are crucial for maintaining gastrointestinal (GI) homeostasis by supporting enteric neuron function, regulating mucosal integrity, and interacting with the microbiome and immune system. Their phenotype is niche-specific and largely determined by micro-environmental cues. In response to injury and intestinal inflammation, enteric glia acquire a reactive phenotype, potentially contributing to GI pathophysiology. Preliminary data indicates that distinct miRNA signatures mark enteric glial identity and suggests the involvement of miR-146a-5p in establishing enteric glial reactivity. Nevertheless, further investigation is required to elucidate the precise role of this miRNA in enteric glia in GI health and disease.

Methods. To discern the role of miR-146a-5p in enteric glial reactivity, we characterized an in vitro model of reactive enteric glia induced by treating primary mouse enteric glia with lipopolysaccharide and interferon-gamma. Transfection of enteric glia was achieved using magnetofection. Through in silico analyses, we investigated the underlying targets and pathways implicated in the association between miR-146a-5p and enteric glial reactivity. To assess the influence of microbiota on intestinal miR-146a-5p expression, various mouse models hoting a specific microbiome were employed. A conditional enteric glia-specific miR-146 knockout model will be used for in vivo analyses.

Results. Reactive enteric glia exhibit increased proliferation ($p<0.0001$), acquire a proinflammatory phenotype ($p<0.01$), and undergo morphological alterations in vitro. Importantly, among several microRNAs, only miR-146a-5p showed significant upregulation ($p<0.05$). Using magnetofection with a pmaxGFP plasmid, we achieved the first successful transfection of enteric glia. Ongoing experiments using a miR-146a-5p inhibitor (miR-146a-5p miRCURY LNA miRNA Inhibitor) will elucidate whether miR-146a-5p is instructive for inducing enteric glial reactivity. In silico analyses suggest an essential role for miR-146a-5p via several target genes, including TRAF6, in enteric glial activity, GI disorders, and host-microbiome interactions. The latter was validated by our findings, revealing a significant downregulation of miR-146a-5p in the gut of germ-free mice ($p<0.001$), contrasting with a significant upregulation in the gut of wildling mice ($p<0.01$). Current in vivo studies utilizing Sox10-CreERT2;R26tdT;miR146flox/flox mice will investigate the impact of miR-146a-5p on GI homeostasis by assessing ENS structure and function.

Conclusion. Together, our findings indicate a role for miR-146a-5p in the reactivity of enteric glial cells, suggest its involvement in GI pathophysiology, and demonstrate that miR-146a-5p expression is affected by intestinal microbiota. Moving forward, we anticipate validation of the impact of miR-146a-5p on enteric glial status and GI health.

12. Abstracts
Oral Presentation Session 2

Advances in Pediatric Care, Anti-Immuno-Aging, Acne Treatment, and HBV

1. Aleksandra Dembowska, Medical University of Lubin
2. Wiktoria Stańska, Medical University of Warsaw
3. Maciej Dubaj, Medical University of Lubin
4. Isabel Bougie, Erasmus University Medical center
5. Mariken Lemmens, Hasselt University
6. Emerance Ishimwe, Hasselt University

Hyperleukocytosis in paediatric patients with acute lymphoblastic leukaemia - a single center experience

Aleksandra Dembowska1, Maciej Dubaj1, Małgorzata Mitura-Lesiuk2

1 Student Student Scientific Society of the Department of Pediatric Hematology, Oncology and Transplantology, Medical University of Lublin, Lublin, Poland
2 Department of Pediatric Hematology, Oncology and Transplantology, Medical University of Lublin, Lublin, Poland

Background. Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer with a frequency rate of 4.32/100,000 paediatric patients each year. Hyperleukocytosis is defined as a white blood cells (WBC) count >50 G/L and is found in 10.2-19.2% of patients with ALL. The condition is associated with an increased risk of leukostasis and secondary: intracranial bleeding, coagulopathy, renal failure and tumor lysis syndrome. It is also believed to significantly worsen the prognosis of patients with ALL. The aim of the following study was to present the demographic characteristics of patients with hyperleukocytosis and the association between WBC count and demographic and clinical features in this group.

Methods. We retrospectively analyzed the medical histories of paediatric patients treated for ALL at the Department of Paediatric Hematology, Oncology and Transplantation in Lublin from 2017 to 2024. Statistical analysis was performed using MedCalc 15.8 software. Continuous variables were compared using Student's t-test (variables with normal distribution) or Mann-Whitney U-test (variables with distribution different from normal). Categorical variables were compared using Pearson's chi-square test. The threshold for statistical significance was set at $p<0.05$.

Results. During the study period, 97 children with ALL were diagnosed and treated at the Clinic. Among them, the majority were girls (52.6%), and the median age at diagnosis was 78 (IQR: 45-143) months. Hyperleukocytosis was found in 10 patients (10.3%). Among them, there were more males (90% vs. 10%; p=0.012) and patients with T-cell ALL (T-ALL)(60% vs. 18.4%; p=0.01). This group was significantly older (119 vs. 76 months; p=00.11) and had a higher mortality rate than patients without hyperleukocytosis (30% vs. 4.6%; p=0.0217). The group of patients with hyperleukocytosis had a significantly higher incidence of petechiae at diagnosis with higher WBC count (290.76 vs. 58.21; p=0.0167). In addition, there was a significant positive correlation between WBC count and LDH level (r=0.798; p=0.0057) and a negative correlation with platelets count (r=-0.661; p=0.0376). A higher incidence of infection as a complication of treatment was also observed with higher WBC counts (290.76 vs. 58.21; p=0.0167).

Conclusion. Hyperleukocytosis accompanies a small percentage of paediatric patients with ALL, but has a higher mortality rate. Patients with hyperleukocytosis are mainly older boys with T-ALL. They have a higher incidence of petechiae at diagnosis and infections during therapy. WBC count correlates positively with LDH levels and negatively with platelet count.

Animal milk oligosaccharides inhibit the biofilm of Cutibacterium acnes - a preliminary study."

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Background. Acne is a global problem, and although associated with adolescence, 12-41% of adults suffer from that life quality-decreasing condition. Although its pathogenesis is not fully understood, recent studies emphasised the key role of a biofilm of Cutibacterium acnes, increasing the virulence of the bacteria and resulting in treatment failure. Therefore, novel antibiofilm substances are being sought. Animal Milk Oligosaccharides (AMOs) may show potential antimicrobial and antibiofilm activity against Gram-positive bacteria. To the best of our knowledge, our study is the first to determine if mare's milk oligosaccharides affect the inhibition of Cutibacterium acnes.

Methods. Reference laboratory strain Cutibacterium acnes ATCC 11827 (American Type Culture Collection) was used in the experiment. AMOs probes came from the collection of the Department of Medical Biology, Medical University of Warsaw. The inhibition of the biofilm formation of the C. acnes was determined after 3-day BHI (Brain heart infusion) cultures diluted to 0.5 McFarland and adjusted to 106 CFU/mL (colony-forming units/mL). The isolates grew in 96-well plates in BHI broth in anaerobic conditions. The ability of AMOs to inhibit the C. acnes strain was provided by a quantitative method using 10-fold serially diluted AMOs and calculating CFU/mL. The 1:2 diluted AMOs in BHI broth, concentrations of the AMOs (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78, 0.354, 0,1529 mg/mL), lactose and BHI broth controls were examined. Each experiment was independently performed in triplicate. We estimated the oligosaccharides' Minimum Biofilm Inhibitory Concentration (MBIC) against C. acnes growth.

Results. In this preliminary study, the characterised mare's milk oligosaccharides showed good potential for antimicrobial activity with MBIC ranging from 0.78 mg/mL to 50 mg/mL. On average, they could inhibit C. acnes biofilm formation from 11% to even 100% in high concentrations. No inhibition effect was observed at lower concentrations of the AMOs. The lactose did not affect the experiment.

Conclusion. Our primary study first demonstrated that mare's milk oligosaccharides might have the potential to be effective against Cutibacterium acnes. The protective properties of the biofilm matrix increase antibiotic resistance, leading to treatment failure and recurring infections. Therefore, finding new antimicrobial agents of natural origin is a promising, cost-effective solution that will facilitate proper future therapeutic strategies and limit antibiotic use.

When you hear hoofbeats, think of horses, but also of zebras. Paraneoplastic syndromes in paediatric oncology

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Background. Paraneoplastic syndromes (PNS) are symptoms or set of symptoms caused by an oncological disease, but not by direct infiltration or metastasis. Their cause is the secretion of hormones or peptides by tumor cells or a cross immune reaction. They occur in 7-15% of adult oncology patients, while the exact statistics in children are unknown. Based on clinical observations, it is known only that they occur far less frequently. However, they are an extremely important diagnostic suggestion. Early detection of cancer enables its early treatment, which determines therapeutic success. The aim of the following paper was to describe the most important PNS in paediatric patients.

Methods. A systematic review was conducted by PubMed, Scopus and Google Scholar databases. Due to the very small number of papers, it was decided not to indicate a time and type criterion. Fifteen original studies, review papers and case reports from 1990-2024 were analyzed.

Results. PNS are estimated to occur in about 1% of children with oncological diseases. The best described are neurological PNS, for which dedicated paediatric recommendations have been created. Most cases have been associated with Hodgkin's lymphoma (HL) (mostly adolescents aged 15-19) and neuroblastoma. Interestingly, only about 70% of the syndromes were classified as certain, while 25% - as probable. About 2-7% of patients with neuroblastoma were accompanied by opsoclonus-myoclonus syndrome (OMS), which accounted for up to 62% of all neurological PNS. Patients with HL had mostly limbic encephalitis or anti-N-methyl-D-aspartate receptor encephalitis. The above cases have been associated with onconeural antibodies (anti-mGluR5, anti-Tr or anti-NMDA), although these are not sensitive or specific enough as a diagnostic tests. Skin symptoms like pruritus, pemphigus and alopecia were also characteristic for HL patients. They preceded the oncological diagnosis by up to 2-5 years. Patients also had gastroenterological syndromes (cholestasis and vanishing bile duct syndrome in 1-3% of patients with HL and neuroblastoma), hematological syndromes (thrombocytopenia and hemolytic anemia in patients with HL, acquired von Willebrand syndrome in 8% of Wilms tumor patients), hormonal disturbances (hypercalcemia in 0.7% of rhabdomyosarcoma cases, precocious puberty, hypertension in 25% of Wilms tumor cases, Cushing's syndrome) and renal ones (nephrotic syndrome in 1% of HL patients).

Conclusion. PNS are extremely rare in paediatric oncological patients. However, any suspicious symptoms that do not respond to standard treatment should trigger an oncological diagnostic path.

Human in vitro models to study the role of pulmonary neuroendocrine cell hyperplasia in the pathophysiology of congenital diaphragmatic hernia

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Background. Pulmonary neuroendocrine cells (PNECs) are a rare cell type, forming up to one percent in the airway epithelium. PNECs are important airway sensors that secrete neuropeptides to regulate pulmonary lung responses. Previously, it was shown that pediatric lung diseases, such as congenital diaphragmatic hernia (CDH), are associated with an increased number of PNECs. Other studies showed that hyperplasia of PNECs may cause changes in the composition of the airway epithelium and cause vascular leakage. Increased understanding of the effects of PNEC secretory neuropeptides will help unraveling the mechanisms behind the role of PNEC hyperplasia in CDH. We hypothesize that altered secretion of neuropeptides by PNECs affects surrounding epithelial, endothelial, and mesenchymal cells, thereby contributing to the pathophysiology of CDH. We aim to optimize in vitro culture models with primary human lung cells to examine the effect of hypersecretion of PNEC related neuropeptides.

Methods. To study the effect of PNEC-related neuropeptides on airway cells, we used human- primary bronchial epithelial cells (hPBECs) at air-liquid interface (ALI) and organoid models. Further, we optimized co-cultures of human endothelial and mesenchymal cells to study the lung vasculature. Retinoic acid (RA) antagonist BMS493 was used to mimic a CDH phenotype in hPBEC cultures as it represses RA induced epithelial differentiation. PNEC related neuropeptides calcitonin gene-related peptide, serotonin, gastrin releasing peptide, gamma-aminobutyric acid, and calcitonin were added to cultures to study the effect of PNEC hypersecretion on epithelial cells.

Results. We detected low numbers of hPNECs in culture by immunofluorescence. Treatment of hPBECs with CGRP and GABA led to increased numbers of goblet and ciliated cells. BMS493 treatment negatively affected differentiation of hPBECs, but interestingly, exposure to neuropeptides seemed to rescue this differentiation defect, suggesting a protective effect of neuropeptides on airway differentiation. Moreover, in mesenchymal and epithelial cultures exposed to PNEC-related neuropeptides, we detected changes in downstream target gene expressions related to CDH. Additionally, we found that human-derived endothelial and pericyte cells cultured in a gel of fibrinogen and thrombin resulted in a sprouting in vitro vasculature network.

Conclusion. PNEC secretory neuropeptides seem to have a protective role in our human in vitro CDH model. In parallel, an in vitro vasculature network model was established that provides the opportunity to study the effect of PNEC hypersecretion on lung vasculature. Furthermore, optimization of our CDH and vasculature models would in the future add to the translation of human in vitro models to new therapies.

Exercise: a powerful tool to counteract immune ageing via IL-15-induced autophagy

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Background. Immune ageing is the gradual decline of the immune system with age. This dysfunction of the immune system, also referred to as immunosenescence, leads to the inefficientive clearance of senescent somatic cells which naturally occur with ageing, exarcebating inflammageing. Consequently, elderly people are more prone to infections and malignancies and age-related diseases continue to rise, which pose major challenges to healthcare systems. Regular exercise is shown to positively influence the immune system's function, however little is known about the underlying mechanisms. Here we propose interleukin (IL)-15, a protein produced by skeletal muscle tissue, as a potential tool to stimulate the immune function via increased T cell autophagy.

Methods. PBMCs of young (25 years old) and old (67 years old) healthy male donors were treated with recombinant human IL-15 for 3 days. Autophagy markers LC3 and P62 were measured by immunocytochemical staining and western blot. Furthermore, young (2 months old) and aged (15-24 months old) male and female mice were assigned to a sedentary or acute exercise group. IL-15 gene expression and protein levels were measured by qPCR and immunohistological stainings. LC3 puncta were counted by immunocytochemical staining and flow cytometry was used to reveal IL-15 binding to immune cells. Lastly, elderly participants (65-85 years old) were assigned to a sedentary or acute exercise group. IL-15 protein levels were measured using immunohistological stainings.

Results. We expect the capability of IL-15 to boosts autophagy to be lower in aged T cells than in healthy young T cells, however autophagy is still presumed to be increased compared to untreated cells. In mice, our results indicate that female mice have increased IL-15 mRNA levels. However, no exercise effect was measured. On protein level, we expect IL-15 to be increased after exercise. It is also suspected that exercise will increase the amount of LC3 positive puncta in T cells and the binding of IL-15 to these cells. Similarly to mice, human participants are expected to have increased IL-15 protein levels after exercise.

Conclusion. Altogether, the present study aims to reveal the potential differences in IL-15-induced autophagy in PBMCs of donors of different age. Furthermore, the data suggest that biological sex influences IL-15 expression in muscle tissue. Overall, the goal of this study is to unravel whether exercise increases IL-15 levels and whether there is a link with increased autophagy. Ultimately, this may offer the potential to reduce age-related diseases arising from immune ageing.

Hepatitis B virus core protein as an antiviral target for functional cure of chronic hepatitis B

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Background. Globally, 296 million people suffer from chronic hepatitis B (CHB), caused by the hepatitis B virus (HBV). Despite the availability of a preventative vaccine, CHB remains the dominant cause of liver disease, including liver cirrhosis and hepatocellular carcinoma. Current antiviral therapies effectively suppress HBV DNA replication; however, they rarely lead to a cure, and consequently, 820,000 individuals die from CHB annually.
HBV infects human hepatocytes and subsequently injects its genome into the nucleus, establishing covalently closed circular DNA (cccDNA) that inherently serves as a template for the production of key HBV proteins. Current therapies do not efficiently eliminate HBV cccDNA, the key contributor to HBV persistence. The HBV core protein (HBc) self-assembles, forming the nucleocapsid that packages viral RNA, yielding newly formed viral particles. HBc is essential in almost every step of the HBV life cycle, making it an attractive target for the development of novel antiviral therapies aimed at finding a functional cure for CHB. Capsid assembly modulators (CAMs) bind HBc dimers, disrupting the correct process of nucleocapsid assembly (HBV RNA encapsidation) and disassembly (HBV nuclear entry). Potent CAMs capable of reducing almost all HBV parameters in vitro have been reported. Moreover, we recently reported on ALG-000184, the first CAM to reduce circulating hepatitis B surface antigen (HBsAg) levels in CHB patients.

Methods. The antiviral activity of various CAMs on HBV DNA was determined in HepG2-NTCP cells using qPCR, and the effect on HBV RNA was determined using RT-qPCR. CAM's inhibitory activity on HBsAg and HBeAg was assessed using HBsAg and HBeAg chemiluminescence immunoassays (CLIA).

Results. Several tested compounds effectively reduced intracellular HBV DNA and HBV RNA in HepG2-NTCP cells. Furthermore, CAM treatment significantly reduced extracellular HBsAg and HBeAg, complementary to the reductions observed in HBV DNA. These results were verified by comparing the EC50 ratios observed between the reductions in HBV DNA and HBsAg, and the ratios were variable. Interestingly, several CAMs showed biphasic curves for HBsAg.

Conclusion. The antiviral activity of CAMs, particularly in reducing HBsAg, depends on distinct features of the CAM compound. By identifying CAMs with increased potency of inhibiting HBV nuclear entry, we develop compounds that indirectly inhibit HBV cccDNA and find a functional cure for CHB.

13. Abstracts Poster Walks 1 - 6

Poster Walk 1

1. Jill Grondelaers, UHasselt
2. Michèlle Hendriks, UHasselt
3. Ana Neves da Fonseca, Maastricht University
4. Emmee Stevelmans, Maastricht University
5. Charlotte Peetersem, UHasselt

Poster Walk 2

1. Jonas Schimmel, UHasselt
2. Inês Santos, Maastricht University - *Abstract could not be published*
3. Amber Theunissen, UHasselt
4. Merel Hufkens, UHasselt
5. Eline Simons, UHasselt

Poster Walk 3

1. Anton Brosens, UHasselt
2. Renée Rita Moonen, UHasselt
3. Zoë Donders, UHasselt
4. Josephine-Elisabeth Pippi, Maastricht University
5. Eva Hensenne, Maastricht University

Poster Walk 4

1. Lisa Steegen, UHasselt
2. Niki Sciarrino, UHasselt
3. Dario Geebelen, UHasselt
4. Cobi Bergsma, Maastricht University
5. Anouar Dlalat, UHasselt

Poster Walk 5

1. Hanne Coenen, UHasselt
2. Elisa Simone, UHasselt
3. Quinn Croughs, UHasselt
4. Amber Delbroek, UHasselt
5. Lien Van Otterdijk, UHasselt - *Abstract could not be published*

Poster Walk 6

1. Bayram Semih, UHasselt
2. Jenny Surholt, Maastricht University
3. Luisa Wensky, Maastricht University
4. Kirsten Poelmans, UHasselt
5. Jule Richartz, UHasselt

Fluoxetine's Anti-inflammatory Potential Hinges on Lipid Metabolism Integrity

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Background. Depression affects nearly 300 million people worldwide, of which one in three develops resistance to pharmacological antidepressants, indicating a need for more effective treatments. Strikingly, many of these patients show elevated levels of pro-inflammatory cytokines in their blood and cerebrospinal fluid, suggesting a potential link between inflammation and pharmacological antidepressant resistance. Previous research showed that lipids play a pivotal role in inflammation, especially in macrophages, which are important immune cells. Therefore, this study assessed if fluoxetine, a commonly used antidepressant, can modulate lipid metabolism in macrophages and whether alterations in lipid metabolism may influence the efficacy of fluoxetine.

