NOVA Medical School

RESEARCH SYMPOSIUM

6-7 OCTOBER, 2025

NOVA MEDICAL SCHOOL

ABSTRACT BOOK

Welcome

It is with great pleasure that we welcome all speakers and participants to the NMS Research Symposium 2025. This year's event will take place over two days, October 6th and 7th, at NOVA Medical School (NMS).

The symposium is divided into four thematic sessions, each focusing on a distinct area of research:

- Session 1 – Stem Cells, Development, and Neuromuscular (Dys)function
- Session 2 – Brain, Vision, and Neurological (Dys)function
- Session 3 – Cardiometabolic and Systemic Health (Dys)function
- Session 4 – Cancer and Health Promotion

Each session will include selected oral presentations, joint discussions, and poster sessions, creating a dynamic platform to share ongoing research, foster collaborations, and promote scientific excellence within our community.

We would like to take this opportunity to thank all the speakers for their valuable contributions and all participants who have submitted their work to be part of this scientific event. We look forward to two days of stimulating discussions, knowledge sharing, and the continued strengthening of ties within the medical and biomedical research communities at NMS.

Organizing Committees:

- **PhD Committee**
  Cristina Demelas, Diogo Sequeira, Jéssica Cabrita, Luana Macedo, Margarida Brotas

- **Post-Doctoral Committee**
  Tatiana Burrinha, Rafael Carecho, Juliana Gonçalves, Luísa Santos

SCIENTIFIC PROGRAM

PROGRAM

6th OCTOBER

9h00-9h45 | Registration

9h45-10h00 | Opening session (Miguel Seabra, Coordinator of the PhD Programme in Health Sciences)

- **Session 1 - Stem cells, development and neuromuscular (dys)function**

10h-11h | Keynote session 1 - **Leonor Saúde, GIMM** (45min + 15min Q&A): "Swimming towards healing: zebrafish as a model for spinal repair"

11h-11h30 | Coffee break

11h30-12h30 | Selected oral communications from abstracts & discussion (4 oral presentations 10min+5min)

- **Ana Rita Colaço**: "From injury to recovery: mechanisms for adaptive motor plasticity upon leg amputation":
- **Tiago Batista**: **"**A Mediator-dependent hypertranscriptional program governs neural stem cell fate decisions *in vivo";*
- **Micael Almeida:** "DAND5 PSCS Models, Bioinformatics discovery and functional validation of downstream enablers of cardiomyocyte differentiation";
- **Dúnio Pacheco: "**Preserving joint line orientation in TKA improves shortto mid-term outcomes: a systematic review and meta – analysis".

12h40-13h | Presentation of NOVA Doctoral School - Catarina Ruivo, NOVA Doctoral School

13h-14h | Lunch break

- **Session 2 - Brain, Vision, and Neurological (dys)function**

14h-15h | Keynote session 2 - **Patrícia Monteiro, FMUP** (45min + 15min Q&A): "Turning Discovery into Therapy: Lessons from Autism Preclinical Research"

15h-16h | Selected oral communications from abstracts & discussion (4 oral presentations 10min+5min)

- **Beatriz Felgueiras**: "In Vitro Modeling of Early Diabetic Retinopathy with Retinal Organoids for Drug Discovery";

- **Luis Sousa**: "Machine learning-based gait analysis identifies insulin-degrading enzyme as key link between diabetes and Parkinson's disease";

- **Inês Almeida**: "Carotid Body Cryoablation as a Strategy to Modulate Parkinson's Related Phenotypes in Thy1-aSyn Transgenic Mice";

- **Ana Rosa Abreu: "**Intranasal administration as a promising route for the treatment of Trigeminal Neuralgia"

16h-18h | Coffee break & Poster Session

7th OCTOBER

9h30-10h | Registration

- **<u>Session 3 - Cardiometabolic and systemic health (dys)function</u>**

10h-11h | Keynote session 3 - **Paulo Oliveira, CNC** (45min + 15min Q&A): "Mitochondrial Follies in Cardiac Aging and Dysfunction"

11h-11h30 | Coffee break

11h30-12h30 | Selected oral communications from abstracts & discussion (4 oral presentations 10min+5min)

- **Inês Ferreira**: "Disrupted Macrophage Homeostasis by Oxidized Lipids: Uncovering Mechanistic Links to Atherosclerosis";

- **Sílvio Leal**: "Unveiling the OPTI-PE Program: Insights into Hemodynamic Outcomes of Catheter-Derived Therapy for Acute Pulmonary Embolism";

- **Adriana Capucho**: "Carotid Bodies: Innovative Players in Feeding Behavior Modulation";

- **Estefania Torrejón**: "EV-Net: Extracellular vesicles-mediated intercellular communication networks in disease pathophysiology".

12h30-14h | Lunch break

- **<u>Session 4 - Cancer and Health Promotion</u>**

14h-15h | Keynote session 4 - **Ana Luísa Correia, Champalimaud Foundation** (45min + 15min Q&A): "Can we keep cancer asleep?"

15h-16h | Selected oral communications from abstracts & discussion (4 oral presentations 10min+5min)

- **Bruna Correia**: “Exploring the role and therapeutic value of immunosuppressive low-density neutrophil subsets in breast cancer”

- **Maria Reis**: “Early detection of hepatocellular carcinoma: A pilot-validation of a liquid biopsy tool”;

- **Isabel Lemos**: “Cell-free DNA (cfDNA) drives metabolic remodeling modulating cancer cell proliferation, quiescence, and chemoresistance;
- **Ricardo Rodrigues**: “Macrophages influence response to combined Dabrafenib plus Trametinib therapy in Anaplastic Thyroid Cancer”

16h-18h | Coffee break & Poster Session

18h-18h20 | Closing session - Miguel Seabra, Coordinator of the PhD in Health Sciences; Paula Macedo, Coordinator of the PhD in Biomedicine, NOVA Medical School

KEYNOTE SPEAKERS

Leonor Saúde, GIMM

"Swimming towards healing: zebrafish as a model for spinal repair"

6th OCTOBER - 10h-11h

Keynote Session 1 - Stem cells, development and neuromuscular (dys)function

Patrícia Monteiro, FMUP

"Turning Discovery into Therapy: Lessons from Autism Preclinical Research"

6th OCTOBER – 14h-15h

Keynote Session 2 - Brain, Vision, and Neurological (dys)function

Paulo Oliveira, CNC

"Mitochondrial Follies in Cardiac Aging and Dysfunction"

7th OCTOBER – 10h-11h

Keynote Session 3 - Cardiometabolic and systemic health (dys)function

Ana Luísa Correia, Champalimaud Foundation

" Can we keep cancer asleep?"

7th OCTOBER – 14h-15h

Keynote Session 4 - Cancer and Health Promotion

ORAL PRESENTATIONS

From injury to recovery: mechanisms for adaptive motor plasticity upon leg amputation

Ana Rita Colaço[1], Ricardo Custódio[1], Raquel Laranjeira[1], Inês Fernandes[1], Marta Santos[1], Anna Hobbiss[1,2], and César Mendes[1].

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[2]Champalimaud Research, Champalimaud Center for the Unknown, Lisboa, Portugal

Coordinated locomotion relies on a conserved neural network involving motor neurons, central pattern generators and higher control centers, enabling efficient movement and responses to the environment. When disrupted by injury, this system undergoes motor recovery to restore function. However, the underlying genetic and neural mechanisms remain poorly understood, although a thorough understanding is essential for neurorehabilitation. To characterize motor recovery, wild-type flies underwent middle-leg amputation and were analyzed in the FlyWalker, a detailed kinematic analysis system. Flies could walk immediately post-injury, gradually adopting a more coordinated gait by refining stepping patterns and improving stability. Recovery was age-dependent with older flies showing reduced motor control and impaired recovery. To explore genetic contributions, we assessed mutants for learning and memory genes. Silencing key CaM-CREB pathway players known for olfactory learning, such as rutabaga, dunce and pka, we observed a reduced recovery, suggesting this pathway also drives motor plasticity. Ongoing work is exploring if activity-based therapies modulate recovery. Using optogenetics to activate neurons that promote or inhibit walking, we are subjecting amputated flies to exercise protocols following injury - or alternatively, prevent movement. We hypothesize that motor training will enhance recovery, while limiting movement will impair neuromuscular adaptation and worsen the outcome

A Mediator-dependent hypertranscriptional program governs neural stem cell fate decisions *in vivo*

Tiago Baptista[1], Catarina CF Homem[1]

[1]iNOVA4Health, NOVA Medical School | Faculdade de Ciências Médicas, NMS|FCM, Universidade Nova de Lisboa, Lisboa, Portugal.

Hypertranscription – a global increase in gene expression relative to differentiated cells – is a hallmark of stem cell identity, but its in vivo regulation and function remain unclear. Using Drosophila neural stem cells (neuroblasts), we investigated the role and regulation of hypertranscription in vivo. Single-cell transcriptomics, chromatin assays, and RNA quantification revealed that neuroblasts have significantly higher transcriptional activity than their progeny. We identified the evolutionary conserved Mediator complex as a key regulator of this hypertranscriptional. Mediator is broadly enriched across neuroblast chromatin, and its loss causes a marked, neuroblast-specific drop in transcription, with minimal effects in differentiated cells. This establishes Mediator as a bonafide driver of hypertranscription. Functionally, Mediator is required for proper lineage progression: its depletion blocks differentiation and leads to stem-like cell accumulation, showing that hypertranscription is critical for exit from the stem cell state. Surprisingly, disrupting metabolism, another known stem cell regulator, has little effect on transcription, indicating that hypertranscription is not simply a metabolic byproduct. Overall, our work identifies Mediator as a central regulator of hypertranscription in both normal and tumorigenic neural stem cells, essential for neural differentiation and stem cell fate transitions in brain development.

DAND5 PSCS models, bioinformatics discovery and functional validation of downstream enablers of cardiomyocyte differentiation

Micael de Jesus Almeida[1], Sofia de Sousa Torres[1], José M. Inácio [1], Matthias Erwin Futschik[2], José António Belo [1].

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[2]Centro de Inovação em Biomedicina e Biotecnologia, Universidade de Coimbra, Portugal.

DAND5 expression in the heart is unique among the Cer/DAN family, acting as an antagonist of Nodal and Wnt signaling and playing a key role in pluripotent stem cell (PSC)-derived cardiomyocyte (CM) differentiation. Reduced DAND5 levels increase CM proliferation, increase the number of cardiovascular progenitor, and prolong their progenitor state. To uncover the mechanisms behind these effects, we performed a bioinformatic analysis of mouse and human cardiac development transcriptomes. Using machine learning, we identified transcription factors (TFs) correlating with known cardiac regulators like Hand2, Gata4, Tbx5, and Nkx2-5, and their involvement in cardiac regulons. By integrating PubMed publication data, we predicted the importance and novelty of candidate TFs. These were cross-referenced with RNAseq data from WT and Dand5 KO mESC-derived CMs. Selected under-characterized candidates were functionally validated through lentiviral-carrying specific plasmid knockdown in mESCs, followed by spontaneous differentiation. RT-qPCR and beating assays showed altered cardiac marker expression and reduced contractile activity, supporting their role in cardiogenesis.

Preserving joint line orientation in TKA improves short-to mid-term outcomes: a systematic review and meta – analysis

Dúnio Jácome-Pacheco[1,2], Tiago Torres[1], Gonçalo Rodrigues[1], Pedro Diniz[3,4], Francisco Guerra-Pinto[1], António Camacho[2,5], João Gamelas[2,6], Romain Seil[3], Michael Hirschmann[7]

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[2]NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal;
[3]Luxembourg Institute of Research in Orthopaedics, Sports Medicine and Science (LIROMS), Luxembourg, Luxembourg;
[4]Department of Bioengineering, iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal;
[5]Centro de Responsabilidade Integrado de Traumatologia Ortopédica (CRI-TO) do Centro Hospitalar Universitário de Lisboa Central (CHULC), Lisbon, Portugal.
[6]Direção Clínica Hospitalar da Unidade Local de Saúde de Lisboa Ocidental, Lisbon, Portugal.
[7]Department of Orthopaedic Surgery and Traumatology, Kantonsspital Baselland, Bruderholz, Switzerland

Purpose

Joint line orientation (JLO) has been identified as a potential factor influencing clinical outcomes following total knee arthroplasty (TKA). This systematic review and meta-analysis aimed to assess whether preserving the JLO according to the individual knee phenotype is associated with improved clinical and functional outcomes. We hypothesized that joint line preserving (JLP) techniques would result in superior patientreported outcome measures (PROMs) and better functional performance compared to non–joint line preserving (nJLP) approaches.

Methods

A systematic search of Pubmed, CENTRAL, and Web of Science was conducted to identify comparative studies evaluating JLP versus nJLP in TKA. Studies reporting PROMs and other clinical indicators with a minimum follow-up of 12 months were included. Risk of bias was assessed using the RoB 2 tool for randomized trials and the ROBINS-I tool for non-randomized studies. Meta-analyses were performed for PROMs and range of motion, with subgroup analyses based on study quality.

Results

Forty-three studies were included in the qualitative analysis, and 38 in the meta-analysis. The Forgotten Joint Score (MD: 7.59), Knee Function – Knee Society Score 2011 (MD: 6.48), Knee Injury and Osteoarthritis Outcome Score (MD: 2.74) and Oxford Knee Score (MD: 1.02) all showed statistically significant differences favoring JLP. Most subgroup analysis of low and low-to-moderate risk of bias studies further supported these effects.

Conclusion

Joint line preservation in TKA is associated with short- to mid-term improvements in PROMs and other clinical outcomes. While the effect may vary across patient populations, these findings support the relevance of JLO in optimizing functional results. A more comprehensive and standardized phenotypic approach could be key to better identifying the subgroups that benefit most from this strategy

In Vitro Modeling of Early Diabetic Retinopathy with Retinal Organoids for Drug Discovery

Beatriz Felgueiras[1], Luana Macedo[1], Daniel Pereira[1], Cláudia Nunes dos Santos[2], Sandra Tenreiro[1]

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[2]NIMBS, Institute for Medical Systems Biology, Universidade Nova de Lisboa, Lisbon, Portugal

Diabetic Retinopathy (DR) is a leading cause of blindness in the working-age population. Before vascular changes manifest, early alterations contribute to vision loss, driven by degeneration of specific retinal neuronal populations and inflammation. We established an early-stage DR model using retinal organoids (ROs) differentiated from human induced pluripotent stem cells (hiPSCs). This model mimics the neurodegeneration, inflammation, and glial reactivity seen in DR-affected human retinas [1] (Pending Patent). We aim to leverage this model to identify novel anti-inflammatory and neuroprotective compounds, exploring promising human phenolic metabolites (HPM). HPMs are found in circulation after the ingestion of fruits and vegetables, are devoid of toxicity at the circulating levels and cross the blood-brain barrier [2]. We observed that the inflammation marker monocyte chemoattractant protein-1 (MCP1) secretion is increased in ROs with 120 days of differentiation exposed to high glucose. However, ROs treatment with HPM07 reduces MCP-1 levels. Increased glial reactivity in Müller cells in glucose induced ROs, was also reduced by HPM07 treatment. These findings indicate ROs as a promising DR pre-clinical model, enabling novel drug testing for early disease intervention. However, ROs have limitations, including the absence of a vascular system and microglial cells. Therefore, in parallel we aim to engineer vascularized ROs (fVROs) incorporating microglia. The incorporation of iPSCs derived microglial cells and the optimization of vascular units (VUs) using retinal cell lines for ROs fusion are underway. These efforts aim to develop a more physiologically relevant DR model.

Machine learning-based gait analysis identifies insulin-degrading enzyme as key link between diabetes and Parkinson's disease

Sousa L.[1], Pascoal S.[1], Luzio B.[1], Chôcho S.[1], Tavares C.[1], Lopes C.[1], Almeida I.[1], Fernandes C.[1], Carecho R.[1], Machado de Oliveira R. [1], Macedo P. [1], Mendes C.[1], Vicente Miranda H.[1]

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Parkinson's disease (PD) involves complex gait dysfunction that emerges before classic motor symptoms, with epidemiological data showing an HR 3.8 in young diabetic adults. Using the MouseWalker system, we analyzed 86 gait parameters in Thy1-aSyn transgenic (Tg) mice - a model of human aSyn overexpression driving PD-like phenotypes - with or without high fat diet (HFD) to assess diabetes contribution. Genotype effects dominated gait changes, with Tg animals showing increased 3-leg swing and altered mediolateral positioning. HFD produced complex non-linear effects where WT mice simplified coordination while Tg mice showed amplified genotype features alongside divergent patterns. Insulin-degrading enzyme (IDE) dysfunction likely links diabetes to PD, as Type-2 diabetes patients present increased pancreatic aSyn pathology. IDE KO mice showed motor impairment in pole test, increased lateral swing and altered spatial limb placement. Neural networks and random forests trained on Thy1 data achieved 82-95% classification accuracy. Cross-model classification revealed IDE KO signatures were partially recognized by our models, with gait changes showing 89.3% similarity to Tg patterns, suggesting IDE underlies aSyn pathology and gait dysfunction. These findings establish IDE dysfunction as a potential PD trigger while demonstrating automated gait analysis as a sensitive biomarker platform with high translational potential for early detection.

Carotid Body Cryoablation as a Strategy to Modulate Parkinson's Related Phenotypes in Thy1-aSyn Transgenic Mice

Inês, F. de Almeida[1], Fernandes C.[1], Carecho R.[1], Pascoal S.[1], Sousa L.[1], Vilares Conde S.[1], Vicente Miranda H.[1]

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Metabolic alterations, including disrupted glucose regulation and insulin signalling, are recognised as early non-motor features of Parkinson's disease (PD). To explore these changes, we performed glucose and insulin tolerance tests (GTT, ITT) in Thy1-aSyn transgenic (Tg) mice, a model of PD-like α-synuclein overexpression, at 9, 22, and 32 weeks, with or without carotid body cryoablation (Cryo) at 24 weeks. WT mice consistently showed greater weight and age-related increases in fasting glycemia, while Tg mice gained weight more slowly and exhibited progressively lower basal glucose. At 32 weeks, Tg Cryo mice exhibited fasting basal glycemia comparable to WT, with the lowest peak glycemia and the fastest return to baseline after GTT. In ITT, Tg Cryo mice exhibited lower peak glycemia compared to Tg sham, along with moderately elevated basal levels, leading to an overall glycemic response (AUC) comparable to WT. These effects suggest that carotid body ablation modulates insulin responsiveness and partially restores a WT-like metabolic pattern in Tg mice. These findings align with reports of reduced body weight, lower leptin/insulin levels, and altered metabolism in Thy1-aSyn mice. Cryoablation further reinforced these changes, supporting the role of the carotid body (CB) in systemic glucose regulation via sympathetic pathways. These results suggest that CB activity modulation may represent a novel strategy to correct metabolic dysfunction in PD mouse models.

Intranasal administration as a promising route for the treatment of Trigeminal Neuralgia

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Trigeminal neuralgia is a chronic and debilitating pain condition, characterised by severe, recurrent pain attacks. The first line of treatment is the anticonvulsant drug carbamazepine which often fails to provide lasting relief and is associated with dose-limiting side effects.

To study the potential of intranasal administration as a treatment for this condition, female Wistar rats were subjected to chronic constriction injury of the infraorbital nerve. Both carbamazepine and a novel compound were administered intravenously and intranasally, and the resulting analgesic effects were quantified through mechanical sensitivity assays. Intranasal administration provided greater and longer-lasting relief, proving to be a viable route for managing trigeminal pain, while being welfare-friendly and non-invasive.

Compared to systemic routes, intranasal delivery offers several pharmacokinetic and anatomical benefits for compound delivery since the compounds are quickly absorbed specifically into the trigeminal system through the trigeminal nerve pathway. These results provide preclinical evidence supporting intranasal administration as an alternative for a more effective and targeted approach to treat this highly disabling and poorly managed pain syndrome.

Disrupted Macrophage Homeostasis by Oxidized Lipids: Uncovering Mechanistic Links to Atherosclerosis

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An early characteristic of atherogenesis is the uptake of lipids in the lysosomal compartment of cells in the arterial intima originating “pathogenic” foam cells. This results from unregulated endocytosis and phagocytosis of poorly digestible modified lowdensity lipoproteins (oxLDL) by macrophages. This event has multiple deleterious effects, including the disruption of lysosome function with consequences to cell homeostasis. We have identified a family of oxidized lipids, cholesteryl hemiesters (ChE), present in the plasma of cardiovascular disease patients. Among these, cholesteryl hemiazelate (ChA) and cholesteryl hemiglutarate (ChG) stand out, with ChG exhibiting heightened toxicity relatively to ChA. Similarly to ChA, ChG induces lysosomal dysfunction in murine macrophage since it promotes a decrease of mature Cathepsin D. However, in contrast to ChA, which has been shown to alkalinize lysosomes, ChG causes lysosomal acidification. Despite these opposing effects on lysosomal pH, both lipids disrupt mitochondrial function, with ChG exerting a more pronounced impact. These findings indicate that lysosomal dysfunction induced by ChA and ChG may occur through distinct mechanisms. Furthermore, the decrease in intracellular iron levels in ChG-treated cells suggests that ChG may trigger dysfunction through a process linked to disrupted iron metabolism. This research may shed light on novel pathways underlying atherogenesis, presenting potential targets for therapeutic intervention in cardiovascular disease.

Unveiling the OPTI-PE Program: Insights into Hemodynamic Outcomes of Catheter-Derived Therapy for Acute Pulmonary Embolism

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Background: Catheter-derived therapy (CDT), including mechanical thrombectomy (MT) and intrapulmonary thrombolysis (IT), offers a novel approach for managing acute pulmonary embolism (PE). However, evidence on its efficacy and safety is limited. The Optimization of Percutaneous Treatment In acute Pulmonary Embolism (OPTI-PE) program aims to enhance understanding and optimize these therapies by evaluating patient outcomes. A sub-study was initiated to assess immediate and long-term hemodynamic effects through invasive parameter evaluations before, after CDT, and during follow-up (FUP).

Methods: Dual-center analysis of patients treated with CDT for acute PE from December 2019 to July 2025. The primary efficacy endpoint was a >15% reduction in the right ventricle to left ventricle (RV/LV) ratio within 48 hours post-procedure. The safety endpoint focused on major adverse events (MAE) within the same timeframe. A systematic hemodynamic sub-study began in mid-2024, involving left and right heart catheterization at baseline, immediately after CDT, and between 6 and 12 months post-procedure.

Results: We enrolled 128 patients (mean age 61±16 years; 58% female). All presented with elevated cardiac biomarkers and right ventricular strain, with 21.3% in cardiogenic shock. A >15% RV/LV ratio reduction was seen in 89.8% of patients (average -28.3%; p<0.001). MAE occurred in 9.4% of patients, primarily due to device-related adverse events (5.5%), including 4 deaths (3.1%). Between the 29 patients submitted to invasive hemodynamic evaluation, cardiac output (CO) and cardiac index (CI) showed no significant changes intraprocedure (COΔ 6.6%, p=0.24; CIΔ -1.9%, p=0.42) but improved at FUP (COΔ 11.0%, p=0.04; CIΔ 3.2%, p=0.27).

Conclusion: CDT for acute PE demonstrated good efficacy and safety. Hemodynamic improvement may not be immediate but rather progressive during follow-up. Completing the OPTI-PE study may reinforce these findings.

Carotid Bodies: Innovative Players in Feeding Behavior Modulation

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A deep understanding of the neurohumoral peripheral-brain networks controlling satiety pathways may lead to new strategies to prevent obesity. Preliminary data from our lab showed that resection of the carotid sinus nerve (CSN) decreases caloric intake in hypercaloric animal models. Moreover, knowing that carotid bodies (CBs) respond to several peripheral metabolic mediators, and that the activity of these organs is integrated in the brain, the goal of this PhD project is to disclose if CBs play a role in the body-brain axis regulating satiety. Herein, it was tested if CBs can respond acutely to different nutritional stimuli. Different nutritional stimuli were administrated via an intragastric catheter in mice: 1) Saline; 2) Fortimel; 3) Smof lipid (20% and 10%); 4) Sucrose; and 5) Glucose, (n=8-10 per group) and CBs output was assessed in vivo, by monitoring ventilatory parameters in sham and CSN-resected animals.

Intragastric injection of smof lipid 20% and glucose leads to an increase in tidal volume and minute ventilation, effects reverse by CSN resection and not observed with the other nutritional stimuli. To elucidate the brain regions regulating satiety associated with these responses, brains were collected at the end of the experiments and activation of AgRP and POMC neurons were assessed using cfos. These results suggest that CBs are able to distinguish between different nutritional stimuli that are integrated in the hypothalamus.

EV-Net: Extracellular vesicles-mediated intercellular communication networks in disease pathophysiology

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Extracellular vesicles (EVs) are key mediators of cell-to-cell communication (CCC) and display distinct molecular signatures across various diseases. Despite abundance of publicly available EVs omics datasets, tailored network-integration tools for studying EVs-mediated CCC remain scarce. We adapted the CCC analysis tool NicheNet to investigate EVs-mediated CCC. Nichenet predicts ligand-target regulatory potential by relying on ligand-receptor interactions. However, EVs engage with the recipient cells via mechanisms beyond ligand-receptor binding. To account for this, we developed EV-Net, applying Nichenet's scoring algorithm not only using ligands as starting nodes, but also including intermediary signaling proteins and transcription factors. As a use case, we investigated the effect of prediabetic-gut derived EVs on Kupffer cells. We used proteomics data from gut-derived EVs in prediabetic and healthy mouse models, and Kupffer cells RNA-seq data from a public atlas. Using EV-Net, we explored a subset of EVs proteins more abundant in the prediabetic condition. From those, Selenocysteine Lyase (SCLY), a protein involved in managing oxidative stress, showed high regulatory potential on numerous Kupffer cell genes, suggesting a role in mitigating liver dysmetabolism in prediabetes. Our adaptation of NicheNet, EV-Net, expands its applicability to EV-mediated CCC, offering a novel approach to study EVs effects on recipient tissues across healthy and disease states.

Exploring the role and therapeutic value of immunosuppressive low-density neutrophil subsets in breast cancer

Bruna F. Correia[1], Daniela Grosa[1], Rute Salvador[2], Telma Martins[1,3], Marina Vitorino[1,3], Carolina X. Sousa1,4, Sofia Braga[1,5], António Jacinto[2] and M. Guadalupe Cabral[1]

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Despite progress in breast cancer (BC) treatment, current options often fail in the metastatic setting, stressing the need for new strategies. Neutrophils, particularly low-density neutrophils (LDN), have emerged as key players in tumor progression and immune evasion. While circulating high-density neutrophils (HDN) exhibit antitumor properties, LDN promote metastasis and suppress immunity. We previously reported significant LDN accumulation in BC patients, associated with advanced disease and poor prognosis. Here we further explored the immunosuppressive and protumor functions of LDN in BC progression. We analyzed HDN and LDN populations isolated from 151 BC patients' blood. Functional assays and 3D co-cultures revealed that, unlike HDN, LDN lack cytotoxicity against BC cells but strongly impair T-cell activation through increased PD-L1 expression and soluble mediators, including arginase. Moreover, LDN-conditioned media enhanced tumor invasiveness, likely via increased MMP-9 expression, highlighting their pro-metastatic role. Notably, we identified within LDN a subset expressing the chemokine receptor X, not previously reported in neutrophils. This X+LDN subset was enriched in metastatic BC patients, displaying higher immunosuppressive capacity, increased migration to tumor-derived chemokines, and association with poor outcomes, suggesting their contribution to BC progression and therapy resistance. Our findings highlight neutrophil heterogeneity and suggest LDN subsets, such as X+LDN, as biomarkers to predict therapy response and disease outcome, and as targets
to improve metastatic BC care through more personalized treatment.

Early detection of hepatocellular carcinoma: A pilot-validation of a liquid biopsy tool

Maria Gonçalves-Reis[1], Migla Miskinyte[1], Luísa Martins Figueiredo[2], Rita Carvalho[2], Joana Carvalho Branco[2], Mariana Nuno Costa[2], Mariana Ferreira Cardoso[2], Gonçalo Alexandrino [2], António Figueiredo[3], Miguel Ramalho[4], Emanuel Gonçalves[1,5], José B. Pereira-Leal[1], Alexandra Martins[2], Joana Cardoso[1]

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Hepatocellular carcinoma (HCC) is the most prevalent liver cancer, mainly associated with cirrhosis. Early detection is essential for better outcomes and survival, but current screening methods have low sensitivity. Blood-based liquid biopsies (LB), particularly tumour-derived cell-free DNA (cfDNA), are a promising alternative with specific methylation patterns showing potential for HCC screening. This study aims to identify a novel DNA methylation signature in cfDNA from patients with cirrhosis for early HCC detection, developing a qPCR tool. A methylation signature based on differentially methylated regions (DMRs) was previously derived through in-silico analysis of publicly available data. A pilot cohort of 60 patients with cirrhosis or cirrhosis-derived HCC was prospectively collected to analyse this signature and identify the top DMRs. Formalin-fixed paraffin-embedded (FFPE) tissue samples were also included in the analysis. A subset of 38 previously identified DMRs capable of detecting HCC in a cirrhotic background was analysed using Next-Generation Sequencing (NGS) on the cfDNA and FFPE samples. Some of these NGS-filtered DMRs were further analysed by qPCR after bisulfite conversion, with cfDNA from cirrhosis and cirrhosis-related HCC patients revealing differential methylation between the two patient groups. Preliminary results indicate that the cfDNA-based LB test shows promise for early HCC detection.

Cell-free DNA (cfDNA) drives metabolic remodeling modulating cancer cell proliferation, quiescence, and chemoresistance

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Cell-free DNA (cfDNA) is a promising tool for cancer diagnosis and monitoring, but its biological role in tumor progression remains unclear. As a potential systemic signal, cfDNA may regulate metabolism and cellular functions that support disease progression. Our aim is to unravel the role of cfDNA in cancer cells metabolic regulation, contributing to cancer establishment and progression. CfDNA was isolated from the conditioned media of breast cancer (MDA-MB-231) and ovarian cancer (ES2) cell lines cultured under varying glucose conditions. Four distinct cfDNA variants, based on glucose availability and culture duration, were assessed for their effect on metabolic profiles and cell features in new cell cultures. In MDA-MB-231 cells, cfDNA modulated metabolic adaptation by altering glucose and glutamine uptake, while in ES2 cells, it induced metabolic profiles resembling the cell-of-origin of cfDNA. Certain cfDNA variants promoted cisplatin resistance in a cell- and condition-specific manner. TLR9 expression declined over time in both cell models, with opposite trends in TLR9-LAMP1 colocalization, increased in ES2 and decreased in MDA-MB-231, indicating cell-specific cfDNA dynamics. Prolonged cfDNA exposure (4 weeks) led to quiescence in a subset of cells, highlighting its lasting influence on tumor behavior. Overall, our findings identify cfDNA as an active player of the tumor microenvironment, driving metabolic shifts, intercellular communication, and phenotypic plasticity.

Macrophages influence response to combined Dabrafenib plus Trametinib therapy in Anaplastic Thyroid Cancer

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Anaplastic thyroid cancer (ATC) is a rare and aggressive malignancy with poor response to treatment. While Dabrafenib plus Trametinib (DT) has improved the outcomes in BRAF+ ATC, resistance remains a challenge. In ATC, macrophages are associated with poor prognosis. Our group identified SPRY4, a MAPK pathway inhibitor, as a potential biomarker in ATC–macrophage interplay. By establishing transwell co-cultures using three BRAF+ ATC cell lines (T235, T238 and T235-DTR - an in-house T235 DT resistant) with THP-1-macrophages, we investigated the role of SPRY4 and macrophages in ATC response to DT. DT significantly reduced the viability of all ATC cells, however, co-culture with macrophages under DT restored the viability to levels comparable or exceeding the untreated monoculture controls. SPRY4 gene/protein and MAPK/ERK signalling (pERK1/2) were significantly downregulated by DT but markedly rescued by macrophages. Macrophages also enhanced ATC invasion, with or without DT, supported by actin cytoskeleton remodelling and vimentin upregulation. DT downregulated PD-L1 in ATC, which was reversed in co-culture. CD68, CD80 and CD163 analyses unveiled macrophage activation and partial M2-like polarization under co-culture, independent of DT. Immunohistochemistry for SPRY4 and tumour-infiltrating immune cells in ATC from patients under DT, is ongoing. Our findings suggest that macrophages may play a pivotal pro-tumoural role in modulating ATC cell responses to DT, highlighting the ATC–macrophage axis as a potential target to improve DT efficacy.

POSTER PRESENTATIONS

Session 1 - *Stem cells, development and neuromuscular (dys)function*

P1: Mechanisms for the regulation of Non-Centrosomal Microtubule Organizing Centres in the Drosophila germ-line

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Microtubules (MTs) are core components of the eukaryotic cytoskeleton, playing essential roles in cell shape, polarity, migration, and division. Defects in the MT cytoskeleton are linked to diseases like neurodegeneration, microcephaly, infertility, and cancer, though the molecular mechanisms remain largely unknown. The geometry of MT networks depends on the localization of sites called microtubule organizing centres (MTOCs), which are responsible for nucleate, anchor, or stabilize MTs. The best-studied MTOC is the centrosome which is considered to be the major MTOC in dividing cells, where it is critical to nucleate the MTs for the assembly of the mitotic spindle. However, upon mitotic exit or differentiation, the activity of the centrosome as a MTOC is generally attenuated and in extreme cases it can be eliminated. This is often associated with remodeling of the MT cytoskeleton into cell type-specific MT arrays, with assignment of MTOC function to other places in the cell. Such non-centrosomal MTOCs (ncMTOCs) can occupy large surfaces, such as the nuclear envelope in muscle cells or the apical surface of the epithelial cells. In contrast to centrosomes, we know very little about the composition and function of ncMTOCs. Using Drosophila germline cells as a model, we investigated ncMTOC assembly after centrosome inactivation. We found that the centrosomal MT regulator WDR-62 localizes ncMTOCs both in oocytes and follicular cells. We performed functional studies in the oocytes, which strongly suggest that WDR-62 is required for the organization of the MT cytoskeleton. Additionally, WDR-62 and the conserved ncMTOC component, Shot, are interdependent for their localization at ncMTOCs, suggesting that WDR-62 is a core ncMTOC component. We are currently investigating the function of WDR-62 at ncMTOCs. Wdr62 is the second most common genetic cause of microcephaly in human patients. Therefore, understanding the role of this protein not only at centrosomes but also at ncMTOCs may provide important insight into its relevance, both in normal development but also in disease.

P2: Mechanisms and role of stochastic muscle contraction during development: the what, the why, and the how

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Coordinated walking is a conserved behavior relying on a neuromuscular system critical for fast, stable, and efficient movement. This system forms during development and allows animals to execute complex motor tasks. Many species, including vertebrates, show rhythmic spontaneous activity during development, thought to be essential for synaptic maturation. However, its origin, regulation, and contribution to walking remain unclear. To study these developmental patterns, we built a long-term imaging pipeline to monitor full pupa development. We designed a 3D-printed cage allowing pupal growth on a flat glass coverslip, enabling high-resolution leg imaging without disrupting development. The setup uses a Raspberry Pi, camera, macro lens, and controlled light/temperature. We also developed a Python script for long-term recording and live detection of movement via pixel change in selected Regions of Interest. To assess synaptic activity, we established a setup for muscle GCaMP imaging. Preliminary data show successful long-term imaging and leg movement detection in wild-type flies, as well as synaptic activity via a GCaMP reporter in muscles. We also demonstrate that leg contraction and muscle activity can be altered by silencing neurons using thermogenetic tools. Future work aims to use the mentioned thermogenetic tools to transiently block neuronal activity at specific developmental periods to examine the role of defined neuronal populations in leg movement and adult walking behavior.

P3: Lactylation in the Drosophila Oocyte Development

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Cell metabolism is essential for energy production, growth, and survival, but metabolites also have nonmetabolic functions as substrates for posttranslational modifications (PTMs) that shape the epigenetic landscape. Histone lactylation, first described in 2019, arises from lactylCoA and modulates gene expression by adding lactyl groups to lysine residues on histones. While its functions in cell fate, embryonic development, inflammation, and cancer have been explored in vitro, in vivo studies remain scarce. Using *Drosophila melanogaster* as a model, we identified robust nuclear lactylation in oocytes throughout oogenesis, with strongest signals when transcription is globally repressed, suggesting a potential role in transcriptional silencing, contrary to what was previously observed for this PTM. We are now performing an RNAibased screen targeting enzymes proposed as "writers" and "erasers" of lactylation to dissect the regulatory machinery controlling this PTM *in vivo*. Our work provides the first characterization of histone lactylation during oogenesis in a wholeorganism model, setting the stage for understanding its physiological relevance in development and establishing Drosophila as a powerful system to study the regulation and function of this emerging epigenetic mark.