Methods. Both human peripheral monocyte-derived macrophages (MoDMs) and murine bone marrow-derived macrophages (BMDMs) were utilized to assess alterations in gene expression levels of inflammatory genes and lipid-related genes after fluoxetine treatment using qPCR. Lipid accumulation in MoDMs and BMDMs was assessed by quantification of the Oil red O staining. Furthermore, deletion of the low-density-lipoprotein receptor (Ldlr -/-), as well as the introduction of oxidized 1-palmitoyl-2-arachidonyl-sn-3-glycerophosphorylcholine (oxPAPC), were employed as models to mimic disruptions in lipid metabolism in MoDMs and BMDMs.

Results. Fluoxetine treatment changed gene expression related to cholesterol and fatty acid metabolism in both MoDMs and BMDMs. Specifically, it altered the gene expression of lipoprotein lipase, ATP binding cassette A1, and lysosomal acid lipase. In addition, fluoxetine increased lipid accumulation in both MoDMs and BMDMs. Furthermore, disruptions in lipid homeostasis, represented by oxPAPC exposure and the Ldlr -/- model, attenuated the anti-inflammatory effects of fluoxetine.

Conclusion. These results imply that fluoxetine alters cholesterol and fatty acid metabolism and causes lipid accumulation in MoDMs and BMDMs. In addition, maintaining lipid homeostasis is crucial for fluoxetine's efficacy. These insights highlight the importance of nutrition and patient stratification in reducing resistance to fluoxetine treatment.

Tackling Cancer Cachexia in Colorectal Cancer Patients by Unraveling Skeletal Muscle Characteristics

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Background – Colorectal cancer (CRC) poses significant global health challenges, ranking as the second-leading cause of cancer-related mortality worldwide. Alarmingly, its incidence is projected to rise substantially by 2040, necessitating urgent intervention strategies. A significant complication of progressive CRC is cancer cachexia (CC), characterized by weight loss due to skeletal muscle and adipose tissue atrophy. This wasting syndrome not only diminishes the quality of life but also disrupts anti-CRC chemotherapy efficacies, thereby exacerbating treatment toxicity and reducing clinical outcomes. Yet, understanding CC mechanisms in humans remains elusive, limiting treatment efforts and increasing mortality. This study aims to elucidate skeletal muscle characteristics in CRC-cachexia patients, focusing on muscle fiber composition, capillary density, myonuclei quantity, and mitochondrial dynamics to unravel novel treatment approaches to preserve muscle mass and function in this population.

Methods – Up till now, 7 cachectic CRC patients, 20 non-cachectic CRC patients, and 12 healthy controls(HCs) were recruited. Fine-needle muscle biopsies from the m. erector spinae and the m. vastus lateralis were obtained. Skeletal muscle fiber cross-sectional area (CSA), fiber distribution, capillarization, and myonuclei quantity were assessed via immunohistochemistry. Western blot was performed to evaluate mitochondrial biogenesis (peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α), fusion (mitofusin 1 (MFN1) and optic atrophy 1 (OPA1)), and fission (fission protein 1 (FIS1), dynamin-related protein 1 (DRP1)) proteins.

Results – Muscle biopsy analysis revealed notable alterations in fiber type composition when comparing HCs with cachexia patients. Cachexia patients displayed significantly decreasing CSA of type II fibers compared to HCs and non-cachexia patients. Furthermore, both cachexia and non-cachexia patients exhibited a consistent pattern, characterized by a reduction in the proportion of type I fibers and an increase in the prevalence of type II fibers compared to HCs. Similarly, relative CSA corroborated these findings in both cachexia and non-cachexia patients. Additionally, Western blot analysis indicated potential changes in mitochondrial biogenesis, fusion, and fission proteins, although further optimization is required to detect significant alterations among cachexia patients, non-cachexia patients, and HCs.

Conclusion – Skeletal muscle analysis in CRC patients reveals potential cachexia markers, including smaller fibers indicative of muscle atrophy, alongside fewer slow-twitch oxidative (type I) fibers and elevated fast-twitch glycolytic (type II) fibers, correlating with reduced endurance and heightened fatigue susceptibility. These findings help develop interventions, like personalized exercise, nutrition, and psychological strategies to enhance specific fiber types and overall muscle mass. Incorporating these interventions into prehabilitation and rehabilitation programs holds promise for improving clinical outcomes and care for CRC patients.

Exploring the roles of ARID4B and BMPR2 in the progression of KMT2A-rearranged Acute Lymphoblastic Leukemia

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Background. KMT2A-rearranged acute lymphoblastic leukemia (KMT2A-r-ALL) is an aggressive form of ALL, which is characterized by a chromosome translocation between the KMT2A gene (chromosome 11) and a fusion partner such as AFF1 (chromosome 4). KMT2A translocations are found in 80% of infants (<1 year of age) diagnosed with ALL, leading to a poor prognosis and high relapse chance. Therefore, a better understanding of the disease mechanism is necessary. Recently, a CRISPR Cas9 library screening between KMT2A-r cell lines and KMT2A-Wt cell lines was performed, leading to the discover of two novel molecular targets: ARID4B and BMPR2. In this study, the roles of ARID4B and BMPR2 in KMT2A-r-ALL were explored.

Methods. To understand the influence of ARID4B and BMPR2 in the progression of KMT2A-r-ALL, ARID4B and BMPR2 knockdown (KD) cell lines were established. Three different shRNA construct targeting ARID4B and BMPR2 were transduced into SEM (KMT2A-r-ALL) and NALM6 (KMT2A-Wt-ALL), via lentiviral vector system. The vector that showed the highest efficiency by western blot (shARID4B) or qPCR (shBMPR2) were further used for experiments. Cellular viability was assessed via flow cytometry (FACS) with 7AAD staining. To identify possible downstream effects, gene expression assays (qPCR) were performed.

Results. In SEM cells, the shARID4B knockdown lead to decrease in proliferation as early as 72h exposure to DOX, and remarkably after 6 days the percentage of cell death reached 83% (affirmed by 7AAD staining). Whereas in NALM6, the shARID4B KD lead to only 30% cell death after 6 days. Further, KD of ARID4B on SEM, lead to an increase in expression of MBD3 (mean expression: 345%) when compared to the -DOX condition (construct not active). BMPR2 KD lead to 45% cell death after 6 days in SEM cells. Further, the mRNA level of BMPR2 decreased to 20% after 6 days, when compared to the non-silencing control.

Conclusion. The collected data reveals that ARID4B is essential for the proliferation/maintenance of KMT2A-r cells, while not notably impact KMT2A-Wt cells.

The glaucoma gambit: rescuing retinal ganglion cells from dysfunction

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Background. The optic neuropathy glaucoma is the second leading cause of vision impairment and blindness. Glaucoma is defined by damage to the retinal ganglion cells (RGCs). RGCs belong to the optic nerve, which is responsible for transmitting visual information to the brain. While treatments primarily target symptoms, they do not reverse RGC damage.
The mitochondria are one important factor involved in the death of RGCs by initiating the apoptotic cascade. Mitochondria can reverse damage in early stages of dysfunction. The RGCs also undergo a period of dysfunction before the irreversible cell death. Consequently, there could be a window of opportunity to rescue dying retinal ganglion cells during this period of dysfunction. Therefore, our study focuses on studying the recovering ability of RGCs.

Methods. A neuronal PC12 rat model was established to mimic the cell death via methanol exposure. The dosage and treatment duration of methanol to induce cell death have been determined. PC12 cells were treated with methanol and evaluated on cell death and neurite retraction. Followed by removing methanol and assessing the recovery potential of the cells. Additionally, the effect of nerve growth factors on the recovery was investigated.
Human retinal ganglion cells (hRGCs) were isolated from a 2D cell culture using embryonic stem cells. The hRGCs were exposed to similar methanol exposures, as described above. The effects of methanol treatment and the recovery potential were assessed using immunofluorescence and electron microscopy.

Results. Pc12 cells exposed to 3-7% methanol exhibited neurite retraction, a sign of cellular dysfunction. When removing methanol, the cells displayed neurite regrowth, suggesting recovery potential. After repeated exposures to methanol for several days, cells continued to exhibit neurite regrowth. The addition of nerve growth factors increased the neurite density and regrowth during recovery phases.
In contrast, hRGCs subjected to 3-7% methanol showed minimal to no cellular damage. Electron microscopy images revealed none to little mitochondrial damage. Immunofluorescence images showed no signs of cytochrome c release, suggesting the absence of mitochondrial-induced cell death.

Conclusion. Methanol exposure (3-7%) induced cellular dysfunction in PC12 cells. After methanol removal, dying PC12 cells were able to regrow neurites indicating a recovery potential after a period of dysfunction.
On the other hand, hRGCs displayed minimal susceptibility to methanol at 3-7%. Further research is needed on the efficacy of methanol to induce cell death in hRGCs. Hereby, optimizing the dosage and treatment duration of methanol to ensure cell death in hRGCs.

The triad between lipids, apolipoprotein E, and microglial activation in Alzheimer's disease explored

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Background. As the global population ages, neurodegenerative diseases like Alzheimer's disease (AD) form a growing health concern with significant societal and individual consequences. Despite substantial progress in understanding ADs etiology, there is still no cure available. AD is characterized by amyloid-β plaques (Aβ) (A), neurofibrillary tangles (T), and neurodegeneration (N), collectively known as the ATN pathology. Another important hallmark of AD is microgliosis, which is the persistent activation of microglia. The strongest genetic risk factor for developing late-onset AD is apolipoprotein E (ApoE)-4. Under physiological conditions, ApoE is involved in lipid metabolism, guiding lipid transportation and distribution through the brain. Furthermore, in AD, ApoE expression is increased in reactive and disease associated microglia, emphasizing its relevance in the disease. However, the precise mechanism by which ApoE affects AD is unclear. Therefore, this research investigates the triad between lipids, ApoE, and microglial activation in AD.

Methods. The neuronal cell line, SH-SY5Y, and microglial cell line, BV2 , were supplemented with an ApoE-related lipid in vitro. In vivo, AD was induced using genetically engineered 5xFAD mice, which were additionally subjected to a 3-month high-fat diet (HFD) to investigate the impact of lipids on disease progression, including ApoE expression. Brain tissues and cells were used for various immunohistochemistry and histological staining protocols.

Results. Lipid supplementation led to a reduction in cell viability in SH-SY5Y cells but did not affect viability in BV2 cells. Additionally, while supplementation with low lipid concentrations increased the intracellular neutral lipid load in BV2 cells, this lipid load decreased again with higher concentrations. Furthermore, the HFD is expected to alter pathological features slightly, including Aβ plaques and microgliosis, and to a modification in ApoE expression in 5xFAD mice.

Conclusion. The increasing incidence of neurodegenerative disorders such as AD, emphasizes the importance for better understanding its etiology and finding new therapeutical alternatives. Our study investigated the impact of lipid supplementation on SH-SY5Y and BV2 cells in vitro, as well as its effects on AD progression in vivo. The findings revealed differential responses to lipid supplementation between SH-SY5Y and BV2 cells, suggesting cell type-specific effects. Additionally, the HFD is expected to induce alterations in pathological features, including alterations in Aβ plaques, microgliosis and ApoE expression in 5xFAD mice. These results enhance our comprehension of AD, highlighting the potential role of lipids in AD pathophysiology. Furthermore, this triad could shed light on potential avenues for further exploration in AD therapeutics.

The influence of extracellular matrix cues on aging retinal pigment epithelium biomechanics

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Background. Age-related macular degeneration (AMD) is a prevalent retinal disorder, affecting nearly 30% of the population over the age of 75. AMD is characterized by retinal pigment epithelium (RPE) degeneration and the remodeling of RPE's extracellular matrix (ECM), including increased collagen crosslinking, reduced elastin content, and the formation of heterogeneous structures termed drusen. Understanding how biochemical cues from the ECM tune RPE mechanics and function during the aging process is crucial for potential therapeutics.

Methods. In this study, we present our novel approach to study the effect of ECM cues in an in vitro model of uncompensated apoptosis in post-mitotic hiPSC-derived RPEs. Non-fouling polyacrylamide hydrogels of 4 and 11 kPa, coated with extracellular matrix proteins laminin-332 or laminin-511, were used as culture substrates providing different physical (stiffness) and biochemical (laminin isoform) ECM cues. RPE cells were transduced with a caspase-8-containing virus, allowing on-demand apoptosis induction to simulate RPE degeneration during aging. Associated biomechanical changes were examined through nanoindentation, shape factor calculations, and movement quantifications, while POS phagocytosis assay and immunolabelling of functional targets shed light on RPE functionality.

Results. Our findings indicate that ECM cues modulate RPE mechanical responses. Physical cues affect cellular stiffness with laminin 511. Furthermore, biochemical cues from laminin 332, are responsible for inducing viscoelastic changes independent of substrate stiffness. Notably, we observed a heterogenous distribution of integrin beta 4 after induced apoptosis, suggesting a shift in substrate adhesion, and restructuring of its associated keratin filaments.

Conclusion. These results provide valuable insights into the role of ECM cues for RPE mechanics in aging and the pathogenesis of pathologies like AMD.

Unraveling the role of reactive oxygen species during epithelial to mesenchymal transition

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Background. Carcinomas represent the most common type of tumors stemming from epithelial cells which form the lining of organs and tissues throughout the body. These cells have a polygonal shape and are sessile. They form adherent and tight junctions with neighboring cells dependent on the expression of E-cadherin. These epithelial cells can transform to a mesenchymal phenotype which is characterized by a spindle-like shape, high motility, and replaced expression of E-cadherin by N-cadherin, a conversion known as epithelial to mesenchymal cell transition (EMT). This change of phenotype gives the cells migratory and invasive properties and also an increased resistance to therapies. Multiple factors can promote this process, such as the transforming growth factor (TGF-). It has been shown previously that reactive oxygen species (ROS) stimulate TGF- which promotes the mesenchymal phenotype. The main objective of this study is to determine the role of ROS in TGF- induced EMT.

Methods. NMuMG epithelial cells were supplemented with TGF- to induce the mesenchymal phenotype and treated with antioxidant N-acetylcysteine (NAC) for 24 h. Gene expression, protein and ROS levels were analyzed, and migration and invasion assays were performed to obtain the results.

Results. Mesenchymal cells upon EMT showed an increased production of ROS which was reduced when cells were treated with NAC. qPCR and western blots revealed that NAC repressed the expression of mesenchymal markers. Moreover, migration and invasion assays showed that NAC inhibits the migratory and invasive properties of cells.

Conclusion. This study reveals insights into the role of ROS during EMT, affecting the expression levels of epithelial and mesenchymal markers, the migration, and the invasion properties of these cells. Further research is still necessary to discover the underlying pathway of how ROS interacts in the EMT process, which could contribute to the development of a more specific therapy.

Uncovering the Differential Roles of Microglia and Macrophages in MS: Foamy or Functional?

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Background. Foamy microglia and macrophages are prominent immune cells in demyelinating disorders of the central nervous system (CNS), such as multiple sclerosis (MS). While early myelin-loaded phagocytes promote CNS reparative processes, previous research demonstrated that sustained intracellular accumulation of myelin-derived lipids shifts their phenotype towards an inflammatory state and thereby exacerbates the disease. However, controversies persist regarding the precise phenotype of these foam cells, leading to ambiguity about their roles in lesion progression. Interestingly, preliminary data revealed a less foamy appearance of microglia compared to macrophages, suggesting some dissimilarities in lipid processing between these phagocytes. This study comprises a comparative analysis of foamy microglia and macrophages, aiming to uncover potential disparities in lipid processing following short-term and prolonged myelin internalization.

Methods. Both BV2 microglial and RAW 264.7 macrophage cell lines and primary cell cultures were used to investigate differences in myelin uptake and lipid storage between foamy microglia and macrophages. For this purpose, immunocytochemistry with lipid-stain Bodipy and ORO as well as the assessment of the uptake of DiI-labeled myelin were performed. Quantitative PCR and flow cytometry were used to characterize and compare the in vitro phenotypes of foamy phagocytes. The impact of intracellular myelin load on pro-inflammatory and anti-inflammatory features of both phagocytes was also evaluated by measuring NO production and arginase enzymatic activity. Finally, the effect of acute and sustained myelin-exposed foamy macrophages was explored on remyelination through macrophage repletion in ex vivo brain slice cultures.

Results. BV2 cells showed increased myelin uptake and foaminess compared to RAW cells. Additionally, lower expression levels of inflammation-related markers suggest that BV2 microglia exhibit a less inflammatory phenotype than RAW cells. Conversely, primary microglia exhibited higher Plin2 gene expression, the main surface protein of lipid droplets, along with elevated gene expression levels of efflux transporters and enzymes involved in fatty acid oxidation compared to macrophages. These findings imply reduced foaminess in microglia and suggest they may be better equipped to manage high myelin content than macrophages, possibly through enhanced lipid efflux and processing. Ongoing experiments with primary cells aim to validate these results and elucidate how differences in myelin load between primary microglia and macrophages influence their respective phenotypic behaviors. Moreover, a current ex vivo brain slice experiment will offer additional insights into how myelin-loaded macrophages affect remyelination.

Conclusion. Thus far, we provided evidence of lipid processing differences in foamy microglia and macrophages. Further experiments with more representative primary cell cultures will refine our findings.

The membrane-associated periodic scaffold regulates endocytosis along the proximal axon

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Background. Endocytosis is a ubiquitous physiological process occurring in several cell types. In the brain, neuronal cells heavily rely on clathrin-mediated endocytosis. This process is mainly studied in somatodendritic compartments and is essential for vesicle cycling at synaptic sites and recycling of ion channels along the membrane. However, this mechanism is poorly studied along axons. It has recently been discovered that the surface of the proximal axon is studded with clathrin-coated pits encased in circular clearings of a submembranous structure. This submembranous structure called the membrane-associated periodic scaffold (MPS), is made up of actin rings connected by a spectrin mesh. Endocytosis along the axon is nevertheless a rare event as the clearing-encased pits are stably stalled at the membrane. Endocytosis can be triggered by stimuli such as elevated neuronal activity induced by NMDA.

Methods. In this research project, we used super-resolution microscopy techniques in combination with uptake assays and MPS-modulating drugs to investigate the underlying mechanism that drives clathrin-mediated endocytosis. We looked at this in the proximal axon of cultured embryonic rat hippocampal neurons.

Results. Results have shown disorganization of the MPS and increased clathrin-mediated endocytosis after NMDA-induced long-term depression. Furthermore, we observed actin polymerization around clathrin pits after NMDA treatment, and preventing this polymerization with latrunculin A inhibited the increase in endocytosis. This suggests that actin polymerization triggers endocytosis from stalled pits along the axon, driving the endocytosis of membrane proteins such as ion channels.

Conclusion. This newly discovered "ready-to-go" endocytic mechanism along the axon offers us new insights into the functions of the axonal MPS and might lead to new understandings of neurodegenerative diseases.

Exploring the role of DNA repair protein APEX1 on the development and longevity of the ENS.

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Background. The enteric nervous system (ENS), our second brain, governs vital gastrointestinal functions. The ENS is mainly comprised of enteric neurons and enteric glia, which are highly susceptible to DNA damage as the ENS is closely located to the lumen of the gut, which contains genotoxins. Therefore, enteric neurons can easily degrade, potentially causing gastrointestinal dysmotility and dysfunction. This suggests a crucial role for robust DNA repair mechanisms in the ENS. One of these mechanisms is the Base Excision Repair (BER) pathway, vital for repairing oxidative damage. It is thought to be most prevalent in enteric neurons due to their high metabolic activity. Apex1, a key protein in this pathway, plays a pivotal role. Noteworthy, whole-body Apex1 knockout (KO) results in embryonic lethality, while CNS-specific KO allows for two weeks of viability before fatality, highlighting its crucial role in early development. However, the impact of Apex1in ENS development and longevity remains unknown. Additionally, unpublished data of 2-month-old longitudinal muscle myenteric plexus (LM-MP) revealed an upregulation of Apex1 in inhibitory neurons compared to excitatory neurons.

Methods. To understand the role of Apex1 in the developing ENS, I performed immunodetection on ex vivo sagittal embryonic sections ranging from embryonic day 12 (E12) -E18. Aside from development, I also performed immunodetection experiments on 24-month-old mouse LM-MP tissues for DNA damage (γH2Ax) and various enteric neuronal subtype markers (e.g. ChAT and nNOS).

Results. Immunoreactivity for DNA damage (γH2Ax) and Apex1 was consistently observed in enteric neuroprogenitor cells from E12 to E18. Similar levels of immunoreactivity were noted across all embryonic days examined. Given the close interaction between glial cells and neurons, immunodetection for DNA damage and Apex1 was also conducted on glialprogenitor cells at E15-E16. These samples exhibited comparable intensity levels for both DNA damage and Apex1 on both embryonic days. Building upon the aforementioned unpublished data, ongoing research involves immunodetection experiments for DNA damage in inhibitory neurons of 24-month-old LM-MPs, given their heightened susceptibility to DNA damage (unpublished data). Concurrently, I am also looking at the dynamics of cellular senescence within enteric neurons as they age.

Conclusion. Currently, Apex1's significant role in neuroprogenitor and glialprogenitor cells has been substantiated due to its consistent expression throughout ENS development. Furthermore, our investigations have highlighted the significance of Apex1 in inhibitory neurons. However, ongoing experiments aim to determine the extent of DNA damage in these neurons compared to excitatory neurons.

EVEN SUPERHEROES NEED THEIR VITAMINS
Investigating Riboflavin's Influence on Planarian Regeneration

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Background. Regeneration, the remarkable ability of certain organisms to restore lost or damaged tissues, remains a subject of enduring scientific interest. Given its exceptional regenerative capacity, the freshwater planarian Schmidtea mediterranea offers an invaluable model for investigating tissue repair mechanisms. Despite significant progress in regenerative biology, including identifying reactive oxygen species (ROS) as important regulators and possible initiators of planarian regeneration, the precise cellular and molecular mechanisms governing regenerative processes remain elusive. Recent observations of the autofluorescence of riboflavin (RF), a significant redox-sensitive compound also known as vitamin B2, have unveiled its abundant presence within the planarian body. Building upon the recognized significance of ROS, this study aimed to delve deeper into the involvement of other redox-sensitive molecules, including RF, in redox-mediated regeneration. Using planarians, we investigated the spatio-temporal dynamics of the RF-related target, riboflavin transporter (RFT), in different physiological states, and the functional roles of RFT and riboflavin kinase (RFK) during regeneration by assessing both the impact of genetic knock-downs on regeneration outcomes and their effects on RF availability.

Methods. Fluorescent in situ hybridizations (FISH) were performed at different regeneration stages for spatio-temporal characterisation. Knock-downs were created using RNA interference (RNAi), and RF levels were assessed via its autofluorescent signal (488nm spectrum). Results were obtained through blastema measurements, morphologic analysis, cognitive tests, and confocal imaging.

Results. Outcomes include transient upregulation of RFT expression in regions associated with active tissue remodelling and cellular proliferation. Additionally, we found that stem cells express RFT. Genetic knock-down of RFK leads to reduced blastema size, eye development, and impaired cognitive function, while that of RFT appears to affect stem cell proliferation. Furthermore, RFK-RNAi affected tyrosine hydroxylase (TH) expression. Lastly, the availability of RF in newly formed tissue was reduced in RFK-RNAi treated animals, whereas RFT-RNAi treated animals showed a disruption of RF distribution, altering its characteristic nerve-like structure.

Conclusion. Conclusively, our study underscores the multifaced involvement of RF in planarian regeneration. Specifically, heightened RF transport during early regeneration stages, probable involvement in stem cell dynamics, and regulatory impact on neural development and behaviour through RF metabolism collectively underscore the importance of RF in orchestrating diverse processes crucial for planarian tissue renewal and repair. Furthermore, its significance extends to blastema formation, eye development, and stem cell proliferation. This study addresses research gaps in the current understanding of redox-mediated planarian regeneration, thereby potentially offering foundational insights into biological processes relevant to the advancement of regenerative medicine.

Downstream PDE4D Signaling: Illuminating the Path to Myelin Regeneration in Multiple Sclerosis

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Background. Worldwide, 2.8 million people experience motor, sensory, and cognitive challenges due to multiple sclerosis (MS), an autoimmune disorder affecting the myelin sheath surrounding neuronal axons. The destruction of myelin-producing oligodendrocytes in the central nervous system (CNS) leads to demyelinated lesions, axonal damage, and disrupted signal transduction. While early-stage remyelination is possible through oligodendrocyte precursor cell (OPC) differentiation, recurrent damage and impaired OPC function result in persistent demyelination and neurodegeneration. Current anti-inflammatory therapies fail to halt disease progression or induce repair, highlighting the unmet medical need for targeted myelin regenerative MS therapies. The second messenger, cAMP, plays a crucial role in cellular differentiation. Our research has shown that elevating cAMP levels by inhibiting its hydrolyzing enzyme PDE4D promotes OPC differentiation and remyelination. However, the precise signaling pathway activated following PDE4D inhibition remains unknown. This project aimed to elucidate the downstream players of the cAMP pathway that mediate myelin regeneration post-PDE4D inhibition in oligodendrocytes using our IP-protected PDE4D inhibitor, RICE01.

Methods. Human induced pluripotent stem cell (hIPSC)-derived OPCs served as an in vitro model. To assess the role of cAMP-effectors EPAC and PKA, OPC differentiation was evaluated by staining cells for oligodendrocyte markers O4 and MBP after RICE01 treatment in combination with EPAC or PKA inhibitors. Moreover, quantitive PCR was used to study PDE4D isoform gene expression and downstream molecule expression upon PDE4D inhibition. Additionally, a pilot phosphoproteomics mass spectrometry analysis was conducted to identify differentially phosphorylated proteins upon PDE4D inhibition.

Results. Short PDE4D isoform expression profiles exhibited similar magnitude and changes during hIPSC-OPC differentiation compared to laser-captured OPCs and oligodendrocytes in MS lesions, validating the hIPSC-OPC model. Moreover, PDE4D inhibition promoted OPC differentiation via both EPAC- and PKA-dependent pathways. Furthermore, RICE01 treatment upregulated Rap, ERK, ATF2, CREB, BDNF, and NGF gene expression levels. In addition, preliminary phosphoproteomic analysis revealed 24 differentially phosphorylated proteins post-PDE4D inhibition, including unexpected proteins relevant to oligodendrocyte development that are understudied in the current literature, prompting novel research hypotheses.

Conclusion. EPAC- and PKA-dependent pathways play a role in myelin regenerative pathways following PDE4D inhibition. Furthermore, an unbiased phosphoproteomic approach can identify downstream players post-PDE4D inhibition, offering novel therapeutic targets for developing MS treatment strategies.

Investigating the therapeutic potential of antibody Candidate 14 in amyloid-β induced neurotoxicity

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Background. Withan improvement of medical treatment options in recent years, age-related neurodegenerative diseases have become increasingly prevalent. Nonetheless, many such diseases remain largely untreatable despite recent developments. A reason for this is the currently insufficient knowledge on disease mechanisms. Recently, the prion protein, a membrane-bound receptor mainly found in the nervous system, has been identified as a potential key player in a sub-group of age-related neurodegenrative diseases called proteinopathies. The prion protein can bind toxic oligomers and induce cytotoxic signalling in cells, causing the neuronal loss commonly associated with neurodegenerative disease. In the proteinopathy Alzheimer's disease, this is reflected in commonly associated symptoms such as memory loss or overall loss of cognitive function. In Alzheimer's disease, the toxic oligomer amyloid-β induces the cytotoxic signalling via the prion protein. As a key player in cytotoxic signalling, the reduction of prion protein may be beneficial for reducing neuronal loss in age-related neurodegenerative diseases. The working group has identified an antibody that is capable of reducing prion protein at the membrane via two separate working mechanisms. This study aims to assess the safety, working mechanism and neuroprotective capacities of the antibody Candidate 14 as a potential therapeutic in an amyloid-β neurotoxicity model thought to mimic Alzheimer's disease.

Methods. To confirm the working mechanism, the two processes expected to lead to reduction in prion protein were verified using western blot and microscopy. To exclude changes in prion protein production, qPCR was performed to assess prion protein mRNA levels. Toxicity assays such as MTT, LDH as well as morphological assessment were perfomed to exclude toxicity. Assessments were performed both in the absence and presence of amyloid-β to investigate the effects in health and disease. Treatments and measurements were perfomed in various cell lines and primary neurons. Cell ines include mHippo, UW473 and N2A cells. Groups were compared using standard one-way ANOVA with a p-value cut-off at $\leqslant 0.05$.

Results. Current results confirm that Candidate 14 is capable of reducing membrane-bound prion protein via two mechanisms. Furthermore, toxicity assays show no toxicity when cells are treated with Candidate 14. mRNA levels are unchanged after treatment with Candidate 14.

Conclusion. While the working mechanism and safety of Candidate 14 has been shown in current results, measurements after amyloid-β exposure need to be performed. Current work focuses on inducing amyloid-β toxicity in primary cells. Measurements assessing safety, working mechanism and neuroprotection will be repeated in amyloid-β exposed cells.

The glutamate/GABA balance as a novel therapeutic target in 22q11.2 deletion syndrome: a clinical trial

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Background. 22q11.2 deletion syndrome is a rare genetic disorder caused by a hemizigous microdeletion of the long arm of chromosome 22. Interestingly, risks of developing psychotic disorders and cognitive dysfunctions are increased among individiduals with this deletion, recognized as the strongest single genetic risk factor for psychotic disorders. Notably, conventional dopamine-targeting antipsychotics demonstrate diminished efficacy among this population and may exacerbate adverse effects beyond their typical manifestation. A hypothesis states that dopamine might not be the most relevant target in 22q11.2 deletion syndrome. Therefore, there is a strong need for novel therapeutics with alternative targets. The glutamate/GABA transmission is a potential candidate since its involvement in psychosis is increasingly demonstrated, and altered glutamate and GABA levels have been reported in 22q11.2 deletion syndrome. This may be caused by the deletion of the proline dehydrogrenase gene since proline intervenes in the glutamate/GABA transmission. Riluzole, a glutamate/GABA modulator used for the treatment of amyotrophic lateral sclerosis, might have the potential to address the lack of effective treatment.

Methods. 16 patients with diagnosed 22q11.2 deletion syndrome, and psychotic disorders and/or cognitive impairments have been recruited. Participants took first a placebo for 8 weeks and then riluzole for 8 additional weeks, after a 2-week washing period. Proton magnetic resonance spectrometry in the anterior cingulate cortex was performed to assess the concentrations of glutamate and GABA after each period. The Positive and Negative Syndrome Scale as well as the Computerized Neurocognitive battery were respectively used to evaluate the severity of the psychotic symptoms and the cognition at baseline, after placebo, and after riluzole.

Results. Regarding cognition, significant improvements in speed were reported after riluzole treatment for some tasks, but not in accuracy. No significant differences were found regarding intraindividual glutamate and GABA concentrations after placebo and after riluzole treatments. In addition, riluzole did not lead to significant changes in the reported psychotic symptoms.

Conclusion. Riluzole did not show a strong effect on the metabolites concentrations, as well as on psychotic symptoms and cognition. The small sample size (only 7 participants for the proton magnetic resonance spectrometry), the heterogeneity of the population (i.e., the use of antipsychotics or not, psychotic disorders or not, ...) and the chosen brain area have certainly played a role in this non-significance. However, riluzole still has potential and further investigations with different perspectives remain crucial.

Echocardiography evaluation to allow early detection of doxorubicin-induced cardiotoxicity in rats

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Background. Doxorubicin (DOX) is a frequently used chemotherapeutic agent that exhibits high antitumor efficacy but shows dose-dependent cardiotoxicity. Early detection of cardiotoxicity is crucial for patient prognosis. This study aimed to determine the onset of DOX-induced cardiotoxicity and alternations in echocardiography parameters over time in rat model of DOX-induced cardiotoxicity. Moreover, we aimed to evaluate the diagnostic performance of different echocardiographic modalities and assess echocardiographic consistency between two researchers.

Methods. Female Sprague Dawley rats (6 weeks old) received weekly intravenous injections with 2 mg/kg DOX or saline (control) for eight weeks. Transthoracic echocardiography of the left ventricle (LV) was obtained at baseline and four, six, and eight weeks after the first injection. Motion (M)-mode, brightness (B)-mode, and four-dimensional (4D) echocardiography were performed, and images were evaluated by two researchers to assess LV function and volumes and compare the echocardiography modes. Bland-Altman plots were created to show the bias and limits of agreement when comparing echocardiographic modalities. Simple linear regression and Pearson correlation were applied to evaluate interobserver variability.

Results. Echocardiography of systolic function showed reduced LV ejection fraction and left radial ventricular fractional shortening (LVFS) compared to baseline six weeks after the first DOX injection. Subsequently, there was a further decline in longitudinal LVFS and LV cardiac index by week eight. Volume parameters significantly increased after week six, with a continued elevation observed by week eight. Furthermore, B-mode echocardiography exhibited lower reproducibility for LV systolic function and volumes compared to 4D and M-mode, characterized by higher bias and broader limits of agreement. Finally, among the two researchers, strong correlations were observed in systolic and volume measurements, except for longitudinal LVFS.

Conclusion. Our study underscores the time-dependent manifestation of DOX-induced cardiotoxicity in a rat model, with discernible changes in LV systolic function and volumes becoming evident by six weeks and intensifying by eight weeks of treatment. Additionally, B-mode echocardiography showed considerable variability, making it less reliable for assessing LV systolic function and volumes compared to 4D and M-mode. Moreover, minimal variation was observed in systolic and volume measurements conducted by two researchers, ensuring reliability. These findings underscore the importance of early cardiotoxicity detection and careful echocardiographic assessment in DOX-induced cardiotoxicity models.

Acyl-CoA-binding protein (ACBP) deletion in brown adipose tissue impairs high-fat diet-induced cardiac remodeling

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Background. Cardiovascular disease (CVD) is the leading cause of death worldwide, often associated with obesity. A novel target in obesity has recently emerged, called acyl-CoA binding protein (ACBP), a secreted protein with a dual role in autophagy and metabolism, coupling fatty acyl-CoA esters to metabolic pathways. Recent studies show that some molecules secreted by brown adipose tissue (BAT) can target the heart and exert cardioprotection. This study examines the cardiac effects of the specific lack of the secretable ACBP protein in brown adipocytes during high fat-diet (HFD)-induced obesity.

Methods. To generate constitutive BAT-specific ACBP knock-out mice (BAT-Acbp-KO), UCP1-Cre mice were crossed with ACBP-flox/flox mice. ACBP flox/flox mice were used as controls. BAT-Acbp-KO and control mice were fed a chow diet or HFD for 12 weeks and cardiac tissue was analysed. Gene expression (real-time PCR), protein quantification (western blot), and histological analyses of the heart were performed.

Results. First, the specific deletion of ACBP in BAT was determined by measuring Acbp mRNA and protein levels in BAT. We did not observe major differences in the general phenotype (body weight, circulating levels of triglycerides,...) between controls and BAT-Acbp-KO mice under chow or HFD conditions. However, when analyzing the myocardium, we found that the heart weight/tibia length ratio (HW/TL) was higher in BAT-Acbp-KO than in control mice upon HFD. Accordingly, genes usually associated with cardiac hypertrophy (Nppa, Nppb, Myh7) showed increased mRNA levels in BAT-Acbp-KO mice compared to control mice. Moreover, HFD did not alter the expression levels of fibrotic genes (Col3A1, Col1A1, Timp1, and Mmp9) in control mice, but they were significantly reduced in BAT-Acbp-KO. Finally, we analyzed fatty acid metabolism and found increased Pdk4 and Acadl mRNA levels and CPT1B protein levels in control mice upon HFD but not in BAT-Acbp-KO. Moreover, a decrease in Mct4 and Mpc1 mRNA expression was observed in the BAT-Acbp-KO models, indicating a reduction in lactate extrusion and reduced glucose mitochondrial oxidation, respectively.

Conclusion. This study shows that a specific lack of ACBP in BAT impacts the myocardium under high-fat diet conditions. Specifically, BAT-Acbp-KO showed more hypertrophy, less fibrosis, and a general reduction of glucose and fatty acid oxidative metabolism upon HFD indicating a more detrimental remodeling of the myocardium. In summary, this data indicates that ACBP is a new cardioprotective factor.

The impact of polystyrene micro- and nanoplastic on neuroregeneration in Schmidtea mediterranea

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Background. Polystyrene (PS) is among the most commonly used plastics in the production of food storage components, including food containers, packing foam, and cups. However, PS plastic can undergo various ageing processes, breaking down into microplastics (MPs; 0.1 µm – 5 mm) and nanoplastics (NPs; < 100 nm). Due to the lack of proper waste management, polystyrene micro- and nanoplastics (PS-MNPs) could be released into the environment, raising concerns about their impact on ecosystems and human health. For thorough toxicity assessment, data from all ecosystem compartments, including the (epi)-benthic zone, is required.However, only limited data is available on the effects of PS-MNPs in freshwater organisms, especially concerning neuroregeneration. Therefore, the present study aimed to investigate the impact of PS-MNPs on dopaminergic and serotonergic neurons during regeneration in the freshwater planarian Schmidtea mediterranea.

Methods. To address this knowledge gap, exposure experiments were conducted in which regenerating planaria (S. mediterranea) were exposed to freshwater medium containing different-sized (2 µm, 1 µm, 200 nm and 50 nm) spherical PS-MNPs (20 mg/L). After seven days of exposure, behavioural and locomotive velocity tests were performed. The planarian central nervous system was visualised via immunohistochemistry. Moreover, fluorescence in situ hybridisation (FISH) was used to visualise dopaminergic and serotonergic neurons after five and seven days of exposure.

Results. Preliminary results show a positive trend in planarian velocity associated with exposure to smaller particle sizes. PS particles of 1 µm were taken up by the planaria and detected in the brain ganglia and ventral nerve cords. After five days of exposure, PS-NPs of 200 and 50 nm significantly reduced the regeneration of dopaminergic neurons. Compared to PS-MNP exposure for seven days, no significant changes were observed in the amount of dopaminergic neurons, indicating a delay in regeneration. However, results show a negative trend in the amount of serotonergic cells with exposure to smaller particles.

Conclusion. Present findings show that PS-MNPs could interfere with neuroregeneration, inducing behavioural effects, in the freshwater planarian S. mediterranea. In regenerating organisms, neurodevelopment was affected, specifically by a reduction in dopaminergic and serotonergic neuron regeneration. The current study highlights the importance of developmental neurotoxicity research using freshwater planaria in future risk assessments. However, more studies are needed to explore the potential underlying toxicological mechanisms and impacts of varying sizes, shapes, and surface groups of PS-MNPs.

Structure-activity relationship between per- and polyfluoralkyl substances (PFAS) and their effects on the NF-κB pathway in innate immune toxicity: a matter of headgroups?

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Background: Per- and polyfluoralkyl substances (PFAS) are a group of synthetic chemicals widely used in commercial products due to their hydrophilic and hydrophobic properties, and high chemical and thermal stability. In its most recent safety evaluation of PFAS, the European Food Safety Authority (EFSA) prioritized immunotoxicity as the primary human health concern based on human data indicating an inverse correlation between plasma concentrations of Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), Perfluorooctanesulfonic acid(PFOS), and antibody titres against diphtheria in one-year-old infants. However, not only is a mechanism of effect lacking, but there is also a lack of data regarding the immunomodulatory effects of PFAS beyond these four types. Aim: The present study focuses on the impact of a variety of PFAS on the innate immune system, using THP-1 cells as in vitro models. The main objective is to determine how functional headgroups of PFAS differently affect the NF-κB inflammatory pathway in macrophages.

Methods: THP-1 cells were differentiated into macrophages and pre- exposed to different concentrations of PFOA, PFOS and 6:2 fluorotelomer alcohol (FTOH) for 24 hours. Afterwards the macrophages were exposed to lipopolysaccharide (LPS) or TNFα to ensure immune activation. The Effects on cytokine release for IL-8 and TNFα were measured by ELISA. Furthermore, the influence of PFAS on NF-κB activation was determined by examining protein levels of phosphorylated and unphosphorylated NF-κB by Western blot.