P4: Exploring innate locomotion in Drosophila Melanogaster

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Locomotion is a central part of an animal's life. Innate locomotion, the instinctive ability to walk without any prior experience, is crucial for an animal's early survival. While this behaviour is "hard-wired" into neural circuits, it's initial form often requires refinement to allow for a full optimized locomotion. The characteristics of this early movement, as well as the mechanisms responsible for this refinement remain poorly understood. Here we show newly eclosed Drosophila melanogaster can be used as a model to investigate innate locomotion. Using high-resolution kinematics, we described the early walking of newly eclosed flies, as well as their motor activity. Furthermore, we explored the synapse maturation after eclosion of a single motor neuron. Here we found that certain parameters, such as speed and swing duration, show a fast optimization within hours, being conserved across sex and genetic backgrounds, although other features, such as duty factor and body displacement ratio exhibited a higher variability. Surprisingly this optimization occurred alongside a sharp decrease in overall motor activity following an initial burst of activity after eclosion. Lastly, we also found a significant post-eclosion synapse maturation of a single motor neuron innervating a muscle on the distal tibia. Our work supports that innate locomotion is not complete but undergoes a rapid optimization process, that seems to be mostly conserved among sex and genetic backgrounds, and may be underpinned by the structural maturation of the motor circuits. This work introduces a new model that provides a foundation for dissecting the physical, genetic and neural mechanisms that underlie innate behaviour.

Keywords - Drosophila melanogaster; innate locomotion; maturation; kinematics quantification.

P5 Transcriptomic profiling of thymic CD45+ immune cells reveals distinct signatures in myasthenia gravis patients

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Myasthenia gravis (MG) is an autoimmune disorder with pathogenic antibodies targeting the neuromuscular junction. The thymus plays a key role, especially in early-onset and seropositive MG. To understand thymic immune dysregulation, we performed RNA sequencing on CD45+ immune cells from MG and control thymuses. Analysis revealed clear transcriptomic segregation, with hierarchical clustering showing robust group separation, driven mainly by increased gene expression in MG samples. Most differentially expressed genes were upregulated, suggesting enhanced immune activation. Preliminary pathway analyses indicate significant changes in immune signaling in MG thymic leukocytes, supporting thymus-intrinsic immune dysregulation in MG pathophysiology. This study focused on immune cells; stromal cells are being sequenced separately. Combined profiling should provide a comprehensive view of thymic alterations. Our findings highlight thymic RNAseq's potential for identifying biomarkers and disease pathways in MG. Further analysis will explore shared and distinct transcriptomic signatures among MG subtypes and validate candidate biomarkers. These results pave the way for better understanding thymic involvement in MG and new therapeutic targets.

P6: Effect of neuronal extracellular factors on synapse formation: how glia contribute to neuronal structure and function during development

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The ability to think, move, or sense the environment relies on the proper development and wiring of the nervous system. A defining feature of neurons is their ability to undergo activity-dependent morphological changes, a phenomenon known as neuronal plasticity. While the molecular mechanisms of synapse formation and plasticity are well studied in neurons, a comprehensive 3D understanding of surrounding cell types—such as glial and muscle cells - and the extracellular matrix (ECM) has yet to be achieved. Recently, we have uncovered a novel mechanism of synaptic bouton formation involving membrane blebbing coordinated with muscle contraction at the Drosophila neuromuscular junction (NMJ). We are using this model to unravel the role of other extracellular factors—such as glial cells—in synapse growth, maintenance, and plasticity. Using subtype-specific fluorescent markers, we characterized the two glial subtypes present at the larval NMJ: subperineurial glia (SPG) and perineurial glia (PG). This analysis revealed distinct morphologies and suggests functional differences between the two subtypes. To assess function, we are performing targeted ablations of each glial subtype and monitoring their effects on activity-dependent bouton formation. This work highlights the importance of glial cells in shaping synaptic architecture and function, contributing to our broader understanding of neuron-glia interactions in neural plasticity.

P7: Exploring intercellular interactions in 3D: contribution of mechanical forces and signalling to synaptic structure and function

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The ability to think, move, or sense the environment relies on the proper development and wiring of the nervous system. A defining feature of neurons is their ability to undergo activity-dependent morphological changes, a phenomenon known as neuronal plasticity. While the molecular mechanisms of synapse formation and plasticity are well studied in neurons, a comprehensive 3D understanding of surrounding cell types—such as glial and muscle cells - and the extracellular matrix (ECM) has yet to be achieved. Recently, we have uncovered a novel mechanism of synaptic bouton formation involving membrane blebbing coordinated with muscle contraction at the Drosophila neuromuscular junction (NMJ). We are using this model to unravel the role of other extracellular factors—such as glial cells—in synapse growth, maintenance, and plasticity. Using subtype-specific fluorescent markers, we characterized the two glial subtypes present at the larval NMJ: subperineurial glia (SPG) and perineurial glia (PG). This analysis revealed distinct morphologies and suggests functional differences between the two subtypes. To assess function, we are performing targeted ablations of each glial subtype and monitoring their effects on activity-dependent bouton formation. This work highlights the importance of glial cells in shaping synaptic architecture and function, contributing to our broader understanding of neuron-glia interactions in neural plasticity.

P8 Mechanisms Contributing to Human Oocyte Spindle Assembly and to Female Infertility

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Female infertility affects over 10% of reproductive-age women worldwide, often due to aneuploidy in Human oocytes, where an abnormal number of chromosomes is present. Human oocytes have high rates of aneuploidy, especially with advancing maternal age, contributing to age-related infertility. Despite its prevalence, the cellular origins of aneuploidy remains poorly understood. A key factor is the intrinsic instability of the Human oocyte meiotic spindle - a microtubule-based structure responsible for chromosome segregation during meiosis to produce healthy, fertilizable eggs. However, the mechanisms regulating spindle assembly, and why Human oocytes are prone to spindle instability and aneuploidy, remain unclear. We hypothesize that infertility may result from mutations affecting spindle-associated proteins, and from age-related changes in protein expression. Thus, I performed a screen to identify proteins as strong candidates to play critical roles in spindle assembly, and I identified several novel components of the Human meiotic spindle. I will now use single-cell proteomics to identify spindle-related proteins differentially expressed between oocytes from young and older women. I will then screen the genome of a cohort of 3627 infertile women to investigate pathogenic mutations in the candidates identified previously. Finally, I will perform functional studies to investigate the candidates' role in spindle assembly, and how their deregulation contributes to infertility.

P9: Identification of Actin-Related Proteins Involved in Oocyte Meiotic Assembly in Drosophila melanogaster

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Female infertility is a growing concern in society as more individuals postpone childbearing. A major biological cause is aneuploidy, eggs with an abnormal number of chromosomes, which is a major cause of infertility and developmental disorders. These errors arise during meiosis, a cell division during oogenesis. Chromosome segregation depends on the meiotic spindle, a dynamic structure that aligns and separates chromosomes. Meiotic and mitotic spindles are formed by microtubules (MTs) and microtubule-associated proteins (MAPs) which assist in spindle assembly and function. Unlike mitotic cells, where centrosomes guide spindle assembly, oocytes lack centrosomes and rely on alternative, poorly understood acentrosomal pathways. Moreover, actin filaments form a spindle-like structure during oocyte meiosis, a feature absent in mitosis. This actin-based spindle has been shown to contribute to accurate chromosome segregation, but how it interacts with MTs is still unclear. We investigated candidate MAPs involved in this interplay using spatially and temporally controlled RNAi in Drosophila. Confocal imaging and fertility assays revealed that knockdown of MAPs such as Shot, KLP3A, and Pigs disrupt actin and MT spindle organisation, leading to reduced fertility. This work advances our understanding on the mechanisms involved in oocyte spindle assembly and function, and how their deregulation may contribute to human female infertility.

P10: The Dark Genome: *Mayhem* Long Non-Coding RNA regulates *Drosophila* Neuroblast Asymmetric Division

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Asymmetric cell division (ACD) is an important process that balances self-renewal and differentiation in neural stem cells, driving diversity in the nervous system. In *Drosophila* neural stem cells (neuroblasts, NBs), ACD segregates polarity proteins and fate determinants unequally into one self-renewing NB and one differentiating cell. While many core components are known, the molecular mechanisms that fine-tune this process are poorly understood. Long non-coding RNAs (lncRNAs) are emerging as important regulators of development because of their higher cell-type specific expression compared to protein-coding genes. We identified *Mayhem*, a previously uncharacterised lncRNA, that is exclusively expressed in cells undergoing ACD. Depleting *Mayhem* reduced type I NBs by 11.5% and increased type II, transit amplifying, NBs by 50%; phenotypes typically linked to defects in cell fate segregation. Consistently, basally localised cell fate determinants Miranda and Prospero were mis-segregated in mitotic NBs, and spindle axes were misorientated, affecting the identity and numbers of the daughter cells. Since we observe that *Mayhem* localises in the cytoplasm, we propose it guides or tethers basal cell fate determinants to the membrane. This work reveals a specific RNA-mediated mechanism in stem-cell maintenance and neural differentiation. We expect to uncover lncRNA-mediated regulatory mechanisms in *Drosophila* neurogenesis and explore new avenues beyond protein-coding genes.

P11: Cardiac signaling via EVs derived from CCBE1 human cardioids in response to cardiac injury

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Cardiovascular diseases are the leading cause of mortality worldwide. Hence, new therapies will benefit patients' well-being. Our previous work identified an important molecule in heart development, CCBE1, which is expressed in the epicardium and cardiofibroblasts. Ccbe1 mutant hearts displayed a thinner compact myocardium and decreased cardiomyocyte proliferation. Extracellular vesicles have been identified as cellcell communication and signaling mechanisms. We recently started implementing selforganized 3D models, cardioids, that allow an integrative study of the different cell types found in the developing heart. Our aim is to investigate cardiac signaling via EV release from WT and CCBE1-KO human cardioids as a means to optimize cardiac repair and regeneration. We have generated WT and CCBE1-KO human cardioids, with different cardiac cell types and beating capacity. CCBE1-KO cardioids exhibited a reduced size and impaired compaction, similar to previous studies. Their EVs have a more heterogeneous and broader size distribution, along with a significantly lower particle concentration, compared to the WT. Proteomic analysis of the EV cargo revealed a distinct profile of EV markers between the two conditions, and a grater abundance of proteins related to metabolism and oxidative stress management in the CCBE1-KO EVs. In the future, we will evaluate the therapeutic potential of these EVs in the context of cardiac injury, using ischemic/cryoinjured human cardioids.

Session 2 – *Brain, Vision and Neurological (dys)function*

P12: Exploring the Renin-Angiotensin System in the Development and Treatment of Diabetic Retinopathy

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Diabetic retinopathy (DR) is a major cause of vision loss and a common complication of diabetes mellitus. Chronic hyperglycaemia promotes oxidative stress, inflammation, and pathological angiogenesis in the retina. The renin-angiotensin system (RAS) was identified in the retina and found to directly contribute to DR pathogenesis. The ACE2/Ang-(1-7)/Mas1 axis, the protective arm of the RAS, counterbalances the harmful effects of the ACE/Ang II/AT1R pathway. In DR, this protective axis, particularly ACE2, is often downregulated, highlighting its potential as a therapeutic target. To test this hypothesis, we overexpressed human ACE2 using pEPito-hCMV-hACE2 plasmid in D407 retinal pigment epithelial cells under normo and hyperglycaemic conditions and in diabetic Ins2Akita mice via subretinal injection to induce retinal ACE2 expression. ACE2 overexpression effects on inflammation and oxidative stress were assessed by immunocytochemistry, Western blot, and flow cytometry. In vitro, ACE2 localized to perinuclear and cytoplasmic regions of retinal cells, increased PEDF and Mas1 levels, coupled with a reduction of VEGF levels, without affecting cell viability or ROS production. In vivo, ACE2 overexpression elevated retinal ACE2 and PEDF levels, with reduced VEGF expression. These findings show that ACE2 overexpression promotes a protective shift of the RAS in the retina, mitigating key DR features, warrant further exploring of its therapeutic potential.

P13: Transcellular proteostasis mediated by small extracellular vesicles in RPE cells

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Age-related macular degeneration (AMD) is a degenerative ocular disease of the macula, which is the central region of the retina. It is a leading cause of vision loss among the elderly in developed countries. Evidence increasingly indicates that AMD stems from the deterioration of the Retinal Pigmented Epithelium (RPE), a monolayer of post-mitotic cells that offers trophic support and phagocytes the shedding Photoreceptor Outer Segments (POS). Our research group hypothesized that proteotoxic stress, which is common in the macula, where photoreceptor density is higher, contributes to RPE dysfunction and subsequent photoreceptor death. We propose that Extracellular Vesicles (EVs), including exosomes, play a crucial role in maintaining tissue-level proteostasis by facilitating the transfer of proteotoxic materials and proteolytic machinery between cells in the macula and periphery, thereby increasing the digesting capacity of the RPE monolayer. Our findings, using ARPE-19 cells as a model for the RPE monolayer, provide direct evidence that EVs mediate the intercellular spread of proteotoxic stress, thereby mediating the accumulation of autofluorescent material that is characteristic of early AMD. In addition, the data indicated that exosome release may be upregulated upon POS exposure. This highlights EVs as critical players in AMD pathogenesis and potential therapeutic targets.

P14: Intermediate Age-Related Macular Degeneration Progression: A 6-year Prospective Study

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Purpose: Age-related macular degeneration (AMD) is a progressive condition that affects the macula, which is responsible for central vision. There are three stages: early, intermediate and late. Rates of progression may vary between individuals.

Predicting individual risk is advantageous for programming timely, more effective treatment and for patient stratification into future clinical trials. Clinical and OCT biomarkers investigation are crucial for a better understanding of the disease progression.

Methods: We have conducted a prospective and noninterventional study for following patients with intermediate AMD during 6 years. Optical coherence tomography parameters related with drusen, hyper-refective foci (HRF), presence of incomplete retinal pigment epithelial and outer retinal atrophy (iRORA) and ellipsoid zone (EZ) status were explored at the baseline. Patients were classified at the end of the follow-up period according to their progression.

Results: A total of 135 patients were enrolled into the study. Progression rate was analysed after 2 and 6 years, 30.4% and 60,7% were the results, respectively. The final study progression for neovascular AMD was 17% and for cRORA 22%. OCT parameters more related to progression were: iRORA, EZ status, drusen area and HRF.

Conclusions: Prospective studies are welcome concerning AMD progression since the combination of clinical and OCT characteristics can help to understand the disease.

P15: Dissecting the circuits and mechanisms underlying mechanosensory-triggered locomotion

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Sensory feedback is critical for stable and coordinated movement. Specifically, previous work from our group has shown that brief optogenetic stimulation of leg Mechanosensory Bristles (MsBs) is sufficient to initiate forward movement in immobile flies, which can be dependent on ventral nerve cord (VNC) circuits (Medeiros et al., 2024). However, how this information is processed and how local motor circuits become engaged remains largely unknown. Understanding the neuronal circuitry downstream of MsBs will help to answer these questions. Taking advantage of the advent of *Drosophila* connectomes, we were able to identify the anatomy of a large set of leg MsBs, as well as their downstream partners using the electron microscopy volume of a Male Adult Nerve Cord (MANC). We found that the most common downstream partners mainly consist of intrinsic neurons, ascending neurons and other MsBs. This information allows us to hypothesize a neuronal network that might be responsible for sensory-triggered locomotion, also sending information to the brain. To functionally test this idea, we searched for sparse genetic tools to label the MsBs and each downstream partner. We have been carrying out optogenetic activation experiments using a closed-loop automatic stimulation behavioural setup. These results will advance our understanding of the functional organization of action selection and engagement through the activity of specific neuronal modules in the context of an universal behaviour.

P16: CD2AP orchestrates synapses via actin and endosomal trafficking

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Genome-wide association studies identified CD2AP as a genetic risk factor for late-onset Alzheimer's disease (LOAD), but its pathogenic mechanism remains unclear. We previously showed that CD2AP regulates lysosomal sorting of APP, and that its loss increases amyloid-β (Aβ) production. Given CD2AP's role in actin dynamics, we also found it modulates F-actin and dendritic spine morphology. Since actin regulates the endosomal trafficking of glutamate receptors, we hypothesized that CD2AP loss could disrupt AMPA receptor (AMPAR) synaptic trafficking. We analyzed how CD2AP knockdown (KD) and overexpression (WT and mutant) affect AMPAR localization in mouse neurons. CD2AP-KD increased dendritic AMPAR levels but reduced synaptic enrichment. CD2AP overexpression lowered dendritic and synaptic AMPAR levels, except for GluA2, which remained retained at synapses. Pulse-chase assays showed AMPAR accumulation in late endosomes upon CD2AP-KD. These results suggest that CD2AP regulates AMPAR retention at synapses and sorting for degradation, likely via F-actin. Live-cell imaging under chemical LTP (cLTP) showed that CD2AP loss limits the spine enlargement required for LTP, demonstrating that CD2AP is essential for synaptic plasticity. We propose that CD2AP is a postsynaptic endosomal protein shaping spine morphology and AMPAR composition. In LOAD, CD2AP dysfunction may impair F-actin organization and endosomal trafficking, reducing AMPAR synaptic expression and contributing to early dendritic spine pathology, potentially as an early driver of AD.

P17: The role of LAMP2A in Endosomal Protein Composition: Implications for Membrane Identity, Endosomal Maturation and Exosome Biogenesis

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Exosomes are widely recognized as critical mediators of intercellular communication, yet the mechanisms governing their selective cargo loading have remained elusive. In our work, we were the first to describe a unique, LAMP2Adependent pathway that directs proteins bearing specific pentapeptide motifs (ExoSignals) into a distinct subpopulation of exosomes. Our findings not only reveal a novel strategy for the selective enrichment of biological content within exosomes but also underscore the broader role of LAMP2A in modulating endosomal dynamics. Through mass spectrometry and functional analyses, we demonstrated that LAMP2A influences endosomal protein profiles, impacting key regulators such as Rab GTPases, cortical actin associations, and phosphoinositide dynamics. LAMP2A deficiency leads to significant alterations, including decreased Rab27A, increased Rab27B, enhanced endolysosomal maturation, and shifts in PI(4,5)P2 and PI4P distributions. By establishing this dual role of LAMP2A, in both directing specific cargo into exosomes and shaping endosomal identity, our work may impact the development of innovative approaches for exosome engineering. The ability to tailor exosomal content holds great promise for advancing therapeutic applications, providing a new level of precision in harnessing these vesicles for intercellular signaling.