Results: A decrease in pro-inflammatory cytokines TNFα and IL-8 was found for all PFAS exposed samples, with the highest effect for PFOS, that seemed to decrease IL-8 release up to 60% and TNFα release up to 40% compared with our control. Furthermore, a decrease of approximately 20% was found in IκBα phosphorylation across all headgroups.

Conclusion: PFAS have an inhibiting effect on the NF-κB pathway, with differences between headgroups. Our results show that the headgroups interfere differently in magnitude at various locations in the NF-κB pathway, including in IκBα phosphorylation and cytokine release. With these in vitro approaches, we hope to gain a better understanding of PFAS' impact on the innate immune system and outline potential key molecular events leading to adverse outcomes on human health.

DNA damage and repair in the spinal cord after traumatic injury

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Background: Every year, around 500,000 people worldwide suffer life-altering spinal cord injuries (SCI), often due to accidents like car crashes or falls. SCI disrupts the vital communication pathways between the brain and the body, leading to paralysis, loss of sensoric perception, and disrupted autonomic functions below the injury site. The impact of SCI extends beyond physical health, affecting the quality of life and posing challenges for the affected individuals. At the moment, there is no definitive cure for SCI, and current treatments mainly focus on alleviating symptoms through surgery, medication, physical therapy, and assistive devices. The pathophysiology of SCI involves two distinct phases: the primary event, which is the immediate result of the injury causing neural tissue destruction, and the secondary phase, which unfolds over hours to days post-injury. The secondary phase is characterized by apoptosis, inflammation, and increased production of reactive oxygen species (ROS) due to mitochondrial dysfunction. Normally, ROS are neutralized by antioxidants, but in SCI, the spinal cord's limited antioxidant capacity leads to elevated oxidative stress. These elevated ROS levels engage with nitrogenous bases and deoxyribose, causing oxidation of DNA. This oxidative damage can lead to DNA strand breaks and mutations, contributing to cell death or uncontrolled growth. Despite the recognition of these processes, there is a notable lack of data regarding the immediate aftermath of SCI, specifically from 1 to 8 hours post-injury (hpi). Current research has primarily concentrated on the period extending from 1 day post-injury (dpi) to 28 dpi. Addressing this gap, this study investigates DNA damage and repair during the early phase of 1 to 8 hpi in Neuroblastoma x Spinal Cord (NSC-34) derived motor neurons. The NSC-34 cell line, a hybrid model, offers a controlled setting to examine neuronal DNA damage and repair pathways.

Methods: In this study, immunocytochemistry (ICC) analyses were conducted on NSC-34 derived motor neurons to investigate DNA damage markers (γ-H2AX, 53BP1) and the DNA repair protein APE1 at 1, 2, 4, and 8 hours post-injury (hpi).

Results: The analyses revealed a progressive increase in DNA damage and repair activities between 1 and 8hpi, indicating a dynamic response to cellular injury.

Conclusion: The observed upregulation of DNA repair mechanisms suggests that targeting these pathways in neurons could be a promising therapeutic strategy for spinal cord injury (SCI), particularly in the early phase following the trauma. This highlights the potential of DNA repair as a therapeutic target in SCI.

The MIF/CD74 Axis as a Driver of Pro-inflammatory B Cell Responses Following Spinal Cord Injury

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Background. Traumatic spinal cord injury (SCI) is a life-changing condition that damages the spinal cord's nerve tissue. Following the primary insult, a pro-inflammatory immune response is initiated, wherein B cells play an important role. Our group demonstrated elevated frequencies of B cells expressing CD74 in the peripheral blood of SCI patients. CD74 functions as a receptor for macrophage migration inhibitory factor (MIF), and in the presence of accessory proteins, including the co-receptor CD44, binding of MIF to CD74 activates a signaling pathway that stimulates pro-inflammatory B cell functions. This project aims to investigate if the axis is differentially expressed in B cells of SCI patients and whether inhibiting MIF/CD74 signaling affects pro-inflammatory B cell functions following SCI.

Methods. Absolute counts of B cells (CD19+), T cells (CD3+), monocytes (CD14+), natural killer (NK) cells (CD56+), and dendritic cells (HLA-DR+) were measured in whole blood of healthy controls (HC, n=2) and SCI patients (n=19), collected at different timepoints (from <1 week to >1 year) post-injury, using flow cytometric analysis. Moreover, proliferation and activation (CD80 and CD86 expression) of primary human B cells from HC (n=3) were determined following in vitro stimulation for 72 hours with or without an anti-CD44 or anti-CD74 blocking antibody by flow cytometry.

Results. An increased cell count was shown for all immune cell subsets at <1 week post-SCI compared to HC, with the highest increase observed in B cell counts, which was nearly ten-fold (102.77 vs. 11.899 cells/µl). No significant correlation was observed between the absolute B cell count and time post-injury; however, a positive correlation was demonstrated for NK cells (P=0.0192). A markedly decreased trend in B cell proliferation was observed upon treatment of in vitro-stimulated primary B cells with an anti-CD44 antibody, demonstrating a more pronounced effect compared to treatment with an anti-CD74 antibody. CD80 and CD86 expression showed minimal alteration with both blocking antibodies, although a greater effect was seen upon blocking of CD44.

Conclusion. These results show increased B cell counts in the acute stages following SCI and the role of CD74's co-receptor CD44 in B cell proliferation. This could point to the early involvement and peripheral activation of B cells in SCI, potentially leading to their migration to the site of injury. These promising outcomes demonstrate a role for B cells and the MIF/CD74 axis in SCI pathology, which could lead to the identification of novel B cell-related targets for SCI treatment.

Interrogating the essentiality of RBBP7 chromatin modifiers in IDH1-mutant glioma.

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Background. Gliomas are the most common malignant brain tumors of the central nervous system. Major advances in cancer genetics have shown that genes encoding isocitrate dehydrogenases (IDH), key enzymes involved in cellular metabolism, are frequently mutated in gliomas. These mutations give rise to a neomorphic activity: the production of an oncometabolite, which, in turn, results in hypermethylated DNA and histone profiles, thereby driving tumorigenesis. Recently, our group conducted a CRISPR/Cas9-based functional genetic screen in vitro and identified retinoblastoma-binding protein 7 (RBBP7) as an essential target gene involved in the epigenetic regulation of IDH1-mutant (MUT) A172 glioma cells. Therefore, this study investigated whether the CRISPR/Cas9-mediated knockout (KO) of RBBP7 decreases cell viability in IDH1-MUT U-87 glioma cells, recapitulating the phenotype previously observed in IDH1-MUT A172 glioma cells.

Methods. First, to achieve the CRISPR/Cas9-mediated KO of RBBP7, U-87 glioma cells were transduced using lentiviruses expressing Cas9 to generate Cas9-stable U-87 cells. Next, these were post-transduced with lentiviruses overexpressing IDH1-wild-type (WT) and IDH1-MUT proteins to achieve a paired cell line. Successful cell line generation was confirmed through Western blot and immunofluorescence analysis, along with RT-qPCR. The IDH1-WT and MUT phenotypes were characterized based on their proliferation and distinctive responses to various treatments, including Temozolomide (TMZ) chemotherapy, irradiation, and inhibitors targeting IDH1-MUT proteins. Treatment response and proliferative capacity were assessed through MTT and clonogenic assays. Next, the KOof RBBP7 was established through lentiviral transduction using viruses expressing two previously validated guide (g)RNAs, along with a non-targeting gRNA serving as a control. Cell viability post-KO was measured using MTT and clonogenic assays.

Results. IDH-MUT U-87 glioma cells exhibit reduced proliferation and colony forming ability compared to their WT counterparts. Importantly, the IDH1-MUT protein sensitizes U-87 glioma cells to TMZ, irradiation and IDH1-MUT inhibitor treatment, reflected by decreased proliferation and colony forming ability, correspondent with the clinical treatment response disparities seen between IDH-WT and IDH-MUT glioma patients. Furthermore, the KO of RBBP7 did not impact the proliferation and colony forming ability of IDH1-MUT U-87 glioma cells as seen in previous findings derived from IDH1-MUT A172 glioma cells.

Conclusion. Our findings imply that the IDH1-mutation renders U-87 glioma cells vulnerable to conventional glioma treatments, reflecting the clinical features in IDH-MUT glioma patients. In contrast to previous observed outcomes, RBBP7 is not an essential epigenetic target gene in IDH1-MUT U-87 glioma cells and therefore does not affect cell viability upon KO.

The fight against head and neck cancer on a nano-scale: ROS-responsive carriers for targeted therapy

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Background. Head and neck squamous cell carcinoma (HNSCC) ranks as the 6th most prevalent cancer worldwide, for which high-dose cisplatin is considered the standard-of-care. The chemotherapeutic agent is associated with systemic toxicity, limiting the therapeutic potential of the drug and severely impacting the patient's quality of life. This highlights the need for a targeted therapy, while reducing the toxic effects for the patient. An emerging class of nanomedicines, so called nanocarriers (NCs), hold promise towards a more targeted treatment approach. These NCs can be engineered to specifically deliver cargo to cancer cells by utilizing the characteristics of the tumor microenvironment (TME). More specifically, reactive oxygen species (ROS) are known to be elevated in the TME. Our research aims to target the TME using ROS-responsive NCs, carrying cisplatin chemotherapy as payload. The objective of this study is to further understand the redox status in cancer by mapping the presence of ROS and glutathione in the TME, which can then be utilized to optimize the NC responsiveness.

Methods. We will characterize the NCs in vitro through cell viability assays, flow cytometry and confocal imaging. Moreover, a range of fluorescent ROS-sensing probes will be used to map the redox status in oral squamous cell carcinoma (OSCC) through live-cell imaging, cell-based assays and flow cytometry.

Results. We expect increased NC payload release and uptake in regions with increased ROS levels. Besides, we aim to identify the levels of both general and mitochondrial ROS and GSH in the TME of HNSCC. Based on those findings, NCs characteristics are further finetuned to optimally react with the TME.

Conclusion. The findings from our study indicate that ROS and GSH levels are altered within the TME of HNSCC and, hence, can aid in targeting a therapy towards the TME through ROS-responsive NCs. In general, our findings may contribute towards a targeted therapy for HNSCC, limiting the severe side effects for the patient and thereby drastically improving the quality of life.

Investigating Microglial Saltatory Migration and Efferocytosis in Disc1 LI Mouse Models

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Background. Microglia, the central nervous system's immune cells, play vital roles in development and brain immune defense. Their migration, essential for their functions, follows a saltatory pattern characterized by discontinuous jumps, yet the underlying mechanism remains elusive. Two potential approaches are actin-myosin-based nuclear translocation and Golgi/centrosome (GC)-based nuclear translocation. Previous research implicated Disrupted-in-Schizophrenia 1 (DISC1), a protein-coding gene, to microglial saltatory migration. This study aims to investigate the mechanism guiding saltatory migration in microglia. We hypothesize that microglial cells will undergo saltatory migration, facilitated by the GC-based nuclear translocation method in in vitro and in situ wild-type (WT) mouse models, while this migration is impaired in Disc1 locus impaired (LI) mouse models.

Methods. To investigate the GC-dependency of murine microglial saltatory migration, primary mouse microglia and embryonic brain slices of WT and LI mice were stained for the Golgi apparatus. Subsequently, the migration was tracked to assess differences in speed between WT and LI microglia. Furthermore, scratch assays were conducted to examine the role of the centrosome during migration. This involved introducing centrinone, disrupting centrosome function, to the cells and analyzing the mean scratch closure. Lastly, apoptotic bodies derived from sushi cells were used to investigate efferocytosis (engulfment of dead cells) by microglia. The pixel intensity of the apoptotic bodies engulfed by microglia was quantified to evaluate the influence of DISC1.

Results. The initial findings of the Golgi staining reveal variability in the number of Golgi puncta within microglia, although not all cells display visible Golgi apparatus. Despite expectations of differing migration speeds between WT and LI cells, initial results suggest that the Golgi apparatus may not play a primary role in guiding microglial migration. Furthermore, analysis of scratch data indicates variance in mean scratch closure between WT and LI cells, yet no significant impact of centrinone, suggesting that the centrosome may not directly influence microglial migration. Finally, we anticipate observing lower pixel intensity of apoptotic bodies engulfed by LI microglia compared to WT cells in the efferocytosis experiments.

Conclusion. In summary, the results reveal variations in the number of Golgi puncta within microglia. Moreover, the findings indicate that the GC-based nuclear translocation method may not be the primary mechanism directing microglial migration. However, both migration and efferocytosis are expected to be impaired in Disc1 LI microglia. Overall, this study contributes to our understanding of microglial migration mechanisms and efferocytosis, aiming to enhance our comprehension of their functionality.

Human dental pulp stem cells as a patient-in-a-dish model for Charcot-Marie-Tooth disease type 1A

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Background. Charcot-Marie-Tooth disease type 1A (CMT1A) is a prevalent inherited neuropathy characterized by abnormal peripheral nerve myelination, affecting approximately 1 in 2500 individuals worldwide. It leads to sensory impairment and diminished motor function, primarily caused by a duplication of the peripheral myelin protein 22 (PMP22) gene, resulting in dysfunctional Schwann cells (SCs) and aberrant myelination. Current research on CMT1A is hindered by the lack of reliable disease models that accurately replicate human pathology. Here, we propose a novel approach utilizing dental pulp stem cells (DPSCs), which have drawn attention due to their neural crest origin similar to SCs, multi-lineage differentiation potential, and easy accessibility. The dental pulp stem cells (DPSCs) are derived from healthy and CMT1A-affected donors, along with induced pluripotent stem cell (iPSC)-derived motor neurons (iPSC-MNs), to create a fully human CMT1A patient-in-a-dish model that accurately mimics the cellular and molecular characteristics of CMT1A in vitro.

Methods. The experimental procedures involved the isolation of DPSCs from human third molars, their differentiation into DPSC-derived Schwann cells (DPSC-SCs), and the differentiation of iPSCs into motor neurons. We aimed to co-culture these cells in both two-dimensional and three-dimensional configurations to investigate SC-neuron interactions under CMT1A conditions. Comprehensive analyses were conducted on gene and protein expression profiles of SCs and myelin markers, with functional assays evaluating cellular proliferation potential. The multi-lineage mesenchymal stem cell differentiation potential of the DPSCs derived from healthy and CMT1A-affected donors was also assessed.

Results. Notable differences in gene and protein expression levels were observed between healthy controls and CMT1A-affected DPSCs in monoculture settings, pre- and post-differentiation into DPSC-SCs. Similar trends were observed in two-dimensional cocultures of DPSC-SCs and iPSC-MNs, where increased expression of mature SC and myelin markers were noted. Lastly, reduced multi-lineage differentiation potential towards adipocytes and osteocytes was observed in DPSCs derived from CMT1A donors compared to DPSCs derived from healthy donors.

Conclusion. The proposed patient-in-a-dish model offers significant insights into the cellular and molecular dynamics underlying CMT1A pathology. Furthermore, the three-dimensional co-culture models have the potential to offer more precise insight into the myelinization defects in CMT1A.

A Two-Edged Sword: Impact of Anticoagulation Treatment on Atherosclerosis

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Background. Atherosclerosis is a chronic inflammatory disease which accounts for the majority of CVD-related deaths worldwide. Vascular calcification is a hallmark of atherosclerosis and contributes to its clinical complications by impairing the physiological structure and function of the arterial wall, leading to reduced elasticity and increased vulnerability to rupture. Patients with atherosclerosis are on life-long anticoagulation therapy to reduce the risk for cardiovascular events, such as stroke or myocardial infarction. Concerns about the long-term effects of warfarin are rising as previous studies demonstrated a link between warfarin, increased vascular calcification and plaque progression due to the inhibition of MGP, an inhibitor of vascular calcification. Novel anticoagulants like rivaroxaban and edoxaban were introduced as an alternative but little is known about their long-term effects on atherosclerotic development and vascular calcification. It is hypothesized that these factor Xa inhibitors have better outcomes on vascular calcification and plaque progression than warfarin due to their different mechanism of action.

Methods. Twelve-week-old female ApoE-/- mice were fed a WTD for 18 weeks supplemented with warfarin, rivaroxaban or edoxaban (protection) or switched after 6 weeks from warfarin to vitamin K2, rivaroxaban, rivaroxaban + vitamin K2, edoxaban or edoxaban + vitamin K2 for additional 12 weeks (reversing). Plaque characteristics were assessed by histochemistry, and vascular calcification was measured using μCT.

Results. Long-term treatment with warfarin showed increased plaque sizes compared to rivaroxaban, edoxaban or the control indicating that warfarin accelerates atherosclerotic development in ApoE-/- mice. Warfarin treatment also increased vascular calcification, while rivaroxaban and edoxaban did not. Switching from warfarin to alternative treatments in the reversing experiment reduced plaque progression, indicating a potential reversal of warfarin's effects. Switching treatments, except for vitamin K2, also resulted in reduced calcification, suggesting potential protective effects against vascular calcification.

Conclusion. Treatment with warfarin increased atherosclerotic development and vascular calcification when compared to rivaroxaban or edoxaban treatment. Changing the intervention from warfarin to an alternative treatment has the potential in mitigating or even reversing the adverse effects induced by warfarin. Future clinical studies are needed to test whether this also applies to patients with atherosclerosis and high risk for vascular calcification.

Assessing genome instability markers in relation to obesity across different tissues and age groups in the ZSF1 hypertensive rat model

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Background. Obesity is a complex disease associated with several comorbidities such as type 2 diabetes, cardiovascular diseases and many types of cancer. The underlying mechanism is thought to be based on low-grade chronic inflammation and the increased production of reactive oxidative species (ROS). Prolonged exposure of DNA to ROS can lead to oxidative DNA damage, which, if not repaired, can destabilize the genome. One of the central mechanisms involved in the repair of DNA oxidation is the base excision repair (BER) pathway, in which the large amount of ROS triggers the activation of DNA glycosylases.
This study aims to further investigate the role of DNA damage and BER capacity in the progression of obesity, comparing the effects on different tissues (liver, skeletal muscle and lung). It is hypothesized that there is an association between obesity and increased genome instability due to increased DNA damage and/or reduced DNA repair, with the magnitude of this association increasing with age.

Methods. For this purpose, obese (n=10/age group) and lean (n=10/age group) Zucker fatty and spontaneously hypertensive (ZSF1) rats of different ages (8-9 weeks, 22-23 weeks and 34-35 weeks) are examined for various markers of genome instability and compared with healthy normative Wistar rats (n=8, 22-23 weeks).
BER activity is assessed using an optimized protocol for the comet assay-based in vitro DNA repair assay. This allows for better comparability of different tissues by normalizing the activity of tissues extracts based on nuclei number rather than the amount of protein. In addition, gene expression of the DNA glycosylases OGG1, APEX1, NEIL1 and PARP1 is studied.
Telomere length, mitochondrial abundance, mitochondrial oxidative damage as assessed by qPCR, and oxidative DNA damage as assessed by the formamidopyrimidine glycosylase modified comet assay are also investigated.

Results. Preliminary data indicate that obese rats have reduced expression of BER genes compared to lean ZSF1 rats and that the expression correlates with triglyceride and glucose levels. This trend is particularly strong in liver tissue and the youngest age group, suggesting an impairment of the repair mechanism in these rats. When assessing the effect on mitochondrial copy number, preliminary data show an increase in obese rats, which may indicate a potential deficit that is being attempted to compensate for.

Conclusion. Based on the preliminary data, it can be concluded that there is a possible association between obesity, impaired DNA repair capacity and mitochondrial dysfunction, particularly evident in liver tissue and younger rats.

Promoting Phospholipid Synthesis via the TGF-β axis as a Potential Strategy to Rescue Remyelination in Demyelinating Disorders

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Background. Failure of remyelination in progressive multiple sclerosis (MS) patients contributes to neurodegeneration and persistent disability. Chronic MS lesions contain abundant oligodendrocyte precursor cells (OPCs) in an immature state, and therefore, failure of remyelination is considered to be secondary to unsuccesive OPC differentiation. Remyelination and OPC maturation are dependent on membrane phospholipid biosynthesis through the Kennedy pathway, as evidenced by an increased abundance of phospholipids within remyelinating lesions and myelinating oligodendrocytes. The transforming growth factor β (TGF-β) signalling axis is proposed to serve as an upstream regulator of phospholipid synthesis during proper myelination. Therefore, we hypothesized that enhancing phospholipid synthesis by stimulating the TGF-β signalling axis represents a promising therapeutic strategy to promote myelin repair in demyelinating disorders such as MS.

Methods. Human-derived induced pluripotent stem cell (iPSC)-OPCs, lysolecithin-induced demyelinating cerebellar brain slices, and the acute cuprizone model were used as representative in vitro, ex vivo, and in vivo models. The impact of TGF-β signaling and pharmacological Kennedy pathway modulation was assessed on lipogenic gene expression, OPC differentiation, and remyelination through quantitative PCR, immunofluorescencent stainings, and electrophysiological testing. In addition, to discern between isoform-specific effects, three different isoforms of TGF-β 1, 2, and 3 were employed in this study.

Results. Here, we report a genetic upregulation of key enzymes of the Kennedy pathway during OPC differentiation and ex vivo remyelination, potentially resulting in increased phospholipid synthesis. Substrates of the Kennedy pathway were found to promote OPC differenitation. However, pharmacological modulation of the pathway activity showed minimal effects on OPC differentiation and remyelination, likely due to nonspecific effects of the pharmacological modulators. Conversely, TGF-β stimulation showed a promising trend towards increased remyelination ex vivo and enhanced OPC differentiation in vitro. TGF-β was found to induce phospholipid synthesis via the Kennedy pathway by elevating gene expression levels of all associated enzymes, with isoform-specific effects, particularly notable with TGFB-β3. However, acute treatment with TGF-β diminished these effects, suggesting downstream mechanisms within the TGF-β axis modulate phospholipid synthesis.

Conclusion. Collectively, these findings suggest that TGF-β signaling can be a potent enhancer of remyelination in demylinating disorders by modulating phospholipid synthesis.

PDE4D enzymes as possible therapeutic target to alleviate dementia symptoms in Alzheimer's disease

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Background. More than 50 million people suffer from Alzheimer's disease (AD), which is the primary cause of all dementia cases. Currently, effective treatment strategies are lacking. Therefore, understanding the molecular processes and mechanisms involved in AD-related memory deficits is crucial. The cAMP-PKA-CREB pathway is essential for synaptic plasticity and memory formation. cAMP is enzymatically degraded by the phosphodiesterase (PDE) enzyme family. It has been shown that inhibition of the PDE4 enzyme family by Roflumilast improves memory performance in humans and rodents. Unfortunately, PDE4 inhibition is impeded by severe side effects such as nausea and vomiting. As PDE4D is identified as the predominant subtype involved in cognitive function, selective targeting of PDE4D on the isoform level (PDE4D1-PDE4D9) could enhance memory consolidation without evoking side effects. This study aims to provide more insights into the role of PDE4D isoforms in AD, potentially elucidating a specific PDE4D target to alleviate dementia symptoms.

Methods. In this study, we utilized the CRISPR-Cas9 tool to selectively knockout human PDE4D isoforms. sgRNAs were designed and evaluated in a cell-free cleavage assay and gel electrophoresis. Next, sgRNAs were cloned into a PX459 vector and transformed into stable E. Coli. Human SH-SY5Y neuroblastoma cells were utilized and their PDE4D isoform expression pattern was analyzed by qPCR, with or without Aβ exposure. Furthermore, the CRISPR transfection and selection protocol was optimized by magnetofection and puromycin. Finally SH-SY5Y cells were transfected with the CRISPR-Cas9 constructs and neurite outgrowth was evaluated by the IncuCyte live cell imager, and PDE4D expression was assessed by means of Western blot.

Results. The SH-SY5Y cells show mRNA expression of all PDE4D isoforms, except for PDE4D1 and PDE4D7. Upon Aβ exposure, PDE4D4 mRNA expression significantly increases. All CRISPR-Cas9 plasmids were succesfully developed, and a selection of four sgRNAs were succesfully assessed by gel electrophoresis. Optimizations of the magnetofection and puromycin selection protocol are currently ongoing based on the NeuroMag transfection protocol in SH-SY5Y cells with the PDE4Dpan plasmid. After obtaining the optimal concentrations of magnetic beads, plasmid DNA, and puromycin, the effect of each specific isoform on PDE4D expression and structural neuroplasticity of SH-SY5Y cells can be examined by morphology (IncuCyte) and Western blot.

Conclusion. The CRISPR-Cas9 tool is a promising selective tool to ablate the different PDE4D isoforms in neurons. Ongoing work aims to clarify the role of each isoform on neuroplasticity, potentially providing novel AD treatment strategies.

14. Abstracts
Oral Presentation Session 3

NeuroInsights: Advancements And Challenges In Neurological Health And Treatment.

1. Ian Meyssen, Hasselt University
2. Elke smeets, Hasselt University
3. Dylan Kidjemet, Hasselt University
4. Femke Cornelissen, Hasselt University
5. Sarah Willems, Hasselt University
6. Yanne van Reusel, Hasselt University

The Real-World Effectiveness and Safety of Cyclophosphamide in Progressive Multiple Sclerosis

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Background. Cyclophosphamide (CYC) holds promise as a treatment option for Progressive Multiple Sclerosis (PMS). Currently, most PMS patients do not have a therapeutic option, as the approved treatments are only suitable for a small portion of patients. A PMS treatment option that is often used off-label is CYC. However, the real-world safety and effectiveness of CYC treatment remain unknown. Hence, this study aims to determine the effectiveness and safety of CYC in real-world conditions.

Methods. In this single-center, retrospective cohort study, we included all PMS patients followed at the University MS Center (UMSC) of Pelt, Belgium, and treated with CYC. Treatment effectiveness was assessed by evaluating disease stability based on the Expanded Disability Status Scale (EDSS) and neurologist-reported stability. Additionally, MRI activity, timed 25-foot walk tests, and walking distance were evaluated. Safety was determined by adverse events (AE) frequency. All data were extracted from the MS data entry portal iMed. Descriptive statistics, time-to-event analysis, and subgroup analysis were performed.

Results. The dataset comprised a total of 169 patients (female n=93; male n=76) diagnosed with either Primary (n=45) or Secondary (n=124) PMS. 123 patients (72.8%) received one CYC treatment cycle, 39 (23.1%) received two cycles, and 7 (4.1%) received three cycles, with a median of 6 (4-6) CYC infusions per treatment cycle. 61 patients (36.1%) were treatment-naive, whereas 43 patients (25.4%) had received one treatment option before CYC, and 65 patients (38.5%) had two or more prior treatments. We found that 78.7%, 52.7%, and 35.5% of patients remained stable after CYC treatment for at least one, two, and three years, respectively. Eighty-two patients (48.5%) reported AEs during their treatment, the most frequent of which were nausea (16.0%), fatigue (15.4%), and urinary infections (12.4%). New MRI lesion activity was observed in 2.5%, 3.8%, and 7.2% of patients and relapses in 3.3%, 11.3%, and 17.7% of SPMS patients after a period of one, two, and three years, respectively. No significant differences in disease stability were found between PPMS and SPMS, maintenance treatments, or the first and subsequent CYC treatment cycles.

Conclusion. Within our cohort, we observed stability in 78.7% of patients. The safety profile is comparable to alternative treatment options like Siponimod and ocrelizumab, suggesting that CYC can be used as an effective and relatively safe treatment option for PMS. The results from this study could help guide healthcare providers and provide them with an extra treatment strategy for patients with PMS.

The good, the fat, the ugly: fatty acid elongation in control of lesion repair in multiple sclerosis

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Background. Multiple sclerosis (MS) stands out as one of the most prevalent demyelinating diseases of the central nervous system (CNS), affecting 2.8 million individuals worldwide with no current cure. A main characteristic is the accumulation of infiltrated macrophages and CNS microglia in the demyelinating lesions. While these phagocytes can exhibit neuroprotective features by clearing myelin debris, our research group has discovered that prolonged accumulation of myelin-derived lipids shifts these phagocytes toward a disease-aggravating state. However, the underlying pathways governing the phenotypic transition in MS remain inadequately understood. Our recent findings have pinpointed disrupted fatty acid synthesis as a fundamental player in these phenotypic alterations. Specifically, we observed reduced gene expression of elongation of very long chain fatty acids protein 1 (ELOVL1) in myelin-laden murine bone marrow-derived macrophages (BMDMs). Therefore, this study aims to elucidate the role of ELOVL1 in shaping the phagocyte phenotype within the context of MS.

Methods. To validate the decreased Elovl1 expression at the protein level, western blot analysis was conducted on BMDMs exposed to myelin for acute (24h) and sustained (72h) periods. The impact of ELOVL1 on the metabolic and inflammatory features of macrophages was assessed using pharmacological ELOVL1 inhibition in myelin-stimulated BMDMs. These features were assessed by qPCR, arginase activity assay, nitric oxide (NO) assay, and BODIPY staining for neutral lipids, and are further being evaluated by phagocytosis assay, flow cytometry and immunocytochemistry. Additionally, we are investigating whether the effects of ELOVL1 are regulated by PPARs. Finally, the innovative ex vivo brain slice culture (BSC) model is utilized to establish the impact of ELOVL1 inhibition on remyelination by immunohistochemistry.

Results. Our findings indicate that acute myelin exposure diminishes ELOVL1 levels, consistent with observations at the gene level. Interestingly, inhibition of ELOVL1 during sustained myelin exposure reduced arginase activity yet increased NO production in BMDMs. No change in neutral lipid load was observed. However, ELOVL1 inhibition reduced the expression of Abca1 and Abcg1, which are lipid efflux transporters that control the lipid load of phagocytes. We anticipate that this decline will be evident at the protein level. Additionally, we expect that ELOVL1 inhibition alters phagocytic capacities and that ELOVL1 is regulated by PPARs within the context of MS. Finally, we expect that ELOVL1 inhibition can impede remyelination in the BSC.

Conclusion. Uncovering the role of ELOVL1 in shaping the phagocyte phenotype in MS could reveal a promising novel therapeutic target, potentially overcoming current therapy limitations.

The isotype-dependent impact of ApoE on miR-146A-mediated autoimmunity in multiple sclerosis

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Background. Recent studies have highlighted the link between defective myelin debris clearance and the lack of remyelination seen in progressive multiple sclerosis (MS) patients. Given its essential role in lipid metabolism within the central nervous system (CNS), ApoE is implicated in various diseases, including MS. Additionally, microRNAs (miRNAs) have been implicated in negatively regulating various biological processes, including lipid metabolism. Moreover, emerging evidence indicates that ApoE modulation affects miRNA expression. However, the intricate interplay between isotype-dependent ApoE modulation and miRNA remains poorly understood in MS.

Methods. To investigate the relationship between miRNA and myelin overload, RNA sequencing was conducted on active MS lesions and phagocytes, more specifically macrophages and microglia, considering their important role in myelin debris clearance. Using state-of-the-art techniques, intracellular lipid load was quantified and visualized in macrophages exposed to myelin debris and transfected with either miRNA mimics or antagonists, alongside inflammatory phenotyping. Complementary phagocytosis and cholesterol efflux assays were performed to determine the impact of miRNA on myelin debris clearance. In addition, lipid load and phagocytotic capacity were assessed in ApoE-deficient macrophages either exposed to exogenous ApoE or not.

Results. Analysis of miRNA expression levels revealed a common hit across active MS lesions, macrophages, and microglia, with miR146A being significantly altered in all datasets. Moreover, miR146A was found to skew macrophages towards a pro-regenerative phagocytic phenotype, potentially promoting remyelination in MS. These effects were especially visible after prolonged myelin exposure. Furthermore, knockout models demonstrate that ApoE-deficient macrophages significantly metabolize less myelin-derived lipid compared to wild-type macrophages. In addition, exogenous ApoE fails to rescue the lack of lipid metabolism seen in ApoE-deficient macrophages.

Conclusion. These findings suggest that exogenous ApoE affects lipid metabolism significantly less as opposed to endogenously-produced ApoE. Moreover, the isotype-dependent effect of ApoE on miRNA, particularly miR146A, holds promise as a therapeutic target for MS. Targeting this pathway may enhance myelin debris clearance and promote a pro-regenerative phagocytic phenotype within the CNS, offering new avenues for progressive MS patients and potentially others with demyelinating pathologies.

Temperature-Dependent Axonal Growth Inhibition via TRPV4 Activation

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Background. Traumatic brain injury (TBI) is characterised by an alteration in brain function caused by an external force. It is accompanied by inflammation in the brain and degeneration of neurons. The increase in inflammation can correlate with an increase in the brain's temperature. Neuronal activity in the brain is highly sensitive to temperature differences, and these differences can lead to functional alterations in the brain. A receptor that plays a distinct role in thermosensation in the brain is Transient Receptor Potential Vanilloid 4 (TRPV4), which is found on the membrane of neurons. It is known that the receptor localizes in the growth cone, where it regulates axonal motility. Very contradictory results have been found concerning the effect of TRPV4 activation. Goswami et al. reported that TRPV4 activation leads to destabilization/retraction of the growth cone and, thus, inhibition of axonal outgrowth. However, Y. Jang et al. stated that activation of TRPV4 is essential for the axonal growth of neurons in early development. To unravel these opposite results, we will study TRPV4 activation in primary neurons that are subjected to increasing temperatures. We hypothesized that the activation of TRPV4 at temperatures above 37°C will lead to deceleration or even inhibition of axonal outgrowth and destabilization of growth cone dynamics.

Methods. Primary cortical neurons were isolated from C57BL/6 mice and cultured accordingly. Afterwards, neuronal growth and growth cone dynamics were visualized with live cell imaging (Incucyte S3) and confocal microscopy (Zeiss LSM880 confocal laser scanning microscope), respectively. In live cell imaging experiments, neurons were subjected to ambient temperatures ranging from 37°C to 40°C. The neuronal growth was analyzed by manually tracing neurites using the ImageJ plugin NeuronJ. In both experiments, a positive (GSK1016790A, TRPV4 agonist) and negative control (GSK2193874, TRPV4 antagonist) was added for comparison. In confocal microscopy experiments, neurons were subjected to increasing temperatures ranging from 37°C to 41°C and imaged. Growth cone dynamics were analyzed using ImageJ.

Results. At the moment, experiments are still running. However, we have already seen that neurons subjected to a temperature of 39°C exhibited slower growth than neurons subjected to 37 °C. This is to be further explored using data analysis and confocal microscopy. All results will have been obtained when presenting at the conference.

Conclusion. The research will show the effect of temperature on neuronal growth and growth dynamics when neurons are exposed to temperatures above 37°C and will demonstrate the association with TRPV4 activation.

The Haunted Treatment Against Stroke, Harnessing the Power of Stem Cell Nanoghosts

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Background. Ischemic stroke, resulting from cerebral artery occlusion, induces neuroinflammation and neuronal damage. Despite existing conventional therapies, their efficacy is restricted by low patient eligibility. Stem cell-derived extracellular vesicles present a novel avenue, yet their therapeutic application is hindered by low scalability. Here, we investigated the potential of dental pulp stem cell nanoghosts (DPSC-NGs), obtained by plasmatic membrane isolation of DPSCs and reduced to nanoscale, for promoting angiogenesis and immunomodulation in vitro within stroke models.

Methods. DPSC-NGs membrane and absence of intracellular proteins were assessed via Western Blot. Nanoparticle tracking analysis (NTA) and Transmission Electron Microscopy (TEM) determined their size distribution and morphology. Cellular uptake was examined using flow cytometry and confocal microscopy in endothelial and macrophage cells. The immunomodulatory and pro-angiogenic potential was evaluated through LPS-induced inflammation and scratch assays, respectively.

Results. DPSC-NG membrane proteins exhibited the expression of CD73, while absent for intracellular nuclear protein histone H3. TEM and NTA confirmed the DPSC-NG size of approximately 100 nm and their vesicular shape. Cellular uptake was confirmed to be dose-dependent in both cell lines studied. Functional assays demonstrated the patient-dependent immunomodulatory potential of DPSCs in reducing the expression of iNOS and CD80 in LPS-induced macrophages. Additionally, DPSC-NGs showed promise in promoting angiogenesis by improving the rate of wound healing as tested by a scratch assay.

Conclusion. In summary, our study thoroughly characterized DPSC-NGs and explored their functional properties. DPSC-NGs are anti-inflammatory entities and promote angiogenesis in vitro, highlighting their potential as a therapeutic option for ischemic stroke. Further research is needed to confirm these findings and explore their clinical applicability.

Turning back time: Can Targeting Cellular Senescence Enhance Recovery After Spinal Cord Injury?

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Background. Spinal cord injury affects millions globally, with current treatments offering limited repair. Notably, the rising mean age coincides with increased activity of the elderly, leading to falls and traumatic spinal cord injury. This population may have an increased predisposition to cellular senescence compared to younger individuals, potentially impairing recovery. Moreover, spinal cord injury itself induces senescence in younger individuals, suggesting its key role in impaired repair. Targeting cellular senescence emerges as a promising avenue to improve spinal cord injury recovery.

Methods. A mouse model was used to investigate the impact of aging on spinal cord injury recovery. Young (12 weeks) and aged (18 months) mice received spinal cord injury via contusion. Functional recovery was evaluated for 28 days post-injury (dpi). Cellular senescence was assessed using immunohistochemistry and qPCR for p16, a senescence marker. The expression of genes associated with aging and/or inflammation (p21, IL-6, TGF-β, chemokines…) was investigated in both lesioned and non-lesioned spinal cord areas. Finally, a senolytic compound was administered to the same mouse model to assess its effect on functional recovery and senescence markers.

Results. Aged mice displayed significantly poorer functional recovery and a higher mortality rate compared to young mice. qPCR revealed an increase of p16 expression following spinal cord injury. Notably, aged spinal cord injury mice exhibited a further increase in p16 compared to young spinal cord injury mice. We anticipate that targeting cellular senescence in spinal cord injury, using senolytic drugs, will improve functional recovery and reduce senescence-associated markers in both young and aged animals.

Conclusion. Our study demonstrates that aging worsens functional recovery after spinal cord injury. Additionally, a higher mortality rate was observed in aged mice, mirroring clinical findings. P16 expression significantly increased in aged spinal cord injury mice compared to its healthy control and young spinal cord injury mice. These findings suggest that age not only influences senescence levels but also that spinal cord injury worsens senescence. Targeting cellular senescence holds promise for improved recovery and reduced senescence-related markers.

15. Abstracts
Oral Presentation Session 4

Advancements in Genotoxicity, Cancer Research, and Machine-Assisted Innovations

1. Anton Changalidi, Maastricht University
2. Oğuzkan İlmaz, Giresun University
3. Jasmijn Timmermans, Maastricht University
4. Sahar Behzad
5. Ozgu Gumustekin, Maastricht University - *Abstract could not be published*
6. Betin Bilkan Karaman, Eskişehir Osmangazi University Medical School
7. Jorn Steeghs, Maastricht University

bioGWAS: a Simple and Flexible Tool for GWAS Datasets Generation

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Background. For the last two decades, an increasing number of genome-wide association studies (GWAS) have been successfully conducted to identify genes, loci, and biological mechanisms associated with complex traits and predispositions to different diseases. A critical challenge in the development of GWAS annotation and interpretation tools is the lack of datasets with predefined biological ground truth. Simulating GWAS results fulfills the need for such datasets for both benchmarking of tools and educational use. Still, no software could simulate datasets with predefined sets of causal genetic variants and/or molecular pathways.

Methods. We present bioGWAS, a simple and flexible Snakemake pipeline that integrates various existing bioinformatics tools (PLINK, HAPGEN2, bedtools, and PhenotypeSimulator) to simulate realistic genotypes (with predefined ground truth: causal genetic variants or molecular pathways), phenotypes, and summary statistics of their association.