P18: Choroideremia Mouse Model Recapitulates Key Features of Retinal Degeneration

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Choroideremia (CHM) is an X-linked inherited retinal disease caused by mutations in the CHM gene, leading to Rab Escort Protein-1 loss of function. CHM is characterized by progressive vision loss, due to gradual degeneration of the choroid, the retinal pigment epithelium (RPE), and the retina. The RPE layer supports photoreceptors by providing nutrients and removing metabolic waste products, being considered the primary site of dysfunction in CHM.

To study the CHM pathogenesis, we employ a *Chm* knockout mouse model. However, in mice, *Chm* null mutations are embryonically lethal in males and in heterozygous females that inherit the mutant allele maternally. To circumvent this lethality, we crossed PGK-Cre females with Chm^{flox} males to generate $Chm^{WT/null}$ heterozygous females.

$Chm^{WT/null}$ females were analyzed at 2- and 6- months by Optical Coherence Tomography (OCT) and Blue Autofluorescence, revealing a reduced retinal thickness and presence of autofluorescent granules as early as 2-months. Additionally, dark-adapted Electroretinogram showed a reduction in the *c*-wave amplitude of $Chm^{WT/null}$ mice, indicating impaired RPE activity. Histological analysis of these animals revealed a reduction in the outer nuclear layer thickness, consistent with the OCT results.

Overall, the $Chm^{WT/null}$ model replicates key features of retinal degeneration and constitutes a useful tool to study CHM. Future studies are required to dissect the exact degenerative pathways occurring in this disease.

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P19: Surgical Outcomes in Intermittent Exotropia: Success Rate, Recurrence, and Predictors of Reoperation in a Pediatric Cohort

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Introduction: Intermittent exotropia (IXT) is a common form of childhood strabismus that often requires surgical correction to achieve ocular alignment and preserve binocular function. Although initial surgical success rates are generally high, reported reoperation rates vary widely in the literature and are influenced by several clinical and surgical factors. This study aimed to determine the reoperation rate in a cohort of children who underwent bilateral lateral rectus muscle recession and to identify perioperative variables associated with the need for further surgery. Methods: We conducted an observational, descriptive, and retrospective study involving 258 children diagnosed with basic-type or divergence excess-type IXT. Clinical and functional parameters were analyzed, including age, pre- and postoperative deviation angles, surgical success, orthoptic and occlusion therapy, and binocular function. Statistical analysis included association testing using odds ratios (OR) and logistic regression, with a significance level set at 5%. Results: The overall surgical success rate was 92.2%. Reoperation was significantly associated with initial surgical failure (OR = 75.03; $p < 0.001$), larger preoperative deviation angle (OR = 1.151; $p < 0.001$), smaller postoperative deviation angle (OR = 0.797; $p < 0.001$), and older age at the time of surgery (OR = 1.261; $p = 0.024$). Orthoptic therapy, occlusion, gender, and preoperative binocular function showed no statistically significant associations. Age at surgery showed a trend toward significance ($p = 0.055$). Conclusion: The need for reoperation in pediatric IXT is strongly associated with failure of the initial surgery, greater preoperative deviation, smaller postoperative alignment angle, and older patient age. These findings highlight the importance of individualized surgical planning and long-term postoperative follow-up to optimize outcomes and minimize the likelihood of reintervention.

P20: Insulin-degrading enzyme as a therapeutic bridge between diabetes and Parkinson's disease

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Parkinson's disease (PD) features dopaminergic neurodegeneration and α-synuclein (aSyn) aggregation. Epidemiological studies show that Type 2 diabetes mellitus (T2DM) increases PD risk. In T2DM, elevated aSyn levels and it's oligomerization status, and reduced levels of insulin-degrading enzyme (IDE), occur in pancreatic β-cells. IDE functions as a molecular chaperone, preventing aSyn oligomerization and toxicity in vitro. We hypothesize IDE exerts neuroprotective effects against brain aSyn aggregation. Adeno-associated virus (AAV)-mediated human IDE overexpression or empty vector (EV) was bilaterally injected into the substantia nigra pars compacta of transgenic mice overexpressing human aSyn (Tg) and WT littermates. Mice received normal chow diet (NCD) or a high-fat diet (HFD), an established model of pre-diabetes. Tg mice showed motor and non-motor phenotypes, both aggravated by HFD. In this exploratory study with low viral titer, no major gross-motor alterations were observed upon AAV-IDE versus AAV-EV. Fine-motor evaluation is ongoing. In contrast, some non-motor protection potential was observed. While current IDE-overexpression evaluation showed limited protection, ongoing studies with higher AAV titer will determine IDE's therapeutic potential in PD and pre-diabetes models. These findings could guide IDE-based therapies for both PD treatment and preventing diabetesassociated PD risk.

P21: Extracellular vesicles as mediators of gut–retina crosstalk in diabetic retinopathy

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Diabetic retinopathy (DR), a complication of diabetes, is the leading cause of vision loss, driven by blood-retinal barrier (BRB) disruption. Since diabetes affects multiple organs, understanding inter-organ communication is essential for preventing complications. Extracellular vesicles (EVs) act as mediators of systemic signalling and disease progression, but their role in the gut–retina axis remains poorly understood. Retinal organoids (ROs), derived from stem cells, replicate the human retinal structure and provide a relevant in vitro model to study DR. We hypothesise that in prediabetes, gut EVs cross the compromised BRB, contributing to disease progression. EVs produced by ROs at differentiation days 100, 150, and 180 were characterised using nanoparticle tracking analysis and western blotting. ROs release EVs at all tested differentiation stages, with the highest concentration observed at 100 days. Size distribution shows a peak at 100 nm, indicating the presence of small EVs. A 22-week diet-induced obese and prediabetic C57BL/6J mice model was characterised to assess BRB disruption using sodium fluorescein. Mice were injected with gut-derived EVs (GDEVs) to understand their role in DR progression. Preliminary results indicate that the model exhibits BRB impairment and GDEVs can reach the retina. Our data demonstrates that after 22 weeks of HFD, BRB disruption occurs, and GDEVs may lead to DR progression by creating a pro-inflammatory niche.

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P22: Impaired right ventricular function as a key contributor to hemodynamic instability in severe hypoxic-ischemic encephalopathy

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Background: Impaired right ventricular (RV) function, in the setting of hypoxic-ischemic encephalopathy (HIE) undergoing therapeutic hypothermia (TH), is associated with death or abnormal neurodevelopmental impairment1,2. The ability to identify RV dysfunction using bedside clinical markers is limited3,4. Since 2019, all patients receiving TH for HIE at the University of Iowa's NICU have undergone mandatory Targeted Neonatal Echocardiography (TNE) evaluation within 24 hours of admission. **Aim:** To evaluate the relationship between HIE severity, cardiovascular support, and initial TNE findings. **Methods:** Retrospective cohort study of neonates ³ 36 weeks' gestation and ³ 1.8 Kg birthweight treated with TH for HIE between June 2019 to March 2024. **Results:** Of 111 patients, 82% had moderate and 18% had severe HIE. Severe HIE was associated with increased use of cardiovascular medications (p=0.025), particularly inotropes such as epinephrine (p<0.001). Infants with severe HIE had lower tricuspid annular plane systolic excursion (TAPSE, p= 0.003) and lower RV 3 chamber longitudinal strain (p= 0.026) suggesting impaired RV performance. There was no difference in índices of left ventricular function between groups. TnECHO-guided care led to more frequent inotrope recommendations in severe group (p= 0.007). **Conclusions:** Severe HIE is associated with marked hemodynamic instability requiring cardiovascular support. The use of inotropes in this group most likely relates to more severe impairment in RV systolic function.

P23: NRF2-based molecular stratification in age-related macular degeneration: An integrative analysis in a Portuguese cohort

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Age-related macular degeneration (AMD) is the leading cause of blindness in adults over 55. It is a complex disease with genetic and environmental components. Current treatments address only late-stage neovascular AMD. Disease progression is driven by oxidative stress, impaired autophagy, and inflammation, with the NRF2/KEAP1 and TFEB/mTOR pathways playing key roles in retinal pigment epithelium (RPE) dysfunction. Elevated systemic levels of IL-6, IL-8, and CRP have been found in AMD patients. Polymorphisms in the NFE2L2 gene (encoding NRF2) have been associated with an increased risk of chronic diseases, whose pathogenic mechanisms involve oxidative stress and inflammation. Our team has shown that activating the transcription factors NRF2 and TFEB is protective in AMD models, and we are validating these findings in vivo using repurposed FDA- and EMA-approved drugs. Progression from intermediate to advanced AMD is variable. While structural and functional biomarkers are being explored, they remain insufficiently established as clinical endpoints. Despite NRF2´s role in chronic diseases, its clinical relevance as a systemic biomarker in AMD remains unclear. No studies have examined circulating expression of NRF2/KEAP1/TFEB-related genes in AMD. Our study aims to assess serum mRNA levels of NFE2L2 and associated polymorphisms across AMD stages in a Portuguese cohort, integrating genetic, inflammatory, clinical, and lifestyle data to evaluate NRF2 as a biomarker and therapeutic target.

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P24: Nanostructured Drug-Delivery Films - Preventing Post-Surgical Fibrosis in Glaucoma Valve Implants

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Glaucoma is recognized as one of the primary global causes of blindness, due to the death of retinal cells, damage to the optic nerve, and frequent elevated intraocular pressure. Implantation of drainage valves facilitates the outflow of aqueous humor, but postoperative excessive proliferation of fibroblasts can lead to the failure of the procedure. To address this issue, a promising approach involves coating the valve with a nanostructured cytostatic drug delivery system. We have created multi-layered thin films, composed of alternating layers of the anti-mitotic drug 5-fluorouracil (5FU), poly(β-amino ester) as a structural layer, and graphene oxide as a diffusion barrier and capping layer. We have tested its biocompatibility and found it compatible with retinal cells. However, given the flow rate of the aqueous humor in vivo, it is of the utmost importance to test this system's biocompatibility and therapeutic potential by simulating the dynamic environment of the eye, where aqueous humor is continuously produced and drained. We have devised a custom-designed microfluidic chip, connected to a peristaltic pump, for a controlled medium flow rate of 2–3 µL/min, closely mimics the physiological flow of aqueous humor and enables the direct exposure of the drug-loaded films to primary human Tenon fibroblasts. Our preliminary data points to a sustained release of 5-FU, capable of halting excessive fibroblast proliferation, validating our proposed approach.

P25: Ex Vivo Porcine Retinal Explants as a Platform to Evaluate Rosmarinic Acid-Releasing Contact Lenses Therapeutic Potential

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Treating diabetic eye conditions is limited by poor patient compliance, low bioavailability from topical formulations, and the invasiveness of intraocular injections. Contact lenses (CLs) with sustained drug release offer a promising, non-invasive alternative. Rosmarinic acid (RA), a natural polyphenol with antioxidant, anti-inflammatory, and neuroprotective properties, has emerged as a candidate for diabetic retinopathy treatment. In this study, we applied an optimized ex vivo organotypic model of porcine retinal explants to evaluate the neuroprotective efficacy of RA after release from previously developed silicone-based CLs. These lenses were engineered to ensure transparency, biocompatibility, and sustained release. Explants were cultured for 2 days in RAsupplemented media (86-400 ng/mL), then cryosectioned and analyzed by immunofluorescence using Brn3a+ as a marker for retina ganglion cells (RGCs) survival. RA-treated explants exhibited higher RGC density compared to untreated controls, which showed neurodegeneration. Quantification was performed using fluorescence microscopy and image analysis using Fiji software. This work demonstrates the feasibility of using RA-loaded CLs for neuroprotection in diabetic retinopathy and supports the relevance of the porcine explant model as a preclinical platform for testing ophthalmic drug delivery strategies.

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P26: Sex-specificity of metabolic mechanisms in Parkinson's Disease: IDE knockout insights and therapeutic implications

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Sex differences in Parkinson's Disease (PD) remain understudied despite documented disparities in incidence and progressio. PD involves α-synuclein (aSyn) aggregation and dopaminergic neurodegeneration with emerging evidence linking insulin resistance to disease pathology. Women appear more protected from both metabolic dysfunction and PD. Insulin-degrading enzyme (IDE) may mediate this connection. In T2DM, reduced pancreatic β-cell IDE correlates with aSyn accumulation, and *in vitro,* IDE prevents aSyn aggregation via chaperone activity . Alzheimer's research suggests sex hormones regulate IDE expression, contributing to sex-specific neuroinflammation. We hypothesize that IDE impairment differently impacts male and females, explaining different PD outcomes. We evaluated mice under normal-chow diet (NCD) vs high-fat diet (HFD) (established model of prediabetes) and IDE knockout vs WT littermates. Only HFD males develop insulin resistance regardless of genotype, while motor and cognitive assessments showed test-dependent sex differences in IDE KO and HFD conditions. Preliminary biochemical analyses revealed no sex-specific differences under HFD. These findings demonstrate sex differentially modulates PD-related phenotypes under metabolic stress. We propose a mechanistic model highlighting convergence of estrogen and brain insulin signaling, positioning IDE as a therapeutic target alongside GLP-1 agonists, glitazones, and metformin.

P27: Krebs cycle associated-metabolic modulation as the basis of retina immune response

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The Krebs cycle is essential to cellular metabolic homeostasis, and its impairment a hallmark of mitochondrial diseases caused by inborn errors of metabolism (IEM). These heterogenous genetic disorders typically manifest in early life with complex symptoms, such as neuromuscular dysfunction, ataxia and vision impairments. Mitochondrial DNA depletion syndromes, particularly those caused by deficiency of succinyl-CoA ligase (SCL) or Coenzyme Q, critically affect retina pigment epithelial (RPE) cell homeostasis and contribute to retinal degeneration and progressive vision loss. Recent studies implicate mitochondrial stress, leakage of mitochondrial DNA (mt-DNA) and RNA (mtRNA) as drivers of chronic inflammation in metabolic diseases. However, the mechanisms of mitochondrial-driven inflammation remain poorly understood. In this study, we investigated how the Krebs cycle shapes innate immunity in RPE cells. To this end, we generated CRISPR-engineered SCL-deficient RPE cells and used metabolic analogues/inhibitors that mimic the buildup/depletion of Krebs intermediates observed in IEM. Through molecular and transcriptomic analyses, we found that Krebs cycle imbalances disrupt mt-RNA homeostasis and activate inflammatory signalling in RPE cells. These data suggest a tight coordination between the Krebs cycle activity and immunomodulation in RPE cells. Understanding this Krebs-immune crosstalk may uncover therapeutic target pathways in retinal degeneration and mitochondrial disease.

P28: Development of a lesion model in retinal organoids: exploring Müller glia regenerative role

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Photoreceptor (PR) loss is a key feature in retinitis pigmentosa and age-related macular degeneration, the leading causes of blindness in working-age individuals and the elderly, respectively. They significantly reduce quality of life and pose a major burden on healthcare systems, and yet, no definitive therapies exist. Interestingly, lower vertebrates such as zebrafish can regenerate retinal cells after injury. This process is driven by Müller glia (MG), support cells that reprogram into progenitor-like cells. In mammals, however, MG respond with reactive gliosis, a non-regenerative process, although they retain latent neurogenic potential that can be experimentally activated. To study this in a human context, we established a UVA-induced lesion model in human retinal organoids (ROs). After injury, we observed increased pyknotic nuclei and degenerating cells, with PRs being particularly affected. MG responded with increased vimentin expression and hypertrophied processes, indicating gliosis. We also observed more proliferating cells, which likely reflect an early response to damage, although their identity remains unknown. No significant changes were found in PR progenitor cell numbers or YAP1 expression, a downstream effector of the Hippo pathway and key regulator of MG regenerative potential. Overall, these results suggest that ROs' response to injury resembles that of other mammalian models, allowing the study of self-regenerative strategies in a human-derived model.

Keywords: Retinal organoids; Regeneration; Müller glia.

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P29: Targeting gut–brain axis dysfunction in Parkinson's disease with a polyphenol-enriched diet

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Dome, enhance short-chain fatty acid production, and reduce gut inflammation. Our previous work showed their potential against neuroinflammation, a key factor in Parkinson's disease (PD), a progressive disorder marked by dopaminergic neuron loss and α-synuclein aggregation. This study evaluated the effects of a (poly)phenol-rich diet on the gut–brain axis in a PD-like mouse model induced by MPTP. Male C57BL6J mice (n=60) received either a standard or 8% berry-enriched diet for 6 weeks. Half were injected with MPTP (15 mg/kg, i.p., 4x, 2 h apart). Behavioral tests, histology, ELISA, flow cytometry, calprotectin assays, and 16S rRNA sequencing were performed. The (poly)phenol-rich diet significantly reduced urinary corti ietary (poly)phenols, abundant in fruits and vegetables, have anti-inflammatory effects and may help prevent neurodegenerative diseases. After ingestion, they are metabolized by the host and gut microbiota into gut-phenolic metabolites (gut-PMs), which modulate the microbi costerone and serum TGF-β1 and CCL22 levels. MPTP-exposed mice on the enriched diet showed improved motor performance, reduced gut inflammation, and calprotectin levels comparable to controls. Microbiota analysis revealed restoration of specific bacterial families. These findings support the potential of (poly)phenol-rich dietary interventions to alleviate hallmark PD symptoms via gut–brain axis modulation.

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P30: Blinding the ear: rethinking the control for transcutaneous auricular vagus nerve stimulation

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Transcutaneous auricular vagus nerve stimulation (taVNS) is a non-invasive neuromodulation technique where electrical pulses are delivered to the auricular branch of the vagus nerve (VN), namely the cymba conchae (CC)1 , and has been proposed as a promising treatment for various neurological and psychiatric conditions2 . However, findings across studies have been inconsistent often due to differences in stimulation protocols3 , inadequate assessment of participant blinding and the absence of biomarkers reflecting VN activation. Therefore, implementing a taVNS protocol that ensures effective blinding and VN activation is essential. We conducted several experiments in which participants received real taVNS (active stimulation in the CC) and control stimulation on different sessions. Sham conditions were first in the earlobe and in the scapha, on two separate control sessions. In a second experiment, sham condition did not deliver any electric stimulation but electrode was placed in CC. In a third experiment, ramp-down sham stimulation in the CC was used as a control. Physiologic measures were collected throughout all sessions. Results indicate that participants differentiated between stimulation sites and could reliably distinguish real taVNS from sham conditions involving no electrical pulses. The usage of a ramp-down approach contributed to effective participant blinding between, but not within subjects. However, analysis of physiological markers showed no significant differences.

P31: Mechanisms through which Infection Impacts the Drosophila Nervous System

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Inflammation has been increasingly linked to the onset and progression of various pathologies. Moreover, sustained glial activation of nuclear factor kappa-B (NF-κB) has been shown to impair neuronal function and trigger cell death, contributing to long-term neurological deficits. Developing strategies to study and modulate glial NF-κB activity is therefore essential for identifying therapeutic approaches that balance inflammation and limit its harmful effects. Using Drosophila larvae, we established a novel in vivo method to assess NF-κB activation across different cell types. We found that peripheral glia respond differentially to peptidoglycan (PGN), a known inflammatory stimulus. Among glial populations in the peripheral nervous system, subperineurial and wrapping glia were identified as immune-responsive, while perineurial glia remained unresponsive. This model also enables the investigation of how prolonged activation of pro-inflammatory pathways affects neuronal morphology and function. Overall, we introduce a versatile and rapid screening platform to study both acute and chronic neuroinflammatory responses. This approach holds promise for testing candidate anti-inflammatory compounds and advancing the development of new therapeutic strategies for neuroinflammation and neurodegenerative disorders.

P32: Lysosomal macular dystrophy is a novel ocular phenotype associated with biallelic variants affecting AP-5/SPG11/SPG15 complex

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Inherited retinal diseases (IRDs) are amongst the most common Mendelian human disorders in humans. Their clinical and molecular diversity is increasingly being unveiled, with more than 346 disease genes and loci identified to date. This study aimed to characterize the phenotypic spectrum of a cohort of patients with IRD associated with mutations in genes encoding different subunits of the vesicular Fifth Adaptor Protein (AP-5) complex, a newly-identified cause of IRD. Retrospective analysis of clinical data focused on ophthalmic findings from a cohort of 22 patients representing 19 families was performed. Younger individuals presented with asymptomatic or paucisymptomatic macular deposits, while older patients demonstrated variable areas of outer retinal and retinal pigment epithelium atrophy, with significant visual acuity loss. Retinal imaging revealed progressive enlargement of the atrophic area in cases with available follow-up data. Additionally, 10 patients exhibited concomitant neurological findings. Ocular findings in our cohort illustrate a phenotypic continuum consistent with both the maculopathy described in Kjellin syndrome and sporadically reported *AP5Z1*-associated retinal findings. SPG11, SPG15, and AP-5 complex are known to interact in order to maintain endolysosomal homeostasis. We therefore propose to designate this novel IRD phenotype, resulting from molecular defects within this functional complex, Lysosomal Macular Dystrophy.

P33: Assessing freezing behavior and its physiological correlates in humans – A virtual reality paradigm

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When in the presence of danger, humans adopt behaviors to avoid injury or death. Such responses are typically to freeze, flee or fight. While flight and fight are well characterized and understood, freezing has a more elusive role. It is thought to anticipate subsequent action choice and decision-making and is mainly characterized by bradycardia and reduced body sway. Although freezing has been extensively studied in non-human animal models, in humans, results remain inconsistent and there is no consensus on the best way to assess it.

We developed an experimental setting to record data on physiological markers of the autonomic nervous system – electrocardiogram, electrodermal activity– and movement, while participants engage in a videogame-based task simulating a dynamic encounter with a threat. Participants can choose to escape, hide in a shelter, or freeze to avoid contact with a fearful animated stimulus. If contact occurs, mild electric shocks are delivered to the wrist.

Recently, we are updating the paradigm by integrating virtual reality (VR), increasing ecological validity and immersion in the threat scenario. With this enhanced setup, we expect clearer expression of threat-induced freezing and corresponding physiological markers, such as bradycardia and reduced movement. This tool may allow us to further investigate individual differences in defensive responses, including comparisons between individuals with neuropsychiatric disorders and healthy volunteers.

P34: Modeling the interplay between dopaminergic injury and inflammation in a Parkinson's disease microphysiological system

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Parkinson's disease (PD), the most common motor neurodegenerative disorder, involves progressive dopaminergic neuron loss in the substantia nigra. Its complexity stems from protein aggregation, oxidative stress, neuroinflammation, and blood-brain barrier (BBB) dysfunction. Current treatments are symptomatic, highlighting the need for disease-modifying strategies. Polyphenol metabolites (PMs), bioactive compounds from dietary polyphenols, show neuroprotective potential due to BBB permeability [1] and their ability to modulate oxidative stress and inflammation [2]. This study aims to evaluate PMs in a brain-on-chip model mimicking the neurovascular unit (NVU) and PD-like pathology. The microfluidic platform includes human brain microvascular endothelial cells (HBMEC) in the vascular channel and LUHMES dopaminergic neurons, astrocytes (HASTR/ci35), and microglia (HMC3) in the brain channel. PD-like conditions are induced with the neurotoxin MPP+ [3] and a pro-inflammatory cytokine mix. MPP+ and inflammation led to increased cytokine release and cell death, underscoring NVU dysfunction. PMs protected LUHMES neurons from MPP+ toxicity and modulated NF-κB translocation in HMC3 microglia. Future work will assess PMs at physiologically relevant levels. This brain-on-chip platform offers a translational approach to study dietary interventions for preserving NVU integrity and slowing PD progression.

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P35: Effect of β -glucocerebrosidase deficiency on lysosomal homeostasis and function

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Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, has been associated with mutations in genes related to lysosomal function. Among these, heterozygous mutations in the GBA1 gene, which encodes the lysosomal enzyme b glucocerebrosidase (GCase), represent the most common PD's genetic risk factor. A genetically modified human H4 neuroglioma cell line with heterozygous GBA1 disruption (H4 GBA+/-) was generated and characterized in comparison to the wild-type (WT) H4 cells (GBA+/+) to evaluate the impact of GCase impairment on lysosomal homeostasis and function. H4 GBA +/- cells showed increased LAMP1 levels compared to WT cells. A trend towards increased total cathepsin D (CTSD) levels and mature to pro-CTSD ratio was observed in the H4 GBA +/- cells compared to WT cells. However, the mature to total CTSD ratio remained unchanged. Autophagic flux was also assessed by measuring p62 levels and the LC3-II/LC3-I ratio in the presence and absence of bafilomycin A1 (BafA1). While p62 levels remained unchanged, the LC3-II/LC3-I ratio increased similarly in both WT and H4 GBA +/- cells upon BafA1 treatment. Taken together, these findings indicate that under these condition reduced GCase activity does not seem to impair overall lysosomal degradative function or autophagic flux. The increased LAMP1 levels may reflect a compensatory expansion of the lysosomal compartment to maintain proteostasis under conditions of altered lysosomal homeostasis.

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P36: Impact of priors and uncertainty on intention inference and associated ERPs in autism spectrum disorders

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Background: Individuals with Autism Spectrum Disorder (ASD) exhibit persistent difficulties in inferring others' intentions — a crucial ability for predicting others' actions. EEG studies have revealed two atypical event-related potentials (ERPs) during intention inference in ASD, namely P200 and P300, yet the underlying neurocognitive mechanisms remain unclear. Two systematic reviews reported predictive impairments in ASD, particularly in neurophysiological measures. Bayesian theories propose two competing hypotheses: a global underuse of prior information ("hypo-prior") or a failure to adjust prediction error precision to contextual uncertainty ("aberrant precision account"). The latter has received more consistent support in recent studies. This study investigates which mechanism better accounts for impairments in intention inference.

Methods: A cross-sectional EEG study using a modified Comic Strip Task with probabilistic manipulation of contextual uncertainty will be conducted in 40 autistic and 40 neurotypical adults. Behavioural accuracy and ERPs (P200, P300) will be recorded under intentional (IA) and physical causality (PCC) conditions.

Expected results: We hypothesize that predictive impairments and ERP differences will be more pronounced in IA under high uncertainty, consistent with the aberrant precision account.

Conclusion: This study aims to clarify the basis of predictive processing alterations in ASD and their contribution to intention inference impairments.

P37: Insulin-degrading enzyme as a therapeutic bridge between diabetes and Parkinson's disease

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Parkinson's disease (PD) features dopaminergic neurodegeneration and α-synuclein (aSyn) aggregation. Epidemiological studies show that Type 2 diabetes mellitus (T2DM) increases PD risk. In T2DM, elevated aSyn levels and it's oligomerization status, and reduced levels of insulin-degrading enzyme (IDE), occur in pancreatic β-cells. IDE functions as a molecular chaperone, preventing aSyn oligomerization and toxicity in vitro. We hypothesize IDE exerts neuroprotective effects against brain aSyn aggregation. Adeno-associated virus (AAV)-mediated human IDE overexpression or empty vector (EV) was bilaterally injected into the substantia nigra pars compacta of transgenic mice overexpressing human aSyn (Tg) and WT littermates. Mice received normal chow diet (NCD) or a high-fat diet (HFD), an established model of pre-diabetes. Tg mice showed motor and non-motor phenotypes, both aggravated by HFD. In this exploratory study with low viral titer, no major gross-motor alterations were observed upon AAV-IDE versus AAV-EV. Fine-motor evaluation is ongoing. In contrast, some non-motor protection potential was observed. While current IDE-overexpression evaluation showed limited protection, ongoing studies with higher AAV titer will determine IDE's therapeutic potential in PD and pre-diabetes models. These findings could guide IDE-based therapies for both PD treatment and preventing diabetes associated PD risk.

Session 3 – *Cardiometabolic and systemic health (dys)function*

P1: Fourier-Transform Infrared Spectroscopy as a Non-Invasive Tool for Biochemical Monitoring in Kidney Transplantation

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P Fourier-transform infrared (FTIR) spectroscopy is a powerful label-free technique for detecting biochemical alterations in biological fluids. This study analyzed serum samples from 37 kidney transplant recipients (74 spectra, collected before and after transplantation) to identify immunometabolic changes associated with the transplant process. Spectral preprocessing included Savitzky-Golay derivatives (1st and 2nd order) and vector normalization. Data analysis combined principal component analysis (PCA) and univariate statistical testing. PCA showed clear group separation, especially after 1st derivative processing. Highly significant differences ($p < 1\times10^{-7}$) were detected in the Amide II (1548–1572 cm^{-1}) and Amide I (1648–1673 cm^{-1}) regions, indicating alterations in serum protein structure and content. Additional significant bands were observed in the 1171–1243 cm^{-1} region, related to phosphates, glycoproteins, and nucleic acids, and in the 1380–1497 cm^{-1} range, associated with lipid-related CH_3 deformations. These consistent, biochemically interpretable shifts support FTIR spectroscopy as a sensitive, non-invasive analytical tool for monitoring molecular changes following kidney transplantation, with potential for early detection of graft-related complications.

P2: Comparative study of the most used growth models applied to weight in infants aged 0 to 2 years

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Monitoring a child's growth through routine assessments over time provides valuable insights into their overall health. Growth is widely recognized as a key indicator for the early diagnosis of potential diseases and in the nutritional status evaluation. Over the years, several models have been proposed to analyze growth patterns. A scoping review conducted in this study—focused on infants aged 0 to 2 years—identified the most used models: Jenss-Bayley, Count, Reed 1st order, fractional polynomials, and SuperImposition by Translation and Rotation (SITAR). These models were compared with the Generalized Additive Models for Location, Scale, and Shape (GAMLSS), which are typically used by the WHO for modeling growth data. For this purpose, data of 414 infants aged 0 to 2 years, collected as part of a birth cohort study conducted in São Tomé Island were analyzed. Model fit and complexity were assessed using Akaike Information Criterion and Bayesian Information Criterion. Predictive accuracy was evaluated using the mean absolute error, the mean squared error, and the normalized mean squared error. Finally, the Wasserstein distance was applied to compare the distribution of estimated weights from each model to the observed weight distribution. Results showed that SITAR provided the best goodness-of-fit, while GAMLSS obtained the best predictive accuracy for both sexes. Regarding the Wasserstein distance, SITAR again performed best in approximating the distribution of observed weights, for both sexes.

P3: Multi-thiol profiling during the Peritoneal Equilibration Test uncovers solute-specific membrane transport characteristics

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Background and aims: Peritoneal membrane (PM) dysfunction limits the long-term efficacy of peritoneal dialysis (PD). The Peritoneal Equilibration Test (PET), commonly used to assess PM function, focuses on creatinine transport and may overlook distinct membrane response patterns. Aminothiols such as cysteine (Cys), homocysteine (HCys), cysteinylglycine (CysGly), and glutathione (GSH) may reflect PM redox status. Extracellular vesicles (EVs), which carry antioxidant molecules, may support local protection; however, their thiol content during PET remains unexplored. We hypothesized that thiol responses to PET vary among patients and that these molecules are present in PET fluid-derived EVs. Methods: A cross-sectional study was conducted in 25 PD patients (Ethics: NMS 120/2023/CEFCM; ULSLO 2024-63). PD effluent samples were collected at 0, 2, and 4 h during PET; plasma was obtained before test initiation. Free aminothiols were quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FD), and the area under the curve (AUC) for dialysate-to-plasma (D/P) ratios was calculated. EVs isolated by filtration and ultracentrifugation were validated through CD63 detection by Western blot and Nanoparticle Tracking Analysis, and aminothiols were quantified using HPLC-FD. Results: Cys and CysGly AUCs were significantly lower than creatinine ($p = 0.001$; $p < 0.0001$), while HCys showed no difference. GSH was detected in EVs. Conclusion: These exploratory findings suggest solute-specific PM transport andindicate that EV thiol content may reflect PM redox status.

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P4: Towards a serum-free 3D human liver model to recapitulate MASLD pathogenesis

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Metabolic dysfunction-associated steatotic liver disease (MASLD) affects over 30% of the global adult population. [1] It ranges from simple steatosis (MASL) to steatohepatitis (MASH) with fibrosis, which can progress to cirrhosis and hepatocellular carcinoma. [2] MASLD pathogenesis involves complex genetic, metabolic, dietary, and inflammatory factors and remains poorly understood. Current preclinical models often fail to replicate human disease features. [3]

Our lab previously developed 3D hepatic spheroids using HepaRG cells alone or with primary non-parenchymal cells. MASH-like conditions were induced using energy substrates, resulting in lipid accumulation, oxidative and mitochondrial stress, inflammation, and hepatic dysfunction. However, control spheroids in fetal bovine serum (FBS)-containing medium also showed lipid accumulation, compromising model sensitivity. To improve baseline specificity, we optimized the HepaRG spheroid model by applying a serum-free medium. This reduced spontaneous lipid accumulation, confirmed by lipid staining and image analysis. Spheroids also formed more quickly and uniformly without FBS. Key hepatic functions, such as, albumin, cholesterol, bilirubin production, ROS generation, and cytokine expression, were preserved. The model responded to MASLD-inducing stimuli, showing MASL and MASH features. This optimization improves the model's reliability and is now being applied to multicellular liver spheroids for enhanced disease relevance.

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P5: Organophosphate Poisoning Impairs Baroreflex in situ in a Strain Specific-Manner

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Cardiovascular complications are common and potentially fatal in organophosphate (OP) poisoning. Previously we showed both in vivo and in situ studies, that acute exposure to chlorpyrifos (CPF), a highly used OP compound, attenuated cardiorespiratory reflexes. Notably, mortality rate by OP is greater in spontaneously hypertensive rats (SHR) than normotensive animals. Therefore, the effects of intoxication in the presence of comorbidities, such as hypertension, are important to be investigated. Using the working heart-brainstem preparation, we recorded heart rate, phrenic and thoracic sympathetic nerve activities in both Wistar and pre hypertensive SHR (4-5 week-old; ethical committee approval number 17/2022). After 15 min baseline recording, the baroreflex was evoked via arterial bolus injection of phenylephrine (1.5 mM, 100□L). Preparations then received Chlorpyrifos-oxon (CPO; 15 mg/Kg), the active form of CPF, or DMSO (vehicle) and reflexe were reassessed. Finally, brainstems were collected to determine cholinesterase activity (AChE). Groups (n = 10 each): Wistar DMSO (WD), Wistar CPO (WC), SHR DMSO (SD), SHR CPO (SC). Data (mean ± SEM) were analysed using two-way ANOVA and generalized mixed models. Both strains exhibited a dysfunctional baroreflex, with normotensive rats showing an attenuated bradycardic response, whereas SHR displayed an increased baroreflex gain sensitivity (DMSO: Wistar □ = 0.977, p = 0.56; SHR □= 1.04, p = 0.66. CPO: Wistar □ = 1.19, p = 0.12; SHR □ = 0.89, p = 0.05; bpm/mmHg.s-1). The sympathetic response, meanwhile, was impaired in both strains, although it was relatively more preserved in Wistar rats. SHR also showed a baseline baroreflex deficit, which was further exacerbated by intoxication (DMSO: Wistar □ = 0.98, p = 0.56; SHR □ = 1.04, p = 0.66. CPO: Wistar □ = 1.19, p = 0.12; SHR □ = 0.89, p = 0.05; µvolts/ mmHg.s-1). AChE activity in the brainstem was inhibited in both strains (WD 5.23 ± 0.31; WC 0.27 ± 0,06; SD 5.83 ± 0.19; SC 0.39 ± 0.08; µmol/h/mg of protein). These findings show that acute OP exposure impairs baroreflex in a strain specific-manner, highlighting the importance of considering underlying

hypertension in individuals' exposure to OP, since different clinical approaches may be needed.

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Keywords: Organophosphate poisoning, Chlorpyrifos, Baroreflex and Hypertension.

P6: Cardiopulmonary exercise test in aortic stenosis: a valuable aid for complex decisions

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Background: Symptomatic severe aortic stenosis (AS) is an indication for aortic valve intervention. However, when symptoms are not clear, it is challenging to determine whether the patient has significant hemodynamic compromise due to the valve disease. Although cardiopulmonary exercise test (CPET) is an objective assessment of patients' exercise tolerance, it is widely underused in this setting. We aim to assess the impact of CPET on selecting the treatment strategy for severe AS patients.

Methods and Results: Retrospective analysis of 40 severe AS patients (mean age 78 years) that performed CPET in our center between 2014 and 2024. Twelve patients (30%) didn´t undergo any valve intervention – conservative group. Nine patients had late valve intervention (at least 1 year after CPET, median time to intervention=3 years). Mean survival of late vs early intervention was similar (37 vs 38 months, p=0.805). The conservative group had higher prevalence of pulmonary disease than the interventional group (42% vs 4%, p=0.002). In the former, CPET parameters showed lower likelihood of circulatory limitation (17% vs 54%, p=0.030) and higher ventilatory efficiency (VE/VCO_2 slope>36 in 8% vs 48%, p=0.0017). Overall, patients in the conservative group had a mean survival of 55 months (vs 71 months in the interventional group, p=0.118).