Results. The validation of bioGWAS, alongside the selection of its default simulation parameters, was conducted by evaluating the precision and recall of detected associations between user-defined causal genetic variants and simulated phenotypes. Generation with optimal parameters yielded an average F1-score exceeding 0.95, indicating a high accuracy of simulation results. Further validation through the simulation of 200 datasets with causal variants from certain biological pathways showcased the capability of bioGWAS in benchmarking tools designed for GWAS results annotation. Additionally, the dataset generated by bioGWAS facilitated the evaluation of a developed tool, Locus-Set Enrichment Analysis (LSEA), addressing the initial challenge of assessing tool performance in the absence of datasets with known biological truth.

Conclusion. bioGWAS represents a significant contribution to the field, offering a versatile tool for the generation of biologically relevant GWAS datasets, with extensive applications in methodological development, bioinformatics software validation, and educational initiatives. Its value was demonstrated through its application in LSEA evaluation.

Machine Learning Approaches Using Complete Blood Count for Diagnosis Chronic Lymphocytic Leukemia

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Background. Chronic lymphocytic leukemia (CLL) is a malignancy of CD5+ B cells characterized by the accumulation of small, mature lymphocytes in the blood, bone marrow, and lymphoid tissues. CLL is the most common leukemia in the Western world, with an incidence rate of 4.2 cases per 100,000 per year. The diagnosis of CLL is primarily suspected based on complete blood count (CBC) results. The diagnosis is confirmed through peripheral smear and flow cytometry analysis. Lymph node biopsy and bone marrow biopsy results are evaluated for definitive diagnosis and differential diagnosis. Often, CLL diagnosis may be missed, or patients may be misdiagnosed with acute leukemia due to incorrect interpretation of hemogram results by physicians. Late diagnosis or failure to administer appropriate treatment may lead to adverse outcomes. Therefore, complete blood count samples can assist in rapid CLL diagnosis. The aim of this study is to compare algorithm performance for CLL diagnosis using a dataset of 154 complete blood count samples and to develop a model for facilitating rapid diagnosis.

Methods.A total of 154 CBC data obtained from Giresun Training and Research Hospital, comprising 77 CLL patients and 77 healthy individuals aged between 20 and 80 years, were included in the study after obtaining ethical approval from the institutional review board. Various machine learning algorithms, including Decision Trees, Logistic Regression, Random Forests, Support Vector Machines (SVM), Light Gradient Boosting Machines (LGBM), K-Nearest Neighbors (KNN), Single Layer Perceptrons, and XGBoost, were employed to assess the predictive performance of CLL diagnosis. Each algorithm was tested with different parameter settings, and the optimal model configurations were identified.

Results.The dataset used in the study comprises 154 CBC samples and provides a comprehensive evaluation of model performance using five-fold cross-validation. Amongst all tested algorithms, the Random Forest algorithm with hyperparameter tuning had the highest performance, achieving an AUC of 0.97, accuracy of 0.91, precision of 0.94, recall of 0.88, and F1 score of 0.91. This algorithm provided an accuracy of over 91% in distinguishing CLL patients from healthy individuals.

Conclusion. Random forest algorithm yielded the highest accuracy in detecting CLL from CBC results. Misinterpretation of hemogram results may lead to missed CLL diagnosis or misdiagnosis of acute leukemia, where our algorithm can assist in rapid CLL diagnosis using complete blood count samples.

MET activity prevents actin cap alignment: implications for cell migration and nuclear morphology

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Background. The perinuclear actin cap (PNAC) is a recently discovered actin-based structure covering the nucleus and crucial for the regulation of nuclear shape. In fibroblasts, it has been found to be important in cell migration where the alignment of the fibers contributes to polarized cell motility. The receptor tyrosine kinase MET, holding an important oncogenic role in many tumor types, has been widely described as regulator of the actin cytoskeleton in the context of cancer cells dissemination, although a direct connection between its activity and PNAC organization has not been described so far. The objective of this research is to understand the effect of MET activity on the perinuclear actin cap alignment in cancer, and the impact on nuclear morphology and cancer cells migration.

Methods. MET gene knock-out (KO) was performed in a colorectal metastatic adenocarcinoma cell line that has a constitutively active MET expression (LoVo). An additional breast-derived non-tumorigenic human epithelial cell line, named MCF10A, was employed as reference for the proper alignment of the actin cap. In these cells, MET hyperactivation was forced by introducing the constitutively active fusion protein TPR-MET.
Live cell imaging, as well super resolution confocal microscopy and RNA sequencing were employed during the experimental procedures.

Results. MET hyperactivated cells displayed dramatic actin aberrations in the perinuclear area, where the perinuclear actin cap fibers collapse into actin patches. It was seen that MET silencing triggers striking morphological changes and cytoskeletal abnormalities found in MET hyperactivated cells were completely reverted upon MET ablation. We also confirmed that proper organization of the PNAC fibers is prompted by the KO of MET, influencing cells movement patterns and nuclear 3D architecture. Finally, MET-mediated PNAC disruption was found to be mediated by YAP inhibition.

Conclusion. MET KO models confirm a direct involvement on MET hyperactivation in the disruption of PNAC fibers. Aberrant MET expression leads to a misalignment of the PNAC fibers and an increased nuclear height, together with YAP1 translocation to the cytoplasm and therefore decreased co-transcriptional activity. The next steps involve investigating the impact of PNAC fibers on the active process of confined migration, particularly focusing on their interaction with MET involvement. This aspect holds particular significance in understanding the mechanisms underlying both extra- and intra-vasation of metastatic cancer cells.

Detection of Low Diastolic Ocular Perfusion Pressure Using Transfer Learning from Fundus Photographs

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Background. Glaucoma is the leading cause of irreversible blindness worldwide. Amongst the primary risk factors for the development of glaucoma is the increased intraocular pressure (IOP). However, reduced optic nerve head (ONH) perfusion is another key player not only in conjunction with increased IOP but also independently of elevated IOP, as in normotensive glaucoma. Notably, several large-scale studies have revealed a notable increase in the risk of glaucoma development, up to six-fold, among individuals exhibiting low diastolic perfusion pressure (DOPP), thus positioning it as an independent risk factor for glaucoma onset. This study aims to predict the presence of low DOPP using a deep-learning algorithm, leveraging a health dataset consisting of 45-degree fundus photographs centered on ONH.

Methods. Data and fundus photographs of 1163 healthy participants (aged ⩾40 years and aged ⩽80 years) that were collected from a previous population-based cross-sectional study were included. DOPP was calculated using the data obtained at the time of fundus photography and computed using the formula; diastolic blood pressure minus IOP, and values with an IOP below 45 mmHg were categorized as low DOPP. A new neural network was trained to classify patients as low DOPP from fundus photographs. Transfer learning techniques were applied over state-of-the-art convolutional neural network (CNN) architecture ResNet18, with no image transformation or augmentation, and the original image resolution of 480x640 pixels. ResNet18, renowned for its depth and efficiency in image recognition tasks, use of transfer learning leveraged its pre-trained weights, and allowed for faster convergence and improved generalization on new datasets with relatively fewer training examples.

Results. The dataset comprised 1163 images, with a training, validation, and test sizes of 0.7, 0.2 and 0.1 respectively: ensuring comprehensive evaluation of model performance. Initial evaluation of our results indicates promising results, with accuracy reaching 0.68, precision at 0.67, recall at 0.69, and an F1 score of 0.68, demonstrating the effectiveness of the trained model even on the small dataset we have.

Conclusion. Although our algorithm showed moderate accuracy in identifying low DOPP from retinal fundus photographs, it shows promise for screening individuals susceptible to glaucoma development in a primary care setting equipped with a simple fundus camera. We will continue our study by training the model with a larger dataset and also implement preprocessing steps to improve accuracy.

Cathepsins Beyond the Lysosome: Extracellular Cathepsin B as a Therapeutic Target for MASH-HCC

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Background. Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver cancer and third-leading cause of all cancer-related deaths worldwide. Metabolic dysfunction-associated steatohepatitis (MASH), characterized by hepatic inflammation and hepatocellular injury, is predicted to become the leading HCC risk factor by 2030. Advanced-stage HCC remains largely incurable and treatments for MASH-induced HCC are limited. Consequently, there is an urgent need for novel therapeutic targets for MASH-induced HCC. An emerging therapeutic target in the context MASH-induced HCC is a group of proteases, known as cathepsins. Cathepsins mostly reside inside lysosomes, where they are crucial in maintaining cellular homeostasis. However, in pathological conditions, cathepsins can translocate extracellularly, where they are thought to contribute to a plethora of diseases, including MASH and HCC. Particularly, cathepsin B has emerged as a key contributor in the pathology of MASH and HCC by affecting cellular processes such as proliferation and apoptosis. Furthermore, cathepsin B has been associated with chemotherapy resistance. Hence, inhibiting cathepsin B is a promising treatment target for MASH-induced HCC. However, due to their vital physiological functions, inhibiting intracellular cathepsins leads to adverse effects. Thus, selective extracellular cathepsin B inhibition is hypothesized as a safer and more effective strategy. Therefore, we aimed to investigate the therapeutic potential of extracellular cathepsin B inhibition in MASH-induced HCC.

Methods. The effect of extracellular and intracellular cathepsin B inhibition on cell viability and spheroid growth was measured in lipid-rich HCC cell lines: Huh-7 and HepG2. Furthermore, the potential of extracellular cathepsin B inhibition as adjuvant to chemotherapy was investigated by assessing its effects on proliferation and apoptosis. To evaluate in vivo toxicity of cathepsin B inhibition, zebrafish larvae were subjected to intracellular and extracellular cathepsin B inhibition and viability was assessed. Additionally, ROS production was measured in PBMC-derived macrophages to evaluate cytotoxicity.

Results. In line with our hypothesis, extracellular cathepsin B inhibition reduced cell viability and spheroid growth compared to intracellular inhibition in HCC cells. Combining chemotherapy with extracellular cathepsin B inhibition, enhanced apoptosis and further suppressed proliferation, highlighting its potential as a combination therapy. In vivo toxicity assessment of both intracellular and extracellular cathepsin B inhibition in zebrafish larvae showed no reduction in viability. However, intracellular cathepsin B inhibition increased ROS production in macrophages, whereas extracellular inhibition did not, suggesting increased cytotoxicity of intracellular compared to extracellular inhibition.

Conclusion. Our findings suggest that extracellular cathepsin B inhibition holds promise as a therapeutic target for MASH-HCC.

16. Abstracts Poster Walks 7 - 11

Poster Walk 7

1. Willem Awouters, UHasselt
2. Alessandra Papitto, Maastricht University - *Abstract could not be published*
3. Birgit Vrancken, UHasselt - *Abstract could not be published*
4. Yara Lambrechts, UHasselt
5. Marie Poncelet, Maastricht University

Poster Walk 8

1. Jens Gielen, UHasselt
2. Vana Stojić, University of Zagreb
3. Hema Felincia Algoe, UHasselt - *Abstract could not be published*
4. Anessa Pijalovic, UHasselt
5. Lucia Malikova, UHasselt

Poster Walk 9

1. Yana Heyvaert, UHasselt
2. Homayoon Yazdanshenas, UHasselt
3. Rombout Moors, UHasselt
4. Emma Geerits, UHasselt
5. Selma Mtoor, University of Duisburg-Essen

Poster Walk 10

1. Elien Mees, UHasselt
2. Thomas Raps, UHasselt
3. Delphine Lemaire, Maastricht University
4. Joke Aerts, UHasselt
5. Iveta Dzivite, Maastricht University

Poster Walk 11

1. Emma Gesquiere, UHasselt
2. Robin Schellingen, UHasselt
3. Freddy Leenders, UHasselt
4. Hanne Eerdekens, UHasselt
5. Miele Martens, UHasselt

Light-induced degradation of N719 in dye-sensitized solar cell-based photovoltaic photographs

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Background. In the pursuit of elegant and efficient solar energy solutions, dye-sensitized solar cell-based photovoltaic photographs present a promising option to integrate next-generation solar panels into various designs, such as buildings, cars, bridges, and more. Here, a light-induced technique is used to create an image in the photoactive layer of the solar cells. The exact mechanism of this photo-induced patterning mechanism, however, remains unclear. This study investigates the influence of light and atmosphere composition on the light-induced degradation of N719 photosensitizer dye bound to titanium dioxide photoanodes.

Methods. N719-stained titanium dioxide photoanodes were exposed to a bright illumination source to induce degradation of the dye molecules. Techniques like Fourier transform infrared, Raman, and UV-VIS spectroscopy were used to gather chemical information about N719 before and after illumination.

Results. From the Fourier transform infrared spectroscopy analysis, it could be seen that degradation of N719-stained photoanodes mainly occurred at the isothiocyanate (NCS) ligands of the N719 molecules. The NCS vibrational band shifted to lower wavenumbers and decreased in amplitude. Multiple mechanisms for this were proposed, including splitting of S to get NC and a substitution mechanism where the ligand was substituted by atmospheric water. The latter mechanism was motivated by an increase of the O-H stretching vibrational band around 3400 cm-1. Changes at the surface were also observed, with a change in the ratio between the vibrational bands from C=O and C-O before and after illumination. No changes were observed in the vibrational bands assigned to the bipyridine components of the molecules, meaning the bipyridine rings remain intact. This was confirmed by the Raman spectroscopy results, where no changes occurred in the bipyridine vibrational bands.

Conclusion. Light-induced degradation of N719 mainly occurs at the NCS ligands and surface, while the bipyridine rings remain intact. The NCS-ligands are thought to be mainly substituted by atmospheric water residues.

Exploring lipid metabolism dynamics during human Schwann cell differentiation for CMT1A therapy

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Background: Charcot-Marie-Tooth disease type 1A (CMT1A) is the most prevalent inherited peripheral neuropathy, disrupting motor and sensory functions in affected individuals. This condition arises from a duplication of the Peripheral Myelin Protein 22 (PMP22) gene, leading to dysmyelination of peripheral nerves caused by dysfunctional Schwann cells.
The differentiation and lipid homeostasis of Schwann cells are crucial for myelination of peripheral nerves and are known to be modified in CMT1A. Hence, this study aimed to explore CMT1A Schwann cell differentiation by examining differentiation marker expression alongside Schwann cell lipid metabolism.

Methods: To emulate CMT1A in vitro, induced pluripotent stem-cell-derived Schwann cell precursors (iPSC-SCPs) from a CMT1A patient were used. To optimize this iPSC cell line, magnetic-activated cell sorting (MACS) was performed to homogenize the iPSC-SCP population. As an analytical approach, immunocytochemistry and qPCR were utilized to assess and correlate Schwann cell differentiation marker expression levels in healthy and CMT1A cells after adding lipid metabolism-altering conditions.

Results: The validation of the model through MACS enhanced the homogenization of the iPSC-SCP population, proving instrumental in refining the differentiation protocol. Moreover, the addition of modifiers targeting the Kennedy pathway or de novo phospholipid synthesis, such as ethanolamine and choline chloride, showed minimal impact on the gene expression levels of differentiation markers in differentiating CMT1A iPSC-SCPs. Likewise, stimulating the Kennedy pathway with glucosylceramide did not significantly affect differentiation marker gene expression. Findings related to the lipid-regulating PPARγ pathway, activated by rosiglitazone, were inconclusive. However, inhibiting SCD1, the rate-limiting enzyme for unsaturated fatty acid synthesis, significantly reduced the gene expression levels of all differentiation markers. Furthermore, lipid-transporter ABCA1 was investigated since RNA-sequencing data highlighted the role of ABC transporters in CMT1A, linked to PMP22. ABCA1 protein expression was significantly higher in CMT1A cells. Additionally, treating iPSC-SCPs with ABCA1 inhibitor, PSC-833, positively impacted the gene expression levels of differentiation markers.

Conclusion: The observed elevation in ABCA1 expression coincides with the hypothesis of reduced cholesterol levels in CMT1A cells. Consequently, targeting ABCA1 inhibition emerges as a promising avenue for rebalancing cholesterol homeostasis. This approach has demonstrated the ability to augment the expression of differentiation markers in CMT1A iPSC-SCPs, thereby enhancing Schwann cell differentiation. Overall, lipid modulation exerts an influence on the differentiation of iPSC-SCPs. However, the results lack consistent linear interpretability, indicating the need for further investigation in this field to draw conclusive insights. Ultimately, lipid modulation offers a promising avenue for therapeutic intervention in restoring dysfunctional Schwann cells in CMT1A.

Exploring Transcranial Alternating Current Stimulation Modulation of Neural Oscillations

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Background. Neural oscillations are crucial for various cognitive functions and are commonly observed via electroencephalogram (EEG) recordings. Recent research has explored the modulation of these oscillations through transcranial alternating current stimulation (tACS). Despite the established impacts of tACS on cognitive and motor functions, the precise mechanism underlying its modulation of neural oscillations remains uncertain. Computational models, such as the Kuramoto model, provide a means to simulate the anticipated effects of tACS on neural oscillations based on the physical characteristics of the oscillator. This study investigates whether the observed online effects of tACS on oscillatory coupling mechanisms in the human brain align with predictions from computational models, using EEG recordings.

Methods. Eleven healthy individuals (21±2.8 years old) were included in the study. Amplitude-modulated tACS and EEG recordings were conducted simultaneously. Intermittent stimulation was administered over the left parietal lobe in a 4x4 design, varying intensity (0.4, 0.8, 1.2, and 1.6 mA) and stimulation frequency (2.5 and 1.5 Hz below and above the individual alpha frequency, IAF, of each participant). The dependent variables of the study were the peak power, the oscillation peak frequency, and the variance of the instantaneous alpha frequency.

Results. Successful artefact removal allowed for the analysis of data during stimulation, making a significant advancement in this research field. Non-significant interactions were observed between stimulation frequencies and intensities for EEG data both during and post-stimulation. Peak power p-values during stimulation were 0.11 and 0.86, while post-stimulation peak power p-values were 0.52 and 0.74, and oscillation peak frequency p-values were 0.41 and 0.89. Despite the absence of statistical significance, notable patterns indicated some interactions between the conditions. The computational model predicted modulations in the variance of the instantaneous alpha frequency (Inst-AF). However, here, the variance of the Inst-AF did not show full entrainment in any conditions. A peak in variance was noted at the highest intensity (1.6 mA) and -1.5 Hz relative to the IAF, partially aligning with the Kuramoto model.

Conclusion. Although the statistical analyses did not yield significant results, the emerging patterns from the data suggested great potential for future research to validate the effects of tACS on the oscillatory coupling mechanism in the human brain. While tACS has many possible clinical applications, understanding its underlying mechanism is crucial, emphasising the importance of this study.

Duration and Quality of Analgesia after Ambulatory Forefoot Surgery under Ankle Block

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Background. Ultrasound-guided ankle blocks provide great anesthesia and analgesia for forefoot procedures. This technique consists of perineural injections of a local anesthetic in proximity to the nerves innervating the forefoot, resulting in a dense sensory block. Ropivacaine is a commonly used local anesthetic that is preferred for its long-acting properties and safety profile. However, there is limited research on the duration and quality of ropivacaine specifically in ankle blocks. This study analyzed the duration and quality of analgesia achieved with 1% ropivacaine.

Methods. A retrospective analysis of adults undergoing forefoot surgery under ankle block with 1% ropivacaine between January 2024 and April 2024 was undertaken. Data were extracted from electronic records and included baseline characteristics, perioperative variables, and postoperative variables. Each patient completed a survey regarding the duration and quality of analgesia. The primary outcome was to define the analgesic duration and quality of the blocks. The secondary outcome was to identify comorbidities that were independently associated with a prolonged duration of pain relief or better patient satisfaction.

Results. Data from 60 patients undergoing forefoot surgery were analyzed. The median age of the patients was 57 [45, 67], with a predominance of female patients (78.26%). The mean duration of analgesia was 22.5h ± 7.23h and a mean visual analog scale satisfaction score of 9.28 ± 1.26 was reported. Analysis revealed significant correlations between age and duration of analgesia, with a longer duration of analgesia observed in an older population ($p = 0.0037$).

Conclusion. Our research contributes to the understanding of factors affecting the duration of analgesia for ankle blocks with 1% ropivacaine. Age emerges as a significant determinant, while further investigations are needed to elucidate the impact of other comorbidities.

Necrotizing fasciitis secondary to retroperitoneal abscess

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Background. Retroperitoneal abscess can be easily misdiagnosed because of its often occult presentation. It can be accompanied by necrotizing fasciitis of the thigh, hip and lumbar area, so skin changes, such as redness and fluctuation of these areas, sometimes suggest the presence of it. It is important for plastic surgeons to be aware of this possible presentation because they encounter these patients and play a role in the management of skin and soft tissue conditions. It can even be related to underlying systemic diseases, like cancer. Early treatment of the abscess and transition to treatment of the cause can significantly improve patient outcome.

Case report. A 62-year-old female patient presented with skin necrosis due to spread of retroperitoneal abscess caused by necrotizing pancreatitis. In her case it was associated with, at that time, undiagnosed intraabdominal cancer. Patient previously had pulmonary embolism and stroke for which she underwent mechanical thrombectomy, so she was already hospitalized when skin changes were firstly observed and treated. Fluctuation and extensive reddening of the skin of her left hip, lumbar and upper leg regions with initial skin necrosis were present. While in hospital patient had rectoraghia. MSCT was preformed and retroperitoneal abscess, as well as an expansive process in her left hemiabdomen infiltrating surrounding organs and suspect metastases in liver and kidneys were found. Patient also had high levels of CA 19-9. Explorative laparotomy verified retroperitoneal abscess lateral to the insertion of the left mesocolon along with retroperitoneal mass medial to mesocolon insertion. Biopsy of the mass was done. Incision, abscess evacuation and drain placement were preformed. Furthermore, skin necrectomy and treatment of the left lumbar wound, as well as incision and evacuation of abscess of upper leg were done. Negative Pressure Wound Therapy was used in conjunction with optimal wound care strategies. Tissues volume and strength were slowly, but surely regained. Local findings were adequate and inflammation resolved. Unfortunately, cancer was already disseminated and in inoperable stage, so the further approach was mostly paliative.

Conclusion. Detecting skin changes is important because they can provide visible signs of an underlying infection or inflammation. Early diagnosis of a retroperitoneal abscess and necrosis is important for prompt treatment and prevention of fatal complications, as well as finding a cause of it. It is important to be aware of this possible presentation because early detection improves patient outcomes.

Generation of Cortical Neurons from Mouse ES Cells to Study Val66Met Polymorphism in the BDNF Gene

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Background: Traumatic Brain Injury (TBI) poses a significant global health concern, exhibiting a mortality rate 3.5 times higher than cancer and heart disease combined among young adults. TBI occurs from sudden head impacts, primarily affecting the cortex and hippocampus of the brain. The condition can be associated with cognitive dysfunction, abnormalities in episodic memory, impaired decision-making, and attention deficits. These events are related to disruptive levels of a key brain factor, Brain-Derived Neurotrophic Factor (BDNF), responsible for neuronal survival and synaptogenesis in healthy individuals. Until today, the prognosis remains challenging due to TBI heterogeneity, limited medication options, genetic variability, and comorbidities. Notably, genetic factors, including single nucleotide polymorphisms (SNPs), are increasingly recognized as contributing to TBI outcomes, adding to the complexity of elucidating the underlying mechanisms. This study investigates the genetic influence of the Val66Met SNP in the BDNF gene. The SNP is suggested to promote neuronal survival, axonal sprouting, synaptogenesis, and differentiation after TBI. However, the underpinning reasons for this beneficial appearance remain unclear.

Methods: This study is unique in that it defines a newly established protocol generating cortical neurons from mouse embryonic stem cells containing the Val66Met SNP. Cell lines with and without the SNP (val/met and val/val) were used for comparison. In vitro injury was performed on the cultured cortical neurons via a scratch assay to mimic TBI, enabling the capture of morphological alterations 24h and 48h post-injury. Hence, this allowed the implementation of a TUNEL assay to quantify neurons undergoing apoptosis. The underlying mechanism was further investigated by testing different concentrations of agonists or antagonists (e.g., 7,8-DHF) to elucidate the effect of the Val66Met SNP on BDNF secretion.

Results: Analysis has uncovered that cortical neurons were generated with a newly established protocol. Upper and deeper cortical layers were characterized through the expression of Tbr1, Ctip2, Satb2, Nestin, Pax6, Otx1, Lhx2/LH2, and B-III Tubulin. It is suggested that in vitro injury causes significantly higher neuronal survival, increased axonal sprouting, and lower apoptotic levels for the val/met cell line in comparison to val/val.

Conclusion. Overall, this study sheds light on the underlying role of the BDNF Val66Met SNP in neural development and provides prospective therapeutic targets to potentially reduce mortality rates among individuals with TBI and neurodegenerative disorders. Further research into the pathway will enrich our understanding and enhance the precision of future pharmacotherapies.

Uncovering the uptake and cytotoxicity of micro- and nanoplastics in human intestinal cells

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Background. Micro-and nanoplastics (MNPs), defined as plastic particles smaller than 5 mm and 1 µm, respectively, are present in our food and drinking water. A recent study detected around 240 000 MNPs, mostly nanoplastics, per litre of bottled water. Following ingestion, the MNPs eventually reach the intestinal barrier. The small intestine, the main site of nutrient absorption, is considered the most likely region for MNP uptake. Since MNPs exist in different sizes, shapes, and materials, it is essential to know how these physicochemical properties affect uptake by human intestinal epithelial cells and cytotoxicity. However, studies have mostly focused on the uptake and cytotoxicity of polystyrene (PS) beads in a monoculture of human intestinal epithelial cells. The uptake and mechanisms of cytotoxicity of other polymers and shapes such as polyvinyl chloride (PVC) fragments in a more advanced in vitro model are currently understudied.

Methods. A co-culture of human intestinal epithelial cells, Caco-2 and HT29-MTX cells (9:1), were exposed to various concentrations (1 - 100 µg/ml) of PS beads (200 nm and 1 µm) and PVC fragments (< 1 µm and 1 - 5 µm) for 4 and/or 24 hours. Lactate dehydrogenase (LDH) and adenosine triphosphate (ATP) were measured with the CyQUANT and CellTiter-Glo assay, respectively, to assess cytotoxicity. Moreover, mitochondrial DNA content (mtDNAc) was measured to determine the mitochondrial response. Cellular uptake of fluorescent particles was studied by confocal microscopy.

Results. Cells exposed to the highest concentrations of PS beads and PVC fragments of both sizes for 4 and 24 hours produced more LDH compared to the controls and cells exposed to the lower concentrations. Additionally, there was no notable difference in the amount of LDH produced between the PS beads and PVC fragments. Moreover, cells exposed to 200 nm and 1 µm PS beads produced more ATP compared to the controls and the ones exposed to PVC fragments (< 1 and 1 - 5 µm). No differences were seen in mtDNAc between the different exposures. Cellular uptake of 200 nm PS beads was observed, but investigation is still needed for the other MNPs.

Conclusion. Our data show the importance of the exposure concentration. Besides, the material and/or shape of the MNPs seem to influence cytotoxicity. Furthermore, 200 nm PS beads can enter human intestinal epithelial cells in the presence of mucus-secretion. Further research is needed to better understand the connection between the mechanisms of toxicity and particle properties.

Conductive MXene-based bioink for integrating biomimetic electronics into new 3D skin model

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Background. The bioprinting of skin tissue using bioink has recently gained significant interest as an alternative approach for treating injuries and facilitating transplantation. However, the inability to monitor and control the internal evolution of engineered tissue remains an issue. Integrating skin tissue with flexible electronics offers a novel platform for continuously monitoring and modulating skin activity, laying the foundation for intelligent tissues that could offer breakthroughs in tissue engineering and healthcare. To establish a fully functional hybrid platform with sensing and tuning abilities, it is essential to render the engineered tissue conductive. In this study, a conductive bioink was formulated by incorporating 2D-nanomaterials called MXenes. These metal carbides have emerged as highly conductive, versatile, and biocompatible compounds with various applications in biomedicine. The primary objective of this work was to investigate the impact of these nanomaterials on the physicochemical and biological properties of the bioink.

Methods. MXene was synthesised by etching of precursor with hydrofluoric acid. Bioink formulations based on alginate and gelatine were developed with different concentrations of MXene. The mechanical properties of these bioinks were assessed through rheology, providing information about the viscoelastic behaviour. Additionally, their printability and structural integrity were evaluated by comparing the relative height of printed constructs. Conductivity was measured in a custom set-up using a potentiostat. Furthermore, the biocompatibility of the bioinks was evaluated by examining cell viability and proliferation of fibroblast cells through appropriate staining assays.

Results. The MXene compound could be easily integrated into the hydrogel formulation due to its hydrophilic nature, eliminating the need for additional functionalisation. Rheological measurements have shown little differences in viscoelastic properties, resulting in bioinks that show similar printability. The obtained structures presented good structural integrity, without severe collapse. Moreover, the presence of MXene improved strand definition. As expected, conductivity increased with increasing concentration of MXene. There was no immediate cytotoxic response observed, indicating the biocompatible nature of the compound.

Conclusion. The ultimate goal was to create a conductive bioink that does not compromise cell viability or the structural integrity of the hydrogel. This study shows that MXene is an excellent candidate for establishing conductivity within a bioink, without negatively altering the mechanical properties of the bioink or affecting cell survival.

Potential Mechanisms of PM2.5 Particulate Transfer via Extracellular Vesicles

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Background: Particulate matter in the air, particularly black carbon, has a profound impact on health, contributing to serious health issues. The effects of black carbon across generations, especially through prenatal exposure, are well recognized, noting its tendency to accumulate within the body. Despite these findings, the detailed processes by which these particles are distributed and moved throughout the body are not well defined. Our study focuses on the role of extracellular vesicles (EVs) as potential carriers for these pollutants, seeking to clarify how black carbon moves through biological systems.

Methods: For this study, we selected 20 plasma samples from the ENVIRONAGE birth cohort, identified based on specific exposure assessments. These samples were immediately collected, processed, and analyzed at Hasselt University. To identify and measure the concentration of black carbon particles, we used a targeted confocal microscopy method across several images. We processed the whole blood from these samples, which showed high levels of black carbon, to isolate EVs. These vesicles were then examined using nanoparticle tracking analysis (NTA) to evaluate their size distribution. Further assessments were conducted using confocal microscopy to detect black carbon within these EVs.

Results: The analysis highlighted a clear relationship between high levels of black carbon and their presence in EVs extracted from whole blood.

Conclusion: This study contributes to filling the knowledge gaps regarding how air pollutants, particularly particulate matter like black carbon, are transmitted within the body. We investigated the movement of these pollutants through EVs in the bloodstream, providing insights into potential pathways for their distribution.

Identification of cellular processes elicited by the defects in ribosome synthesis that cause Diamond-Blackfan anemia

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Background. Diamond-Blackfan anemia (DBA) is a rare congenital bone marrow failure syndrome. The syndrome presents with anemia, congenital malformations, growth retardation, premature birth, and cephalic anomalies. Most common mutations are found in ribosomal protein (RP) S19 (25%) and RPL5 (7%). Impaired production of erythroid lineage transcription factor GATA1, p53 activation, and radical oxygen species accumulation are linked to the pathogenesis of DBA. However, eliminating p53 to rescue DBA in animal models does not always ameliorate the DBA phenotypes. Additionally, loss of GATA1 is not always seen in DBA patients. This suggests that there are other mechanisms leading to DBA. Preliminary data has identified gene A, the expression of which is strongly downregulated in human colorectal cancer cell lines (HCT116) undergoing reduction of RPS19 or RPL5. Surprisingly, gene A mRNA levels increase after RPL depletion but decrease after RPS depletion. We hypothesize that the upstream open reading frames in the 5′ untranslated region in the mRNA of gene A are responsible for this behavior. In this work, other genes and the implication of diminished expression of gene A in cell death are investigated.

Methods. First, transfection of silencing RNAs (siRNA) for RPS19 and RPL5 in HCT116, HCT116 p53-/-, and K562 cell lines were performed. mRNA levels of the genes were analyzed by RT-qPCR and protein levels by Western blot. Second, a knockout model for RPS19 and RPL5 was made using CRISPR/Cas9. Third, the implication of gene A in cell death was investigated by overexpression following lentiviral transduction of an inducible cDNA viral vector. Cell viability and Western blot assays were carried out.

Results. 1) Knockdown of RPL5 increases gene A mRNA levels but only produces a minor protein increase at 24 hours after siRNA transfection. RT-qPCR data of p53-/- cell lines corroborated these results for gene A but not other candidate genes. 2) The creation of an RPS19 and RPL5 knockout model undergoes validation. Preliminary data indicate that RPS19 mRNA is decreased in one candidate clone. 3) Overexpression of gene A diminishes the viability of HCT116 cells in preliminary experiments.

Conclusion. 1) The candidate genes tested in this work do not follow the gene A specific expression pattern. 2) Validation of the RPS19 and RPL5 heterozygous deletion must be finalized before studying the different mRNA expressions in RPS and RPL deficiencies. 3) Gene A can be overexpressed in an inducible manner. The effect of this overexpression is currently under investigation.

Decoding tumor heterogeneity: cyclic staining to visualize the tumor microenvironment.

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Background. Lung cancer is, with a predicted 5-year survival rate of 15.9%, a leading global cause of cancer-related deaths due to treatment failure. A delayed or ineffective detection of the tumor, as well as the heterogeneity present in the tumor microenvironment (TME) play an important role in this low survival rate. The tumor heterogeneity refers to a broad spectrum of differences in cell types within the TME and impacts treatment efficacy, influences the development of treatment resistance, and serves as a prognostic indicator. Therefore, visualizing the tumor heterogeneity can help develop better treatments, leading to improved outcomes for patients. This research focuses on visualizing the tumor heterogeneity by employing a combined approach of multiplex immunofluorescence (mIF) and cyclic staining for precise cellular identification, along with hematoxylin-eosin (HE) staining for morphological characterization.

Methods. Lung tumor tissue was produced by and harbored from genetically modified KrasG12D+/-/Lkb1-/- mice. This paraffin-embedded tumor tissue was used to perform mIF stainings to map the TME. Specifically, markers characterizing the tumor subtype, its immune component, and metastasis potency were used. Subsequently, hematoxylin and eosin (H&E) staining was performed on the same tissue section. Additionally, different methods were tested to allow mIF cyclic staining on the same tissue, such as antibody elution and photobleaching techniques. Tissues were digitized with the Axioscan.Z1 (Zeiss), generating whole slide images.

Results. Tumor formation in the Kras/Lkb1 mouse model was observed nine weeks post-induction. Furthermore, three mIF panels followed by an HE staining on the same tissue were successfully performed, giving a first indication of the TME composition and retrieving both cell-specific and morphological features. Additionally, we were able to perform stainings of all panels on the same tissue in three cycles using the antibody elution method based on a strong reducing agent (2-mercaptoethanol) and a detergent (sodium dodecyl phosphate). Signal loss was observed after antibody elution, confirming effective antibody removal between each mIF staining cycle.

Conclusion. We successfully optimized cyclic mIF staining followed by H&E staining on lung tumor tissue of the Kras/Lkb1 mouse model, gaining both morphological and cell-specific information on one tissue section. The mapping of the TME and utilization of whole slide imaging represent pivotal steps toward establishing a fundamental resource for future artificial intelligence algorithm development addressing tumor heterogeneity.

Roles of apical ectodermal ridge and zone of polarizing activity in chicken embryo limb development

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Background. The chicken embryo has served as a vital model for evaluating developmental biology and understanding human limb development and associated pathologies. During chicken embryo limb development, the apical ectodermal ridge controls the growth of the proximodistal axis through fibroblast growth factors (FGF), especially FGF4. The zone of polarizing activity determines the anterior-posterior axis through Sonic hedgehog (SHH). We evaluated how removal of the apical ectodermal ridge and the zone of polarizing activity affects formation of limb axes, examined phenotypes resulting from zone of polarizing activity transplantation, and investigated gene function during skeletal and limb development using RCAS retrovirus expressing green fluorescent protein (RCAS-GFP) and Indian hedgehog (RCAS-IHH).

Methods. Fertilized chicken eggs were incubated for 3.5 days at 38°C and 80% humidity in a rotating incubator to reach Hamburger-Hamilton stage 20-21. For removal of the apical ectodermal ridge or zone of polarizing activity, respectively, the distal ectodermal tip of the limb or the posterior part of the limb bud were removed with fine forceps. Transplantation of the zone of polarizing activity was performed by removing the posterior part of the limb bud from one embryo and transplanting it into the anterior part of the limb bud of another embryo. 10 µL of RCAS-GFP (control) or RCAS-IHH virus solution mixed with 1% Fast Green dye was injected into the anterior part of the wing bud. Embryos were stained with Alcian blue and photographed under fluorescence microscope.

Results. Early apical ectodermal ridge removal led to limbs with only stylopod elements, while later removal allowed more distal structures to form. Removal of the zone of polarizing activity resulted in limbs with shorter bones and reduced anterior-posterior character, forming only one digit instead of the normal complement. Transplantation of the zone of polarizing activity led to mirror-image duplication of skeletal patterns. RCAS-GFP injection showed efficient infection of cells in the injected anterior limb bud; however, the virus also spread systemically through the bloodstream, infecting other tissues like the heart and liver. RCAS-IHH injection resulted in maintained proliferation of distal and columnar chondrocytes and repressed hypertrophic differentiation.

Conclusion. Our results confirmed the roles of the apical ectodermal ridge and the zone of polarizing activity in limb development and the functions of IHH in regulating chondrocyte development. These findings support further research exploiting FGF4 and SHH signaling pathways in the treatment of human conditions such as brachydactyly or ectrodactyly or in future regenerative strategies.

Phenotypic and functional characterization of IgD-CD27- double negative B cells in multiple sclerosis pathology

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Background. Multiple sclerosis (MS) is an autoimmune disorder characterized by inflammation, demyelination and axonal damage within the central nervous system (CNS). Pro-inflammatory age-associated immunoglobulin (Ig)D-CD27- double negative (DN) B cells are increased in the peripheral blood (PB) and cerebrospinal fluid (CSF) of MS patients. DN B cells can be divided into three subsets: IgD-CD27-CD11c-CD21+CXCR5+T-bet- DN1, IgD-CD27-CD11c+CD21-CXCR5-T-bet+ DN2 and IgD-CD27-CD11c-CD21-CXCR5- DN3. This study aimed to identify the DN B cell subsets in relapsing-remitting (RR)MS patients and to further reveal their pro-inflammatory phenotype and activation potential.

Methods. Precision count beads were used to determine the absolute number of B cell subsets in PB samples from untreated RRMS patients (n=8). Expression of Ig isotypes (IgM/IgG/IgA), pro-inflammatory chemokine receptors (CXCR3/CXCR5), adhesion molecules (LFA-1/VLA-4/ALCAM), and activation markers (CD80/CD86) were measured on DN B cells of paired PB and CSF samples from newly diagnosed RRMS patients (n=3) using flow cytometry. DN B cell activation was investigated by measuring B cell receptor (BCR) signalling in a phosphoflow assay. The phosphorylation of downstream BCR signalling proteins (SYK/ERK/PLCγII) after BCR stimulation was analyzed in DN B cells from 1 healthy control and 1 MS patient, and was compared with IgD+CD27+ non class-switched memory (NCSM), IgD-CD27+ class-switched memory (CSM) and IgD+CD27- naive B cells.

Results. The absolute number of DN B cells (7.36±5.28 cells/µl) in the PB of RRMS patients was lower compared to NCSM (39.84±37.02 cells/µl), CSM (30.96±20.96 cells/µl) and naive (81.07±68.65 cells/µl) B cells. DN1 cells were identified as the predominant subset in both the PB (83.3% of total DN B cells) and CSF (63.3%) of 1 RRMS patient. Furthermore, the frequency of DN3 cells was increased in the CSF (33.3%) compared to PB (11.9%) Phenotypic analysis suggests that DN B cells have an activated and migratory phenotype with increased frequencies of IgM+, CXCR3+ and CD80+ cells in CSF compared to PB. For both donors, phosphorylated SYK, ERK and PLCγII were detected in DN B cells after BCR stimulation. However, BCR signalling was the lowest in the DN B cells compared to the other B cell subsets.