Conclusions: The integration of CPET in severe AS assessment aids in the safe selection of patients for late valve intervention or conservative treatment.

P7: Artificial intelligence in shoulder surgery: a pathology-segmented systematic review

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Background: Shoulder disorders account for ~30 % of musculoskeletal consultations and more than 250 000 rotator-cuff repairs annually worldwide. Despite advanced MRI, CT and ultrasound, clinicians still struggle to predict tear reparability, graft failure or recurrence after stabilization. Artificial-intelligence (AI) models, particularly deep learning can mine subtle clinical-imaging patterns beyond human perception, but existing evidence is fragmented across pathologies, algorithms and endpoints, hindering clinical uptake. **Objective**: To synthesize and critically appraise AI models for the full spectrum of shoulder pathology. **Methods**: A PRISMA-guided search of PubMed, Web of Science and Google Scholar (Jan 2005–Jan 2025) identified original adult studies that used AI for diagnosis, prognosis or treatment of rotator-cuff tear, instability, osteoarthritis or peri-articular shoulder diseases. Pre-specified inclusion/exclusion criteria were applied independently by two reviewers; 156 records were screened and 39 included. **Results:** Rotator-cuff tear dominated the type of pathologies where more studies were performed. Convolutional neural networks on MRI or ultrasound achieved pooled good results (estimated AUC 0.88 average for best models) for tear detection. In imaging analysis results obtained by AI models were not compared to human experts' evaluation. Multimodal clinical-imaging models reached relevant median accuracy but lacked external validation. Only few studies prospectively predicted treatment response. Risk of bias was high because of small, single-center cohorts and opaque ground-truth labelling in several studies. **Conclusion:** AI research in shoulder surgery is most mature for image-based rotator-cuff diagnosis, yet rigorously validated multimodal tools for other conditions remain scarce. Large, multicenter prospective studies with transparent reporting are essential for safe integration of AI into shoulder decision-making.

P8: Genomic and epigenomic characterization of the HepaRG cell line

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The HepaRG cell line is a well-established in vitro model for studying hepatic metabolism and liver diseases, including metabolic dysfunction-associated steatotic liver disease (MASLD) and its progressive form, steatohepatitis (MASH). However, its genetic and epigenetic landscape remains poorly characterized. A detailed understanding of a cell line's genomic architecture improves its experimental utility and interpretation in disease modeling. To address this, we performed whole genome sequencing and DNA methylation profiling of HepaRG cells in four states: undifferentiated, differentiated, metabolically stimulated, and metabolically stimulated with MASH induction, using Oxford Nanopore technology. Sequencing achieved ~99% chromosomal coverage at ~30x depth. Structural variant analysis identified ~35,000 events, primarily 50–400 bp insertions and deletions in non-coding regions, along with trisomy of chromosome 7. Variant calling revealed clinically relevant MASLD-associated polymorphisms, including the heterozygous presence of variants I148M in PNPLA3 and E167K in TM6SF2. CpG methylation profiles remained largely conserved across all tested states, suggesting genomic stability and susceptibility of HepaRG cells to metabolic liver disease. The minimal DNA methylation variation among the 4 states implies that other regulatory mechanisms may play a dominant role in differentiation and disease progression in this model.

3 Keywords: HepaRG; Genomic profiling; Liver disease modeling.

P9: Impact of time-restricted eating on metabolic health in postmenopausal women with type 2 Diabetes

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The decline in estrogenic levels that characterizes the menopausal transition has been demonstrated to intensify insulin resistance and inflammation in women diagnosed with diabetes, increasing the probability of cardiovascular complications and metabolic dysfunction-associated-steatotic liver disease (MASLD). Metabolic syndrome and insulin resistance, both closely linked to MASLD, are especially concerning in postmenopausal women with type 2 diabetes (T2D). Time-Restricted Eating (TRE), a promising non-invasive dietary strategy, may offer therapeutic benefits in this population who often present elevated levels of fasting glucose, insulin, adiponectin, leptin, and body weight. We hypothesize that synchronizing dietary intake with circadian rhythms – through early or evening TRE – may enhance metabolic, cardiovascular, and liver health, as well as quality of life. Our aim is to develop tailored therapeutic strategies to improve adherence to the study and, with the integrated data acquired we will develop a mobile app and a digital wristband to monitor and integrate data of physical activity, sleep, circadian hormone patterns, glycemia, hydration, food intake, while also providing compliance reminders to enhance adherence to the study. Overall, we expect to elucidate the potential of TRE as a comprehensive intervention for improving metabolic health outcomes in postmenopausal women with T2D and elucidate its therapeutic potential.

P10: Exploring the implications of sex differential immunity in persistent Long COVID

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Five years on COVID-19 reemerges as a public health crisis in the form of Long COVID (LC). LC is defined by the WHO as a post-infectious condition of COVID-19 characterized by symptoms persisting for at least 3 months from onset, and not explainable by other diagnosis. LC exhibits a strong female bias in prevalence and symptomology, with women experiencing higher rates of headaches and neurological sequelae. Gaining insight into the biological underpinnings contributing to the strong women bias may further help us understand LC and identify therapeutic strategies. We have started to address the immunological underpinnings of sex bias in a cohort of persistent LC (pLC), i.e., people who have experienced LC for at least a year. We have recruited 34 individuals diagnosed with pLC and 26 age/sex matched recovered individuals. There were no significant demographic differences between the 2 groups. The interval between SARS-CoV-2 infection and sample collection was higher for individuals diagnosed with pLC than for controls, with most study participants only having been infected once at the time of sample collection. Next, we determined whether our cohort displayed sex differential LC symptomatology. Women with pLC displayed higher symptom burden than their male counterparts and reported a higher frequency of neurocognitive symptoms. We found that the immune responses were also sex differential with pLC women displaying a higher concentration of sCD40L and sFAS compared to controls.

P11: Peroxisome proliferator–activated receptor alpha and delta agonist impacts inflammatory, fibrotic and symptom-associated markers in patients with primary biliary cholangitis

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Keywords: Cholestatic diseases, primary biliary cholangitis

Background and Aims: Primary biliary cholangitis (PBC) is a rare, cholestatic liver disease, characterised by inflammation, fibrosis and destruction of intrahepatic bile ducts. Elafibranor, a peroxisome proliferator-activated receptor (PPAR) agonist exerting effects on PPAR alpha and delta, was efficacious and well-tolerated in patients with PBC in the phase III trial (NCT04526665). Here, longitudinal protein expression profiles were evaluated in serum samples from patients in the trial, to provide insights into the immunoregulatory mechanism of elafibranor in PBC.

Method: For patients in trial who consented, serum samples were collected at baseline, Week 26 and Week 52. Longitudinal protein expression profiles were analysed using Olink® Proximity Extension Assay technology. Two Olink® panels, Target 96 Immune Response and Explore HT, were used, covering >5,500 proteins. Quality control and data normalisation were conducted by Olink®. Linear mixed models were fitted to each protein from baseline to Week 52. Statistically significant changes in protein expression were identified using the Benjamini-Hochberg method with a 5% 2 false discovery rate. Proteins with statistically significant changes in expression were assessed in elafibranor-treated patients with and without biochemical response, according to the primary endpoint of the study. Pathway analyses were conducted using QIAGEN® Ingenuity Pathway 5 Analysis (IPA®).

Results: Serum samples were analysed from 121 patients, of whom 87 received elafibranor; 46 with and 41 without biochemical response at Week 52. Expression of control proteins, gamma-8 glutamyl transferase-1 (GGT1) and 5'-nucleotidase (NT5E), decreased with elafibranor treatment when measured by Olink® and standard methodologies, validating the use of Olink®.

A network of >20 proteins involved in hepatic inflammation had statistically significant changes of expression in elafibranor-treated patients with biochemical response at Week 52. IPA® revealed a novel inflammatory signature, including downregulation of intercellular adhesion molecule 1 (ICAM-1), dipeptidyl peptidase 4 (DPP4) and SerpinA3. This network is known to be implicated in immune cell adhesion and inflammatory regulation, and maps to immunomodulation and fibrosis pathways. Similarly, biomarkers associated with itching and fibrosis, such as bone marrow stromal cell

antigen 2 (BST2) or solute carrier family 39 member 14 (SLC39A14), were significantly downregulated.

Conclusion: This is the first longitudinal proteomic analysis to characterise proteins with modulated expression in patients with PBC treated with Elafibranor, a peroxisome proliferator–activated receptor alpha and delta agonist. The findings provide novel mechanistic insights into the anti-inflammatory effect of this new class of drugs in PBC, as well as impact on proteins associated with PBC symptoms, through effects on PPAR alpha and delta.

P12: Effects of Digoxin in modern Heart Failure treatment: Rationale and design of the DIG-Mod HF trial

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Aims: Even though it is the oldest Heart Failure (HF) medication still in use, Digoxin has not been evaluated alongside the updated algorithm for treating HF with reduced ejection fraction patients. This could help explain the low use of Digoxin in recent HF studies. The main objective is to assess the effectiveness of Digoxin in functional capacity and cardiac function when combined with modern HF therapy. **Methods:** An interventional, open-label, randomised, crossover trial (EU CT Number: 2024 513448-26-00) among adults with chronic Heart Failure and reduced ejection fraction (≤ 45%), was designed to allocate 50 patients for 24 weeks in each arm (48 weeks of study). The arms are daily intake of Digoxin 0,125mg plus standard of care versus standard of care alone. Both patients with sinus rhythm and atrial fibrillation were enrolled. At the end of each 24 weeks arm, patients will undergo the co-primary outcome measurement of the peak oxygen consumption, evaluated through Cardiopulmonary Exercise Test, and the Global Work Index, measured by transthoracic echocardiography. Key secondary outcome includes a safety composite of death, need for urgent heart transplantation or mechanical assist device, total hospitalisations and sustained ventricular arrhythmias. The study completed recruitment in April 2025 (Table 1 for baseline information) and will complete follow up in March 2026. **Conclusions:** The use of Digoxin could be significantly impacted by evidence that it still has a considerable effect on functional capacity and cardiac function in HF patients.

P13: Development of a Predictive Model Based on Ultrasonography for Successful Ventilatory Weaning in ICU Patients (PredictIVE-US)

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Abstract: This study aims to develop a predictive model using integrated pulmonary, cardiac, and diaphragmatic ultrasonography for ventilatory weaning failure risk stratification in critically ill patients. Weaning failure is associated with adverse clinical outcomes, including prolonged mechanical ventilation, extended hospital stays, and increased morbidity/mortality. Ultrasonography is a promising, non-invasive, and cost-effective diagnostic tool for comprehensive cardiopulmonary and muscular function evaluation, aiding weaning prediction. This prospective, observational, multicenter cohort study is conducted across four ICUs in João Pessoa-PB, Brazil, targeting 156 patients selected by predefined criteria. Patients will be randomized to Pressure Support Ventilation (PSV) or T-tube weaning modalities. Data collection includes detailed ultrasound exams (pulmonary, cardiac, diaphragmatic) performed before, during, and after the Spontaneous Breathing Test (SBT), plus 48-hour post-extubation follow-ups. Concurrently, clinical, ventilatory, and gasometric data are gathered. Statistical analysis will use multivariate logistic regression and Receiver Operating Characteristic (ROC) curves to identify predictors and assess model accuracy, with machine learning techniques for optimization. The model's efficacy will be compared against traditional methods like the Tobin Index. Expected benefits include improved weaning prediction, reduced reintubation rates, and optimized mechanical ventilation management. **Study Progress:** The study is currently on schedule, with data collection actively initiated following comprehensive training of all involved centers. To date, 56 patients have been included in the ongoing recruitment process, out of a planned total sample of 156. Preliminary findings, based on a cohort of 44 valid patients for whom outcomes have been assessed, indicate: extubation success in 75.0% of cases, extubation failure (reintubation) in 18.2%, occurrence of new respiratory insufficiency treated with NIV (without reintubation) in 11.4%, and Spontaneous Breathing Test (SBT) failure without extubation performed in 2.3%. A more comprehensive partial data analysis is planned upon reaching half of the target sample size (78 patients). Concrete data and insights derived from the first half of the recruited sample are anticipated to be available by the time of presentation.

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P14: Serum PON1 activity: Genetic determinants and dysmetabolism risk

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Paraoxonase1 (PON1) is an antioxidant and anti-inflammatory enzyme primarily circulating with HDL. It plays a crucial role in protecting against oxidative stress and metabolic dysfunction. Reduced PON1 activity has been linked to type 2 diabetes (T2D), dyslipidemia and metabolic dysfunction-associated steatotic liver disease (MASLD). We hypothesized that serum PON1 activity and genetic variants could help identify distinct metabolic profiles associated with increased risk of dysmetabolism. We analyzed serum PON1 activity in the PREVADIAB2 cohort and performed a genome-wide association study (GWAS) using PLINK v1.9, adjusting for age, sex and BMI. Genetic analysis confirmed PON1 variants as the primary determinants of PON1 activity, with the strongest association observed at rs2057681 under both additive ($Padj=3.99\times10^{-48}$) and dominant ($Padj=2.04\times10^{-57}$) models. The G allele at this SNP was associated with increased PON1 activity in a dominant manner. Conditional analysis on rs2057681 revealed that the SNP rs854572, located in the PON1 promoter region, remained significantly associated with serum PON1 activity ($Padj=7.87\times10^{-3}$). Preliminary analyses suggest that genetic variation at the PON1 locus may contribute to individual susceptibility to liver fibrosis and T2D. Overall, our findings support a model in which independent variants at the PON1 locus jointly modulate serum PON1 activity, with potential implications for genetic stratification in metabolic dysfunction.

P15: Characterization of cellular interactions between equine colonic epithelial cells and immune cells

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Colitis is a major medical challenge with high morbidity and mortality rates especially in equines. The pathophysiological mechanisms remain poorly understood, particularly the crosstalk between intestinal epithelial cells and immune cells, which is essential for understanding disease mechanisms. This study investigated interactions between equine colon enteroids (eqCE) and peripheral blood mononuclear cells (PBMC) via secreted factors.

EqCE from N=5 horses and PBMC from N=5 different horses were isolated according to established protocols [1,2] and preserved at -80°C. Two-dimensional monolayers were derived from enteroids. After three days of cultivation, 50% supernatants were exchanged between cell types, with half receiving 1 µg/mL lipopolysaccharide (LPS). Controls used unconditioned media. Cell growth was monitored for 78 hours at three-hour intervals using live cell imaging (Incucyte S3).

Both cell types grew normally in respective media mixtures. However, PBMC-conditioned medium decreased eqCE proliferation, aggravated by LPS addition, while LPS alone had minimal effects. PBMC remained unaffected by eqCE supernatants.

Results suggest asymmetric interaction where immune signaling impairs intestinal epithelial cell growth while PBMC are inert to intestinal factors. Future research should include more biological replicates and molecular mechanism investigation, including differentiation and apoptosis markers.

P16: Cholesteryl hemiglutarate induces lysosomal dysfunction and senescence in murine vascular smooth muscle cells

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Stress-induced premature senescence occurs when cells exposed to stressors stop dividing and enter a senescent state. While senescence prevents malignant transformation, the accumulation of senescent cells contributes to aging and age-related diseases, as Atherosclerosis. Atherosclerosis, a chronic and progressive inflammatory disease and the leading cause of cardiovascular disease (CVD), is characterized by the atherosclerotic plaques formation in the arterial wall, where cells like vascular smooth muscle cells (VSMCs) are exposed to an array of LDL lipids, particularly those resulting from oxidation of polyunsaturated fatty acids in cholesteryl esters (PUFA-CEs). We identified cholesteryl hemiesters (ChE), including cholesteryl hemiglutarate (ChG), as the oxidation end-products of PUFA-CEs, in tissues from CVD patients. ChG impairs lysosomal activity and induces senescence marked by an increase of cell cycle inhibitors, loss of lamin B1, and an increase of senescence-associated β-galactosidase enzyme, indicative of lysosomal mass increase and dysfunction observed in senescence. These cells remain metabolically active, as shown by mTORC1/S6K activation, with consequent senescence-associated secretory phenotype secretion. Notably, lysosomal dysfunction appears before senescence markers, suggesting a possible causal link. Further research is required to assess whether restoring lysosomal function can prevent or delay senescence and atherosclerosis appearance and progression.

P17: Preoperative anemia in thoracic surgery: prevalence and impact in a Portuguese cohort

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Preoperative anemia is a risk factor for adverse surgical outcomes [1], yet real-word data in thoracic surgery, especially in Portuguese settings, remain scarce. This study aimed to assess its prevalence and its association with short-term outcomes in patients undergoing elective lung resection.

We retrospectively analyzed patients who underwent elective lung surgery at Hospital de Santa Marta, a tertiary center in Lisbon, in 2022. Data were extracted from electronic records. Anemia was defined per WHO criteria. Ethical approval was obtained.

A total of 239 patients were included (mean age 61 ± 15 years; 65% male). Histopathological analysis confirmed malignancy in 69% of cases. Preoperative anemia was identified in 26% of patients. Age was negatively correlated with baseline hemoglobin and hematocrit, and positively with red cell distribution width.

No major differences in baseline comorbidities (COPD, hypertension, diabetes, coronary artery disease) or smoking status were observed between anemic and non-anemic groups. Anemic patients had higher intraoperative transfusion rates (30% vs. 2%, $p < 0.0001$), longer Intensive Care Unit stays ($p = 0.007$), and longer median hospital stays (7 vs. 5 days, $p = 0.001$). Two-year mortality did not differ significantly.

In conclusion, preoperative anemia was common and associated with increased healthcare utilization. These findings support early identification and management of anemia in thoracic surgery patients.

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P18: Modulation of carotid body activity in obese dysmetabolic mothers prevent dysmetabolic and anxiety and cognitive decline phenotypes in the offspring

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Introduction: Maternal obesity and gestational diabetes impact future generations' metabolism. Lactation is crucial for metabolic development. Maternal obesity causes dysmetabolism, anxiety and cognitive decline in offspring. The carotid body (CB), traditionally recognized for sensing oxygen, carbon dioxide, and pH, is now understood to play a role in glucose and energy homeostasis. Carotid sinus nerve (CSN) resection reduces weight gain and body fat in obese rat models of type 2 diabetes, suggesting CB modulation as a therapeutic target for metabolic diseases. We hypothesize that surgical resection of the CSN will prevent the onset of dysmetabolic conditions and anxiety and cognitive decline in the offspring of mothers submitted to an HF diet Methods: Female Wistar rats were fed either an NC or HF diet for 12 weeks. After this period the groups were randomly divided and half underwent bilateral CSN resection or sham surgery. After a 1-week recovery period post surgery, the females were paired with control male Wistar rats and maintained under their respective diets until weaning of their offspring. Metabolic parameters of the offspring (both females and males), as well as behaviour test to evaluate anxiety, cognition, memory, stress, food behavior were assessed at 60 and 120 postnatal days. Results:. Maternal HF diet leads to pronounced metabolic dysfunction and behavioral impairments in the offspring, including increased anxiety-like behavior and cognitive decline. CSN resection in mothers prevented the dysmetabolic and anxiety-like behavior and cognitive decline phenotype. Conclusions: The findings suggest that targeted interventions to modulate CSN function could potentially mitigate the transmission of dysmetabolism from HF diet-exposed mothers to their offspring.

P19: Core temperature and heart rate responses to exercise in hot-humid and temperate conditions in female football athletes

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The study aims to assess the effects of hot-humid (HEAT) and temperate (TEMP) conditions on the core temperature (T_c) and heart rate (HR) in female football athletes. Six female football athletes [30.5(5.0) years; mean(SD)] performed a 90-minute exercise protocol with two 45-minute halves separated by a 15-minute rest period ($SAFT^{90}$), in HEAT [30°C, 79% relative humidity (RH), 28°C wet-bulb globe temperature (WBGT)] and TEMP (22°C, 62% RH, 19°C WBGT) conditions. Three athletes were excluded from analysis due to not completing the study [injury (n=1) or exhaustion (n=2)]. The study was conducted in a controlled environment. Menstrual cycle (i.e., menstruation and ovulation) and hormonal contraceptive use (i.e., type, formulation, and usage schedule) were tracked, and sessions were scheduled accordingly. T_c (via ingestible telemetric capsules) and HR (via radio telemetry) were recorded continuously. Linear mixed model analyses were performed in Jamovi. Data presented as mean(95% CIs).

Peak T_c was higher in HEAT [38.7(38.4–39.0)°C] than TEMP [38.3(38.0–38.6)°C; p=0.023] and in the first half (p=0.024), regardless of the condition. Mean T_c did not differ between conditions (p=0.096) but was higher in the first half (p=0.014). Peak HR (p=0.736) and mean HR (p=0.619) did not differ between conditions, but both were higher in the first half (p=0.019 and p=0.045, respectively).

Thermal and cardiovascular strain were higher in the first half, with peak T_c greater in the HEAT condition.

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P20: Impact of hydroxytyrosol (OHTyr) treatment on Chronic Intermittent Hypoxia-induced changes in renal AHR Signaling and Cysteine metabolism

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Chronic intermittent hypoxia (CIH) is responsible for drug-resistant hypertension (HTN) development in obstructive sleep apnea (OSA) patients. We linked aryl hydrocarbon receptor (AhR) overactivation and altered cysteine redox dynamics with CIH-induced elevated blood pressure. We also found that hydroxytyrosol (OHTyr), the most bioactive phenolic compound in extra-virgin olive oil, reverts the CIH-induced HTN and decreases renal AhR activation. Herein, we aim to investigate the impact of OHTyr treatment on the renal AhR-HIF interplay and cysteine metabolism under CIH-HTN conditions. Briefly, Male Wistar rats (n≥5/group) underwent CIH (21-5% O_2, 5.6 cycles/h, 10.5h/day) for 21 days, followed by oral treatment with OHTyr (15mg/kg/day in vegetable oil) for 14 days under CIH. Animals under normoxia or CIH alone were controls. The kidney was used to assess changes in AHR-HIF signaling (*e.g.*, PON1, HIFs, VEGF) and cysteine metabolism (*e.g.*, CBS, MST) markers by Western Blot and changes in cysteine-related thiols (*e.g.*, GSH, Cys) by HPLC-FD. This study had ethical approval from DGAV and NMS. OHTyr treatment reversed, at least partially, the changes in renal AhR-HIF signaling and cysteine metabolism, besides normalizing oxidized cysteine levels. Our findings suggest that OHTyr impacts the CIH-induced changes in renal AhR-HIF signaling, in addition to modulating cysteine metabolism/H_2S production. Herein, OHTyr is presented as an *in vivo* putatively relevant approach to treat OSA-HTN.

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P21: Spatiotemporal Mapping of Gut Microbiome-Metabolite Dynamics and Systemic Host Responses to Dietary Berry (Poly)phenols

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The intestinal microbiome critically impacts host health. Diet shapes gut microbiota, influencing microbial metabolite distribution. We fed C57BL/6 mice berry-enriched or standard diets for one week. Temporal/local analyses revealed microbial diversity and (poly)phenol metabolite changes within three days, peaking at one week (ileum/cecum most affected). LC-MS identified gut-PMs and phase II metabolites in plasma/liver, excreta, and lumen, revealing metabolic dynamics. Concurrent multi-organ transcriptomics showed widespread gene alterations: >10% changes in blood, pancreas, ileum, esophagus, liver, bladder, gallbladder; 5-10% in proximal/mid-intestine and proximal colon; <5% in spleen, stomach, mid/distal colon, lungs, heart, kidney, brain. Notably, >300 brain genes changed despite the blood-brain barrier. Pathway enrichment identified 50 altered pathways, with oxidative phosphorylation most impacted. Blood had maximal gene changes but minimal pathway impact. Berry (poly)phenols rapidly remodel regional gut ecology and metabolite flow, triggering systemic transcriptomic responses, demonstrating diet-microbiome-host interplay.

P22: Investigating Lipid Metabolism in Influenza A virus-host interactions

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Influenza A Viruses (IAV) continually threaten animal and human health. Their rapid adaptation underpinning antiviral resistance call for continuous research on the virus-host interactions. These viruses are highly dependent on the host's lipid synthesis throughout their lifecycle, and we explore how infection disrupts sphingolipid homeostasis. Preliminary data shows increased levels of glucosylceramides and lactosylceramides alongside a reduction of GM3. This lipid has drawn attention to the viral community due to its single sialic acid and can be degraded by the host enzyme Neuraminidase3 (NEU3). [1] We have observed NEU3 expression to decrease during infection, but no changes in GM3 synthase or galactosyltransferase were recorded. However, given that IAV also encodes for a neuraminidase in its genome (NA) and that the hosting lab observed that it can cleave the sialic acids of host cell factors, we are investigating the factors involved in GM3 cleavage. This would constitute a second example whereby the viral neuraminidase could regulate the function of syalylated host molecules. [2] We are validating our findings using HPLC and performing LC-MS with standards for GM3. We are also using Eliglustat, a glucosylceramide synthase inhibitor, NA inhibitors, and a mutant virus (NA E229A) that retains only ~20% NA activity. These approaches aim to clarify whether the observed sphingolipid alterations support viral replication or reflect a host response to infection.

P23: Liver-derived extracellular vesicles modulate skeletal muscle insulin response

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Obesity and prediabetes lead to insulin resistance and compensatory hyperinsulinemia. Insulin's action depends on its binding to the insulin receptor (InsRec) and subsequent internalization, a mechanism crucial for insulin clearance, happening mainly in hepatocytes. Recent studies highlight extracellular vesicles (EVs) as key mediators of inter-organ communication, modulating metabolism and insulin sensitivity. We hypothesized that liver-derived EVs (LEVs) carrying InsR modulate skeletal muscle response to insulin, offering a novel strategy to counteract insulin resistance.

We found that plasma EVs carry insulin on their surface, accounting for up to 25% of circulating insulin in both in humans and mice. Interestingly, LEVs are taken up by myocytes and accumulate in the perinuclear region after 24 hours. LEV from prediabetic and obese mice showed higher surface levels of InsR, increasing its insulin-binding capacity. Exposing myocytes to these LEVs altered insulin-induced metabolomic responses, enhancing intracellular glucose and gluconate, while blunting the increase of 25 other metabolites, mainly amino acids and related intermediates. Total glucose transporter 4 (GLUT4) protein increased stepwise in cells incubated with control and prediabetic LEVs under insulin stimulation, despite unchanged mRNA, supporting a post-transcriptional effect.

In total, these findings reveal a LEV-induced metabolic shift with potential therapeutic relevance, which we aim to further investigate.

P24: Gut Microbiota and Inflammatory Response are modulated by Blackberry Intake in Humanized Obese Mice

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Background: Gut microbiota dysbiosis, common in obesity, contributes to the obesity characteristic chronic low-grade inflammation. Anthocyanin-rich fruits like blackberries are known bioactives, wtih impact on gut microbiota and systemic inflammation. Thus, this study aimed to evaluate the impact of blackberry supplementation on gut microbiota and inflammatory markers in a humanized obese mice model. **Methods:** Male C57BL/6J mice were humanized with fecal microbiota from obese patients after 8-day antibiotic regimen and fed a high-fat, high-sucrose diet for 5 weeks. Two groups were formed: FMT_HF (non-supplemented) and FMT_HF_BB (blackberry-supplemented, 0.4 g/day). Body weight, energy intake, serum cytokines, and fecal microbiota (16S rRNA gene) were analyzed. Blackberry composition was determined by HPLC-DAD. Results: Blackberry powder contained 3.6 ± 0.2 mg/g anthocyanins, 93% of which was cyanidin-3-glucoside. FMT_HF_BB mice reduced weight gain, without changes in energy intake. The microbiota composition differed significantly between FMT_HF and FMT_HF_BB groups ($p = 0.035$). FMT_HF_BB showed a significant increase in Bacteroidota ($p = 0.0051$) and a reduction in Proteobacteria ($p = 0.0076$). FMT_HF_BB had reduced IL-1α and MCP-1, increased IFN-β and showed a trend towards higher IL-27 levels (FMT_HF: 58.56±51.71 pg/mL; FMT_HF_BB: 229.80±134.27 pg/mL). **Conclusions:** Blackberry supplementation modulated gut microbiota and reduced inflammatory markers in humanized obese mice, supporting its potential in mitigating obesity-associated dysbiosis and inflammation.

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P25: Exploring the PDE-thiolome as a biomarker of early peritoneal membrane fibrosis

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Background and Objectives: Peritoneal membrane (PM) integrity is key for effective peritoneal dialysis (PD). However, strategies to prevent PM fibrosis remain limited, partly due to the lack of early markers. While oxidative stress is known to trigger fibrosis, most studies focus on radical species. We hypothesized that non-radical pro-oxidants, such as oxidized thiols (e.g., cysteine), may contribute to PM damage. This study aimed to characterize the thiol profile, *thiolome*, in peritoneal dialysis effluent (PDE) and its link to PM fibrosis.

Methods: We conducted a cross-sectional study of incident PD patients at ULSLO (Ethics: NMS 50/2019; ULSLO 2024-63). PM biopsies were obtained at catheter insertion, and submesothelial thickness (STM) was measured. Serum α-Klotho was quantified by ELISA, whose levels <742 pg/mL indicated fibrosis risk. Cysteine (Cys), glutathione (GSH), and cysteinylglycine (CysGly) in PDE were analyzed by high-performance liquid chromatography with fluorescence detection (HPLC-FD). Univariate analysis and Principal Component Analysis (PCA) were performed.

Results: Among 41 patients (mean age 55 ± 2; 30% female), 39% showed fibrosis. Total GSH (free + protein-bound forms) and free total GSH, and the protein-bound forms of all thiols showed >50% variability. PCA did not differentiate fibrotic from non-fibrotic profiles. However, total Cys was positively correlated with STM ($r = 0.33$, $p < 0.05$). Patients with low α-Klotho had higher total and oxidized Cys ($p = 0.03$; 0.02) and CysGly ($p = 0.03$; 0.01).

Conclusions: Altered thiol metabolism, especially involving Cys and CysGly, might signal early PM fibrosis. These findings support their potential as biomarkers of PM vulnerability in PD.

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P26: The impact of climate change on anemia in Cape Verde

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More than 2 billion people worldwide suffer from anemia, with over 60% cases in sub Saharan Africa. This scenario is aggravated by malnutrition, as insufficient (micro)nutrient intake increases the susceptibility to infectious outbreaks. Nutritional anemia is the most common form of anemia. It often coexists with anemia of inflammation. Both compromise the ability of the immune system to cope with the infection. Anemia of inflammation affects nearly 40% of sub-Saharan population. Its prevalence is mainly related to the high incidence of vector-borne diseases, which geographical transmission is influenced by climate change. Environmental alterations affect mosquito ecology, increasing the proliferation and competence of these vectors to spread illnesses causing anemia of inflammation. Pathogen adaptation to evolving climate conditions represents a major health threat to countries where specific infections have been eradicated or remain at low incidence. This research aims to assess the prevalence of anemia of inflammation in Santiago Island, Cape Verde, by determining the influence of climate change on mosquito proliferation and the transmission of vector-borne diseases triggering anemia. Preclinical data from our laboratory support this hypothesis and will be used to foresee potential reality scenarios. The impact of environmental variables, e.g. temperature, humidity, and extreme weather events, on human health will also be analyzed and used to confirm our prediction.

Session 4 – *Cancer and Health Promotion*

P27: Person-centred Care: A Compassionate Approach to Palliative Care

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Compassion is increasingly recognised as central to person-centred palliative care, yet its translation into community-based clinical practice lacks structured evidence. This PhD research aims to design, validate, and pilot a complex intervention to enhance compassionate care during the first home visit of Community Palliative Care Support Teams (ECSCP). Following the Medical Research Council Framework, methodology includes five phases: scoping review, qualitative data collection, modified Delphi consensus, protocol co-development, and a pilot study in contrasting urban and rural settings. Outcomes will be assessed using the Compassionate Engagement and Action Scales. The sample includes ECSCP professionals and family caregivers of patients in pre-terminal or terminal stages. Expected results: improved emotional resilience, therapeutic relationships, and care satisfaction. Findings will contribute to ethically grounded models, compassion-focused professional training, and culturally adapted practices for community palliative care.

P28: Telemedicina Assíncrona Suportada por IA para Cuidados Pediátricos: Um Estudo de Avaliação Multicêntrico (TelePedIA)

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Introdução: Em Portugal, a maturidade digital das instituições de saúde é heterogénea. No quadro europeu, o Regulamento da inteligência artificial (IA) impõe obrigações a sistemas de alto risco, como os da saúde. Antes da sua aplicação, os algoritmos devem provar a sua conformidade e eficácia. Objetivo: Avaliar a eficácia, segurança, satisfação e eficiência operacional de uma plataforma de telemedicina assíncrona melhorada por IA para cuidados pediátricos no Serviço Nacional de Saúde português. Métodos: Metodologia mista para um estudo piloto organizacional de 18 meses em duas unidades de saúde locais (Lisboa e Almada-Seixal), com aproximadamente 800.000 habitantes. A plataforma, Usawa Care, imita a comunicação por WhatsApp e integra IA para estruturar os dados enviados pelos doentes e sugerir ações clínicas. Os pediatras irão rever, validar e responder aos casos assincronamente. Os dados quantitativos provêm de métricas da plataforma; os dados qualitativos serão obtidos através de entrevistas semiestruturadas analisadas com apoio do MAXQDA para codificação temática. Relevância: O projeto visa gerar evidência sobre a telemedicina assíncrona nos cuidados pediátricos, com foco na redução da pressão sobre os serviços de emergência, melhoria do acesso e tomada de decisões através da IA, mantendo a segurança clínica e a supervisão humana.

P29: The Role of Platelet-Rich Plasma in Conservative Management of Rotator Cuff Tears – a Scoping Review

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Objective: To evaluate high-quality evidence on the role of platelet-rich plasma (PRP) in rotator cuff tears, identifying trends, gaps, and offering an updated synthesis.

Introduction: Rotator cuff tears are common and cause significant pain and dysfunction. Conservative treatments aim to relieve symptoms and restore function. PRP, a biological therapy, may enhance healing and improve outcomes, but existing literature shows mixed and heterogeneous results regarding its efficacy and safety.

Inclusion Criteria: Systematic reviews, meta-analyses, and recent RCTs on PRP as a conservative treatment for adult rotator cuff tears (≥18 years) will be included.

Methods: We will search PubMed, Scopus, Web of Science, Cochrane Library, Epistemonikos, ClinicalTrials.gov, CTIS, EU Clinical Trials Register, ICTRP, and ISRCTN. Citation tracking and hand searching will supplement. No language or date limits apply for systematic reviews/meta-analyses. RCTs will be searched from Jan 2024 to Mar 2025 in any language. Two reviewers will screen studies independently; discrepancies will be resolved by discussion or a third reviewer. Data will be extracted into a structured chart, summarized in tables/graphs, and critically analysed to map current evidence and gaps.

P30: Unraveling Griscelli's syndrome hypopigmentation using an in vitro model

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Griscelli syndrome (GS) is a rare disorder characterized by hypopigmentation of the hair and skin. Skin pigmentation relies on melanin, which is produced and stored in specialized organelles termed melanosomes, within melanocytes. Melanin is transferred to keratinocytes, where it accumulates in supranuclear caps and exerts a photoprotective effect. GS is caused by mutations in MYO5A, RAB27A or MLPH, which encode for three proteins that form a tripartite complex required for the positioning of melanosomes in melanocyte dendrites. Strikingly, it is not understood why GS patients display hypopigmentation, as there is no significant difference in melanin transfer between co-cultures of keratinocytes and Rab27a-deficient melanocytes. This opens the need for an in vitro model that mimics GS patients' skin pigmentation defect. Also, we hypothesize that only such a model can reproduce the deficient melanin transfer that occurs in the skin of GS patients, and the defects in melanin transfer caused by Rab27a depletion that are not apparent in co-cultures. We successfully established a GS in vitro model and confirmed that melanin is transferred by a different mechanism when Rab27a is depleted. Electron microscopy studies showed that Rab27a-silenced melanocytes secrete large globules from the dendrites and cell body, laden with melanosomes. Thus, the creation of a disease model that recapitulates GS etiology can shed light on the mechanisms of melanin transfer in pathological conditions.