Conclusion. DN B cells demonstrated an activated and migratory phenotype in the PB and CSF of RRMS patients with DN1 cells as the predominant subset. In addition, DN B cells showed the potential to become activated following BCR signalling. Thisstudy provides novel insights that underlie the potential contribution of DN B cells to MS pathology.

Evaluating suicide gene therapy with human dental pulp stem cells for oral squamous cell carcinoma

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Background. Oral squamous cell carcinoma (OSCC) is the most prevalent form of head and neck cancer. However, current treatments do not significantly improve the patient's survival rate. Moreover, these approaches are associated with severe side effects. Hence, a more local and targeted strategy is acquired to enhance the therapeutic efficacy and the patient's quality of life.

Methods. In this project, a suicide gene therapy based on the herpes simplex virus type 1 – thymidine kinase/ganciclovir (HSV1-TK/GCV) system will be examined. Human dental pulp stem cells (hDPSC) will be utilized as an HSV1-TK carrier in vitro. This enables the cells to convert the prodrug GCV into its cytotoxic form. Through gap junctional intercellular communication, the cytotoxic GCV will induce OSCC cell death. Additionally, various cancer types will be examined in vitro and in vivo to expand the proposed suicide gene therapy to various cancer types.

Results. The efficacy of the targeted strategy will be evaluated in 2D and 3D co-cultures of OSCC and HSV1-tk+-hDPSC. The killing efficiency of HSV1-tk+-hDPSC after GCV administration will be examined with an alamarBlue analysis. Here, a significant reduction of cell viability is expected with a GCV concentration starting from 10 µg/ml and 600 µg/ml in a 2D and 3D culture, respectively. Furthermore, gap junctional intercellular communications will be identified by connexin 43 immunocytochemistry in colon, kidney, lung, mammary, pancreatic, and skin cancer. Additionally, xenograft models containing HSV1-TK+ tumor cells will be monitored with BLI. After GCV administration for 21 consecutive days, a decrease in photon emission is expected, indicating cell death.

Conclusion. In summary, our findings will demonstrate the potential of HSV1-tk+-hDPSC as a novel targeted strategy for OSCC and the potential expansion of this therapy to other cancers.

Deciphering D-Amino Acid Dynamics in Cancer Cachexia: From Plasma Profiling to Muscle Cell Responses

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Background. Cachexia, a devasting syndrome marked by progressive weight loss and muscle wasting, poses a significant obstacle in the comprehensive care of individuals affected by cancer. This multifaceted condition, often accompanied by adipose tissue loss and systemic inflammation, strikingly diminishes anti-cancer treatment effectiveness, patient quality of life, and survival rates. Occurring in approximately 80% of cancer patients, cancer cachexia has been identified as the cause of over 20% of cancer-related deaths, marking the urgent need for effective treatment options. Innovative therapeutic targets are essential to address this pressing medical issue. Given the recent scientific interest in D-amino acids due to their physiological roles and implications in disease pathogenesis, including cancer, these molecules hold promise for uncovering the underlying mechanisms of cancer cachexia.

Method. To investigate the potential role of D-amino acids in cancer cachexia development, their plasma levels in both cachectic and non-cachectic pancreatic cancer patients were measured using liquid chromatography coupled with tandem mass spectrometry. Furthermore, the impact of D-serine, D-alanine, D-glutamine, and D-aspartate on skeletal muscles was investigated using the C2C12 mouse skeletal muscle cell line. Cell morphology was assessed via live cell imaging, and gene expression of 19 relevant genes (including those related to skeletal muscle function, D-amino acid metabolism, and cachexia) was analyzed using qPCR. Each D-amino acid exposure experiment was conducted three times with triplicates per condition in each experiment to facilitate statistical analysis of the results.

Results. Data analysis and statistical tests are currently underway, with preliminary findings yet to be determined. However, based on existing literature, we hypothesize that certain D-amino acids, particularly D-serine, D-alanine, D-glutamine, and D-aspartate, will exhibit significantly higher plasma levels in cachectic patients compared to non-cachectic patients. Furthermore, we anticipate that exposure to these D-amino acids will induce skeletal muscle cell wasting, characterized by reduced myotube width and altered gene expression profiles.

Conclusion. By elucidating the molecular mechanisms underlying cancer cachexia progression, this study strives to pave the way for the development of targeted interventions that improve patient outcomes and quality of life.

The role of inflammasome activation for T cell migration in Multiple Sclerosis

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Background. Disruption of the blood-brain barrier (BBB) is one of the major pathological hallmarks of multiple sclerosis (MS), facilitating the migration of myelin-specific T cells and promoting neuroinflammation. Adaptive immune responses, mediated by T cells and auto-antigens, form a central aspect of MS pathology. However, alongside the adaptive mechanisms, innate immune responses involving inflammasomal reactions are also thought to be a significant contributor to the pathogenesis. Inflammasomes can be activated via the interaction of Jagged1 (Jag1) with one of its ligands, such as CD46, a protein expressed on BBB endothelial cells. Studies have already explored the connection between inflammasomes in T cells and the effect of inflammasome activation on T cell migration across the BBB. To date, however, the precise relationship between inflammasome activation within T cells and their migration over the BBB remains elusive.

Methods. To examine whether direct contact between T cells and endothelial cells alone induces inflammasome activation or if migration is necessary, inflammasome activation was compared between in vitro migration assays and co-culture setups. Inflammasome activation was studied using ASC specking, flow cytometry, ELISA, and qPCR. Additionally, IL-1β production and Jag1 expression were investigated using immunohistochemistry. Finally, the effect of inflammasome activation in MS pathology was studied in vivo by inducing experimental autoimmune encephalomyelitis (EAE) in wild-type (WT) and NOD-like receptor protein 3 knockout (NLRP3 KO) mice.

Results. Here, we demonstrate that Jag1 and IL-1β are expressed in the brains of relapse-remitting MS patients and EAE mice. Furthermore, there is also an increased Jag1 expression in inflamed brain endothelial cells in vitro. Additionally, the actual migration process over endothelial cells is necessary for inflammasome activation in T cells instead of contact with inflamed endothelial cells alone. Lastly, NLRP3 KO mice showed less severe EAE scores and experienced less weight loss than WT mice, again indicating that the inflammasome plays a pivotal role in EAE.

Conclusion. Collectively, these findings demonstrate that inflammasome activation is imperative for T cell migration across the BBB and aggravates MS severity. Targeting the inflammasome may, therefore, offer a novel therapeutic approach for MS, meriting further research.

In vitro differentiation profile of human periosteum-derived cell aggregates

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Background. Osteoarthritis is a highly prevalent degenerative joint disorder, affecting 500 million individuals worldwide. Osteochondral defects, in which both the cartilage and the subchondral bone are affected, represent a significant risk factor for osteoarthritis. Under physiological conditions, osteochondral defects in long bones can be repaired by a process called endochondral ossification, in which a cartilage template is formed and later replaced by bone. However, when the damage is too severe, this repair mechanism is compromised. In these cases, cell therapy strategies can be employed to overcome this limitation. Previous research has demonstrated that transplanted human periosteum-derived cells (hPDCs) have the potential to repair osteochondral defects, making them a promising cell source for autologous therapies. However, variability in cell behaviour has been observed, limiting their therapeutic implementation. The aim of this study is to investigate the in vitro differentiation profiles of hPDCs from various donors, to further assess the feasibility of using these cells in autologous therapies.

Methods. Cells of 15 patients (15 - 45 years) were isolated from surplus periosteum excised during routine orthopaedic surgeries. Cells were assembled into aggregates and differentiated to simulate the endochondral ossification process. Aggregates were collected at four time points (days 0, 7, 14 and 21) for histological, immunofluorescence and gene expression analysis.

Results. The presence of glycosaminoglycans, collagens and proteoglycans confirmed the successful differentiation of 12 of 15 donors. However, the onset and degree of the extracellular matrix deposition indicate that the differentiation dynamics vary among donors. This was further confirmed by RT-qPCR analysis, where COL2A1, ACAN, ALPL, IBSP and OPN transcripts were also highly variable in their expression levels across donors and time points.

Conclusion. Donor aggregates exhibited diverse profiles of differentiation. Further analyses are warranted to evaluate if this heterogeneity is relevant in the context of the clinical application of hPDCs in autologous therapies.

The neuroregenerative potential of IGF-II as a therapeutic strategy in ischemic stroke

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Background. Ischemic stroke, the second leading cause of global death, claims 3.3 million deaths annually. This condition impairs cerebral blood flow through thromboembolic occlusion of cerebral arteries, causing cell death in the affected area. Despite two existing thrombus removal therapies, patient reach is limited due to stringent eligibility criteria and a constraint treatment timeframe. When therapy is given, many individuals still experience disabilities that significantly impact their quality of life. Consequently, there is an urgent need to explore alternative therapeutic strategies to broaden patient access. Neuroregenerative strategies promote brain repair by enhancing neurogenesis, where neural stem cells (NSC) generate new neurons. Efficient circulation is vital for the viability of newly formed brain tissue, emphasising the importance of angiogenesis, the formation of new blood vessels. Insulin-like growth factor II (IGF-II) exhibits neuroregenerative promise, suggesting IGF-II as a potential therapeutic agent. However, the cellular mechanisms driving this potential remain elusive. This project explores IGF-II's involvement in cellular processes crucial for neuroregeneration following ischemic stroke. It is hypothesised that IGF-II enhances angiogenesis and NSC proliferation and migration in vitro. Moreover, IGF-II is expected to reduce infarct size in a distal middle cerebral artery occlusion (dMCAO) stroke mouse model.

Methods. A proliferation assay determined the effects of IGF-II on NSC proliferation. A scratch-wound-healing assay and a chemotactic transwell assay were used to investigate the effect of IGF-II on NSC migration. The angiogenic potential of IGF-II was investigated in vitro via the tube formation assay using endothelial cells and in ovo using the chick chorioallantoic membrane (CAM) assay. Finally, an in vivo dMCAO mouse model investigated whether IGF-II reduced the infarct size.

Results. This project has found that IGF-II stimulates NSC proliferation and enhances migration via chemotaxis, as demonstrated by the transwell assay. However, the scratch assay indicates that IGF-II did not stimulate direct migration of NSC. Furthermore, IGF-II stimulates tube formation of endothelial cells and increases blood vessel formation in the CAM assay. Finally, it was demonstrated that IGF-II reduced the infarct lesion size in dMCAO mice.

Conclusion. This study assessed the cellular processes influenced by IGF-II to stimulate neuroregeneration after ischemic stroke. While findings suggest that IGF-II is suited as a therapeutic agent for ischemic stroke, further research is required to ascertain its efficacy in stroke treatment.

Unraveling the Brain's Cleanup Crew: TRPV4 in Mitochondrial Movement during Microglial Phagocytosis.

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Background. Microglia, the immune cells of the brain, are dynamic players essential for brain development and health. To maintain brain homeostasis or when the central nervous system is injured, microglia are responsible for phagocytosis of particles such as apoptotic cells. In order for microglia to perform their function, movement is required through cytoskeletal rearrangement, which is mediated by calcium for process motility and extension. In this process, the Transient Receptor Potential Vanilloid 4 (TRPV4) channel, a calcium-permeable cation channel, plays an important role to increase local calcium concentration. As phagocytosis is a dynamic process, a lot of energy is required to facilitate movement of the phagocytic cup, which is the part of the plasma membrane that extends around the particle to engulf the particle. This energy is provided by mitochondria, maternally inherited organelles that have essential roles in energy metabolism and cell survival. Mitochondrial dysfunction can contribute to the development of many neurological diseases such as Alzheimer's disease and epilepsy. Until now, the typical movement of mitochondria during microglial phagocytosis remains elusive. It is necessary to consider the importance of mitochondria in microglia, as they comprise a significant portion of cells in the CNS and perform essential functions in the development and function of the brain. Our research group found that by blocking the microglial TRPV4 channel, cytoskeletal dynamics were decreased. As TRPV4 is a calcium channel, the inhibition of TRPV4 could have a possible effect on mitochondrial dynamics and microglial phagocytosis, offering an opportunity to study the molecular mechanisms underlying mitochondrial movement during phagocytosis.

Methods. Microglia will be isolated from wild type and TRPV4 knockout mice. We will investigate the mitochondrial distribution in homeostatic and phagocytic microglia and whether TRPV4 is present at the phagocytic cup. Additionally, mitochondrial dynamics and movement in homeostatic microglia will be investigated using live cell imaging. A TRPV4 antagonist will be used to block the TRPV4 channel to investigate the role of TRPV4 on the mitochondrial movement. Dynamic parameters will be analysed, including speed, motility, and movement directionality.

Results. We expect TRPV4 and mitochondria to be present at the phagocytic cup site. A significant difference in mitochondrial distribution between homeostatic and phagocytic microglia is expected, and when TRPV4 is blocked, mitochondrial dynamics and movement will decrease in homeostatic microglia.

Conclusion. By understanding the molecular mechanisms of mitochondrial movement in microglial phagocytosis, valuable insights can be obtained into potential disease mechanisms associated with mitochondrial and microglial dysfunction.

Forever Young – The Epigenetic Clock of Oligodendrocyte Precursor Cells in Multiple Sclerosis

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Background. As with many other regenerative processes, aging detrimentally impacts the differentiation capacity of oligodendrocyte precursor cells (OPCs), resulting in a low density of mature oligodendrocytes and significantly contributing to remyelination failure. Progressive multiple sclerosis (PMS) represents the phase of MS characterized by irreversible neurological disability, with age-related OPC differentiation failure linked to disease progression. Nonetheless, despite extensive unraveling of the transcriptome and proteome of aged OPCs, the underlying causes of the aging process largely remain elusive. However, research has revealed global changes in methylation and histone modification patterns in aged OPCs, suggesting a role for epigenetics in the aging process. Yet, the methylome of aged OPCs largely remains undisclosed. Therefore, this study aimed to investigate the role of DNA methylation in the aging process of OPCs and its relevance to PMS.

Methods. An epigenome-wide association study (EWAS) was performed using OPCs derived from newborn and aged (22 months old) mice to study epigenome-wide differences in methylation patterns. Differential methylation was validated using human post-mortem MS samples that were also used for transcriptomic profiling of target genes. This validation process included (i) bulk brain samples analyzed via EWAS, and (ii) lesion-derived OPCs isolated by laser capture microscopy (LCM) and analyzed by pyrosequencing. Furthermore, to study aging at the protein level, immunohistochemistry (IHC) was employed across various types of human post-mortem MS lesions and healthy control brain samples.

Results. Principal component analysis revealed an age-dependent methylomic signature, with nearly 50% of the investigated genetic positions showing differential methylation between young and aged mouse OPCs. Furthermore, aged OPCs generally displayed a higher number of hypermethylated probes, while hypomethylation was more common in young OPCs. Gene ontology analysis revealed differential methylation in senescence-associated genes, such as Cdkn1a, Cdkn2a/2b, and myelin genes, such as Mbp, that were previously linked to OPC aging. On the protein level, IHC showed increased expression of CDKN1A/2A in lesions compared to normally appearing white matter (NAWM) derived NG2+ cells, including active, remyelinated, and chronic inactive MS lesions. LCM experiments and bulk EWAS analyses are ongoing; results will be reported at the conference.

Conclusion. Based on these findings, we have pinpointed new targets linked to aging that might be involved in impaired OPC differentiation. Gaining a deeper understanding of the aging process might reveal new opportunities for targeting and improving remyelination strategies in the context of MS.

NANO-ARD: Utilizing NANOvesicles for accelerating angiogenesis in Acute Radiation Dermatitis

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Background. Radiotherapy (RT) is crucial in treating approximately two-thirds of all cancer patients worldwide. However, it often leads to high rates of adverse events, with acute radiodermatitis (ARD) being a prevalent complication affecting 95% of RT patients. ARD is primarily characterized by excessive reactive oxygen species (ROS) release, damaging skin blood vessels and impairing wound healing. The absence of a universal treatment underscores the objective of this study, which focuses on developing a novel treatment strategy that enhances wound healing post-RT by using ROS-responsive nanocarriers (NCs) loaded with Vascular Endothelial Growth Factor (VEGF) to promote new blood vessel growth. This study aims to analyze the biocompatibility, cellular uptake, and cellular stress response of VEGF-loaded NCs. Moreover, it seeks to evaluate the ROS producer tert-butyl hydroperoxide (tbHP) as a positive control - defined as a control ensuring maximal ROS production without inducing cell death-to develop an in vitro ROS model for evaluating the responsiveness of NCs to ROS.

Methods. To evaluate the ROS production by tbHP, human umbilical vein endothelial cells (HUVECs) were exposed to various concentrations of tbHP. ROS levels were quantified using an H2DCFDA assay, followed by an alamar blue assay to measure cell viability. Moreover, immunofluorescent staining and an Ellman's reagent assay were performed on HUVECs treated with varying concentrations of tbHP, aiming to measure the antioxidant response. HUVEC internalization of Rhodamine B-loaded NCs was evaluated using immunofluorescent staining and FACS, while cytotoxicity of both Rhodamine B and VEGF-loaded NCs was assessed through the Alamar blue assay. Additionally, the effect on cellular stress of both NCs was measured using an H2DCFDA assay.

Results. HUVEC treatment with 50 μM tbHP for three hours produced very high ROS without causing significant cell death. Both the IF staining and Ellman's reagent assay revealed a concentration-dependent increase in signal intensity, indicating a robust activity of the cellular antioxidant system in counteracting ROS. Additionally, HUVECs demonstrated effective uptake of the RhoB-loaded NCs. Furthermore, when administered at low concentrations, both RhoB and VEGF-loaded NCs did not induce cytotoxicity or trigger cellular stress.

Conclusion. The results of this study demonstrate excellent biocompatibility of NCs, with efficient uptake and minimal cellular stress production. Additionally, our findings establish the foundation for developing an in vitro model to assess the ROS responsiveness of the VEGF-loaded NC utilizing tbHP as a potent positive control. These findings provide essential groundwork for advancing a targeted therapy for ARD.

Synergistic sensing:
combining thermal and impedance sensors into the microwell format.

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Multiparameter sensing offers the potential to monitor complex biological processes, such as cell proliferation, by integrating two distinct measuring techniques into the same microwell sensor format. This study addresses the combination of thermal and impedance sensing and the opportunity it provides for performing continuous measurements that are more accurate and sensitive than what could be accomplished with single-parameter sensing. Thermal sensing utilizes the Transient Plane Source (TPS) method to evaluate the thermal properties of a sample. The TPS method was validated in the microwell format by monitoring cell count and metabolic activity. In comparison, impedance sensing employs Electrochemical Impedance Spectroscopy (EIS) to assess the electrical properties of a sample. Previous studies have confirmed EIS's potential to offer insights into cell viability, morphology, metabolic activity, medium condition, and responses to external stimuli. While these applications hold promise, they are considered advanced and require validation within this multiparameter sensing platform. The integration of thermal and impedance sensing for the continuous monitoring of the suspension medium (NaCl) concentration, independent of yeast (Saccharomyces cerevisiae) cell count, will be explored. Continuous monitoring could offer a higher measurement resolution than existing point measurement techniques and provide real-time insights. It is intended to combine thermal and impedance sensing data by deploying multivariate modeling to develop a predictive model for independently estimating suspension medium concentration or yeast cell count. This approach allows for incorporating multiple dependent variables, and their influences, on multiple independent variables, thereby providing predictive capabilities to the monitoring system while using both sensors in a synergistic manner. This proof of application study lays the foundation for applying this multiparameter sensing platform with human cells, possibly providing a cheap, label-less, and non-invasive technique for continuous cell culture monitoring in various fields, such as biotechnology, pharmaceuticals, and medical research.

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