P31: Development of Fibrillar Hydrogels to Study the Mechanical Stiffness of Breast Cancer Subtypes and Therapeutic Responses

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This project aims to develop fibrillar hydrogels with tunable mechanical stiffness to biomimic the breast cancer tumor microenvironment. This study aims to investigate the influence of matrix stiffness on cellular behavior and therapeutic responses of breast cancer subtypes. This research seeks to contribute to the development of enhanced therapeutic strategies for breast cancer. Hydrogels were developed by integrating human platelet lysate (PL) with aldehyde-cellulose nanocrystals (a-CNC), synthesized through periodate oxidation. These 3D models were produced using different concentrations of a-CNCs (0-0.61 wt%) and crosslinked with thrombin and $CaCl_2$. Mechanical and morphological characterization are conducted using Atomic Force Microscopy (AFM), Rheology, Confocal, and Fluorescence Microscopy. Furthermore, MCF-7 spheroids were encapsulated within the hydrogel to study migration and invasion responses. Preliminary data indicate successful synthesis of CNC and a-CNC, exhibiting rod-shaped morphology and colloidal stability. These data also show that higher a-CNC concentrations (0.61 wt%) improve hydrogel stability. Hydrogels are expected to reproduce stiffness values representative of native tissue, approximately 1.8 kPa for normal tissue and up to 5.7 kPa for TNBC. Increasing hydrogel stiffness will affect the proliferation of encapsulated cell lines, with TNBC demonstrating more aggressive responses. These findings are expected to anticipate the differences in stiffness and support the application of biomimetic 3D models in the mechanobiology of breast cancer research.

Keywords: 3D models; biomimetic models; extracellular matrix.

P32: LDH and Metabolic Heterogeneity in Brain Tumors

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Brain tumors, as Glioblastoma, are aggressive and lethal diseases, with treatment failures predominantly attributed to tumor heterogeneity. Genetic heterogeneity has been well-studied, but the role of metabolic heterogeneity in tumor progression and recurrence remains unclear. As metabolic reprogramming is a hallmark of cancers, this becomes particularly relevant. The aim of my PhD project is to understand how metabolic heterogeneity supports Neural stem cell-derived tumors (tNSCs) and tumor progression. Using *Drosophila* tNSCs we discovered that a subset of tNSCs overexpresses Lactate dehydrogenase (LDH) during tumor development, which we found to be essential for normal tumor growth. LDH converts pyruvate to lactate and is therefore a key enzyme in the orchestration of anaerobic glycolysis and the Warburg-effect. Our results show that LDH overexpression leads to larger tumors compared to control tumors, putting LDH as a linchpin in the generation of metabolic heterogeneity and Warburg-like metabolism induction in a subset of cells that could sustain tumor growth. However, it remains unknown how LDH heterogeneity is regulated, how it impacts tumor metabolism and how it correlates with tumor progression. I hypothesize that tumors modify metabolism in a heterogeneous way, upregulating LDH and possibly other metabolic pathways, and that this is a relevant and unexplored strategy to sustain tumor growth and might provide with the basis for new therapeutical approaches.

P33: Uterine inflammatory myofibroblastic tumor: clinicopathological and molecular study emphasizing p16 absence and CDKN2A deletion association with aggressiveness

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Uterine inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal neoplasm with frequent 'leiomyoma-like' morphology associated with *ALK* gene rearrangement. Clinical outcome is uncertain, but *CDKN2A* deletion and genome complexity have been shown to be prognostic relevant. We aim to evaluate clinicopathological and molecular features of a small series of uterine IMT.

Four cases of uterine IMT were diagnosed between 2020-2025 in our archives. Clinicopathological data was reviewed. p16 and p53 IHC were performed as well as FISH for *ALK* and *CDNK2A* and CGH.

Patients were aged 53-75 years old (mean age:65 years). One case presented with extrauterine disease. During a mean follow-up of 14.3 months, none of the cases recurred. Tumors' mean size was 12.2 cm. 3/4 cases (75%) showed expansive borders. An exclusive compact, leiomyoma-like, pattern was present in 2/4 of cases with the remainder showing a myxoid pattern (ranging from 60-90%). A diffuse and severe lymphoplasmacytic infiltration was present in 3/4 of cases. Ischemic-type necrosis was present in 3/4 of cases and 2/4 had moderate/severe atypia. Mitotic mean count was 2/10HPF. Based on the risk stratification score, 1 case was classified as high-risk and 3 as intermediate-risk. All cases were ALK IHC positive (diffuse or multifocal) and smooth muscle actin and desmin expression ranged from multifocal to diffuse in all cases; caldesmon was negative in one case. All cases were wild-type p53. 3/4 cases had *ALK* gene rearrangements by FISH. Only the tumor with extrauterine disease had p16 expression in <1% of cells and *CDKN2A* deletion. P16 was patchy in the remaining tumors and no *CDKN2A* deletions were found. A complex profile was found by CGH in 3 cases.

Some IMTs can have an exclusive 'leiomyoma-like' morphology. ALK IHC staining may not correlate with *ALK* fusions, especially using FISH. P16 IHC and *CDKN2A* deletion may be prognostic useful.

3 Keywords: ALK; inflammatory myofibroblastic tumor; CDKN2A

P34: Edible proteins in the treatment of rectal cancer

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Background: in the treatment of localised rectal cancer, patients who achieve a pathological complete response in the surgical specimen after neoadjuvant treatment have a better prognosis than the patients who do not. Legume seeds contain deflamin, a potent matrix metalloproteinase (MMP) inhibitor. MMP inhibition by deflamin stops cancer cells' migration and causes tumours to shrink. A deflamin enriched legume seed extract has been tested in preclinical models with promising results. The legume seed extract is compatible with human consumption.

Methods: this is a prospective, investigator-initiated, first-in-human, unicentric, randomised, double-blind, placebo-controlled, dose-escalation, phase 1-2 clinical trial comparing standard-of-care + placebo versus standard-of-care + legume seed extract, in patients with localised rectal cancer. The primary endpoint is the pathological response. The secondary endpoints are progression-free survival at 6, 12 months post-surgery, and the changes in the plasma levels of proinflammatory cytokines. The planned sample size is 50 patients, 25 patients for each arm (experimental versus control). Longitudinal blood samples will be obtained. Patients will be followed up from screening visit until 12 months after surgery. The follow-up of all patients is expected to be completed 2-3 years after the first patient's screening visit.

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P35: Keratinocyte responses to ultraviolet radiation-modified melanin

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Melanin is produced by melanocytes and transferred to keratinocytes, where it exerts its photoprotective effect against UVr-induced damage. However, it can also cause harmful effects upon UVr exposure, promoting cellular toxicity and behaving as a "double-edged sword". Therefore, melanin persistence must be tightly regulated to balance its protective and toxic effects under UVr exposure. Autophagy has been implicated in this regulation, even though melanin seems to resist degradation in moderately acidic and poorly degradative organelles termed melanokerasomes. Therefore, we hypothesize that UVr-induced alterations in melanin, along with the resulting ROS production, trigger autophagy as a protective mechanism. To test this hypothesis, we optimized UVr dosages that induce ROS production with low cell death, mimicking environmental conditions. Moreover, melanocores irradiated with the same UVr dosages and then fed to keratinocytes led to increased cell death, compared with keratinocytes fed with non-irradiated melanocores. Additionally, we confirmed the increased ROS production in keratinocytes fed with UV-irradiated melanocores. This supports that altered melanin can induce oxidative stress and cell death. We are now analyzing autophagic flux to understand the mechanisms underlying melanin turnover within keratinocytes. This can pave the way for novel skin cancer prevention strategies, therapies for pigmentary disorders, and cosmetic applications.

P36: Decoding the role of a new subset of low-density neutrophils in triple negative breast cancer progression and immunotherapy resistance

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Triple-negative breast cancer (TNBC) is an aggressive subtype with poor prognosis and limited treatment options. Although immunotherapy has emerged as a promising approach, most patients fail to respond, likely due to tumor microenvironment (TME) mediated resistance. Low-density neutrophils (LDN), a heterogeneous population with immunosuppressive features, are increasingly recognized as key mediators of immune evasion. Our team recently identified a novel subset of LDN expressing the chemokine receptor X (X^+LDN), enriched in metastatic breast cancer patients and associated with worse outcomes. These cells exhibit enhanced migration toward tumor-derived chemokines and potent immunosuppressive activity. This project aims to deeply characterize X^+LDN phenotypically and functionally, investigate their metabolic and transcriptomic profiles, and clarify their role in immunotherapy resistance. We will employ advanced techniques, including spectral flow cytometry, metabolomics, single cell RNA-seq, and dynamic tumor-on-chip models to explore neutrophil–tumor interactions under physiologically relevant conditions. Additionally, we will assess the clinical relevance of X^+LDN and test novel therapeutic strategies to modulate or block their activity. Ultimately, we anticipate establishing X^+LDN as prognostic biomarkers and therapeutic targets, supporting the development of more effective and personalized immunotherapies for TNBC.

P37: Identification of colorectal malignancies enabled by phasor-based autofluorescence lifetime macroimaging and ensemble learning

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Significance: Colorectal cancer (CRC) remains one of the most frequent cancers and a leading contributor to cancer-associated mortality globally. CRCs are often diagnosed at an advanced stage, which leads to high mortality and morbidity. This outcome is exacerbated by high rates of recurrence and postoperative complications that contribute substantially to poor prognosis. Advancements in endoscopic assessment have improved CRC prevention, early detection, and surveillance over the years. Yet, CRC remains one of the most significant health challenges of the 21st century. Label-free optical spectroscopy methods have long been explored as potential partners to endoscopy, not only to enhance diagnostic accuracy but also to confer predictive capabilities to endoscopic evaluations.

Aim: We investigated the potential of time-resolved autofluorescence measurements excited at 375 nm and 445 nm to correctly classify benign and malignant tissues in CRC surgical specimens from 117 patients.

Approach: Multiparametric autofluorescence lifetime data were collected in two distinct datasets, which were used for training (n = 73) and testing (n = 44) a supervised ensemble learning classification model, with standard histopathology assessment serving as ground truth.

Results: Using 5-fold cross-validation, we achieved 82.6 ± 0.02% sensitivity, 90.4 ± 0.01% specificity, 87.4 ± 0.01% accuracy, and 0.941 ± 0.004 area under the curve (AUC) for training data. Evaluation on unseen test data yielded similar results, with 85.2% sensitivity, 84.5% specificity, 84.8% accuracy, and 0.915 AUC.

Conclusions: While preliminary, our findings underscore the potential impact of AI-assisted autofluorescence lifetime measurements in advancing CRC prevention, early detection, and surveillance efforts.

Keywords: autofluorescence lifetime, colorectal cancer, machine learning, phasors, label-free, diagnosis, disease monitoring.

P38: Contribution to validation and implementation of a tailored digital risk management tool by clinical research teams on the quality of clinical trials

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Background: Clinical trials (CT) face increasing complexity and resource demands, necessitating performance improvement strategies to achieve desired outcomes with fewer resources. The safety and effectiveness of a trial relies on stakeholders managing risks effectively. Challenges such as low patient referral rates, investigator shortages, stringent rules, and inadequate infrastructure hinder clinical research performance. Traditional risk management techniques often fall short in addressing these complexities, and specific guidelines or resources for clinical research sites are lacking. In the dynamic landscape of clinical research, risk management is mandatory to ensure the successful execution of CT. The significance of identifying and mitigating potential risks at clinical trial sites cannot be overstated, as several of these risks have the potential to impact not only the integrity of the study but also the safety of participants and the credibility of the trial outcomes. The purpose of this project is to contribute to the development, validation and implementation risk assessment and mitigation measures for clinical teams during clinical trials, promoting a rigorous method of assessment in health technologies, to attest to differentiated quality and capable of cementing decisions in health policies. Based on the hypothesis that implementing a structured risk management model in clinical trials may reduce the occurrence of protocol deviations, improves the quality of data collected and increases the operational efficiency of the study. Methods: The project will have three tasks that foster the project design. Task 1 - Characterization of clinical trials in Portuguese research sites, for which a descriptive study will be developed; Task 2 - Validation and acceptability of the use of a digital clinical risk management tool, for which a methodological study will be developed; Task 3 - Assess a tailored risk management digital tool by clinical research teams on the quality of clinical trials, for which a longitudinal cohort study will be implemented. This project will be conducted under a joint collaboration between NMS-UNL and CoLAB Trials.

P39. Turning Vitamin D3 into a Nanocarrier: A Synergistic Strategy with Paclitaxel Against Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with limited therapeutic success and high resistance to conventional chemotherapy. To address these challenges, enzyme-responsive micelles based on vitamin D3–polyethylene glycol (VD3-PEG) conjugates were developed for the controlled delivery of paclitaxel (PTX). Two conjugates using PEG chains of different lengths (600 and 2000 Da) were synthesized and self-assembled into sub 100 nm micelles with high PTX encapsulation efficiency and colloidal stability. These micelles exhibited esterase-triggered drug release, mimicking the enzymatic profile of the PDAC tumor microenvironment. In vitro studies using 2D and 3D models based on human PDAC cells (BxPC 3 cells) demonstrated superior cytotoxicity of VD3-PEG micelles compared to free PTX, attributed to the dual therapeutic action of PTX and VD3. Notably, the PTX-loaded micelles showed enhanced tumor growth inhibition in an in ovo CAM model compared to Abraxane®, the clinical standard nanoformulation. Overall, VD3-PEG micelles may have a promising role in PDAC therapy, since VD3 could act not only as the hydrophobic core of the micelles but also as a therapeutic agent that provides synergistic effects with the encapsulated PTX.

3 Keywords: Vitamin D3-based micelles; Paclitaxel delivery; Pancreatic ductal adenocarcinoma.

P40: Resistance in Legionella: Portuguese findings

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Legionnaires' disease is a severe form of pneumonia caused by Legionella, a waterborne bacterium. Transmission occurs through contaminated aerosols released by showers, cooling towers, etc. If the correct antibiotic is administered promptly, the disease can be successfully treated. However, therapeutic failures related to resistance mechanisms have been reported. The lpeAB gene, a resistance-associated gene that encodes the efflux pump LpeAB and found predominantly in Lp serogroup1 (Lp sg1), has been associated with decreased susceptibility to azithromycin. This study aims to investigate the presence of the lpeAB gene in Legionella clinical (n=178) and environmental (n=245) isolates and for a subset of environmental isolates, determine the pattern of susceptibility to 5 antimicrobials. The lpeAB was screened using PCR and the EUCAST microdilution method was used to assess the susceptibility patterns of 5 antimicrobials for 208 environmental isolates. The lpeAB gene was present in 21% (87/423) of the total collection of isolates. It was found in 10% of clinical isolates, 82% of which belonged to Lp sg1, and in 29% of environmental isolates, 66% of which belonged to Lp sg1. These findings raise concerns about potential future risks to public health. The susceptibility pattern identified in the 208 environmental isolates recovered in Portugal was greater than that reported by EUCAST. These results can lead us to a possible early phenomenon of resistance in the environment. Keywords: Legionella; Resistance mechanisms; lpeAB.

P41: Rab7a regulates lysosome exocytosis and triple-negative breast cancer cell invasion

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The small GTPase Rab7a plays a central role in late endocytic trafficking by regulating endolysosomal transport through the interaction with effectors, including RILP for retrograde movement of late endosomes and lysosomes, and FYCO1 for the respective anterograde movement. Moreover, Rab7a mediates endolysosomal maturation and fusion [1]. Beyond these roles, Rab7a was shown to have a dual role in cancer, acting both as an oncosuppressor and an oncoregulator, depending on the cellular context. In this study, we investigated the role of Rab7a in lysosome exocytosis and assessed its impact on the invasive behavior of triple-negative breast cancer (TNBC) cells. We found that Rab7a silencing in MDA-MB-231 cells significantly enhances lysosome exocytosis and TNBC cell invasion. We also observed an accumulation of enlarged LAMP1-positive vesicles in the perinuclear area, suggesting that Rab7a depletion leads to the impairment of endolysosomal fusion, resulting in hybrid late endosomal/lysosomal compartments. Conversely, RILP silencing was observed to decrease both lysosome exocytosis and TNBC cell invasion. This phenotype suggests opposing roles for Rab7a and its effector RILP, supporting the hypothesis that Rab7a promotes lysosome exocytosis and invasion via another effector, potentially FYCO. Therefore, we are exploring the role of FYCO in endolysosomal dynamics to clarify the mechanism by which Rab7a regulates lysosome exocytosis and TNBC cell invasiveness.

P42: Improving fertility care – oocyte ageing and its impact on gamete quality

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Ageing influences all cells. Oocytes are strongly affected by that, and investigation on the molecular composition of follicular fluid and cumulus cells has the potential to shed some light on whether there are molecular signatures that enable doctors to better identify patients at risk for IVF failure due to this issue. During the course of this project, we intend to conduct an exploratory analysis of the molecular constitution of the oocyte's microenvironment, using the cells that surround it. In order to achieve this, we will collect follicular fluid and cumulus cells from patients undergoing ovarian pick up of oocytes in a public hospital and then analyze them using bioinformatics tools. Once identified the most relevant molecules, we will attempt to verify whether the same pattern is present in peripheral blood samples from those patients. The final goal is to identify something that could be used as a biomarker of oocyte quality, during infertility workup and prior to treatments. This would undoubtedly lead to better results in assisted reproduction treatments, in a shorter period of time.

P43: Evaluating Large Language Models (LLMs) as a Screening Tool in Breast Biopsy Diagnosis: A Feasibility Study

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Background and Objective: Workloads in pathology have been increasing in volume and complexity. Advances in artificial intelligence (AI) promise increased productivity; adoption remains the exception, however. We investigated the performance of generative algorithms in breast biopsies, using different prompting strategies and retrieval augmented diagnosis (RAG), assessing their potential as screening tools for preliminary review, report pre-filling, and immunohistochemistry (IHC) recommendations.

Methods: Representative images of lesions were extracted from breast biopsies gathered from our archive and fed to GPT4o and o1 large language models (LLMs), alongside clinical information. Two different prompts were tested with each model: a short one, defining only the task – breast biopsy diagnosis – and a longer one detailing common differentials and a diagnostic approach. Each model and prompt were tested with and without RAG.

Results: Our series consisted of 65 cases, all female (ages 18-95). Twenty-three were benign and 42 malignant. Overall diagnostic accuracy varied between 35.38% and 53.85%. For the detection of malignancy, however, this value increased to 73.21-81.54%. Differences between runs did not show statistical significance. The best performing combination was GPT4o with the longer prompt, achieving a sensitivity of 83.33%, a specificity of 78.26%, a PPV of 87.50% and a NPV of 72.00% for the detection of malignancy. IHC studies for estrogen, progesterone, HER2 and Ki67 were correctly ordered in between 67.69% and 83.07% of cases; when these were ordered, however, they were done so correctly in 92.68-95.07% of cases.

Conclusion: Our results highlight the potential of LLM use in breast biopsy screening and first look. Although the algorithms failed to identify lesions correctly in a significant proportion of cases, they showed good sensitivity and specificity in identifying malignancy and proved accurate in the ordering of additional IHC studies. Therefore, even with current limitations, productivity gains may be possible through screening prior to pathologist evaluation.

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