

A B S T R A C T B O O K

GIGA DAY 2025

12.09.25

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GIGA DAY

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LIÈGE université GIGA institute

INVITED SPEAKERS

Michael Heneka, University of Luxembourg
Frédéric Kerff, ULiège
Valentin Fischer, ULiège
Olivier Malaise, CHU Liège
Jingjing Zhu, UCL

Invited Speakers

Michael Heneka
Frédéric Kerff
Valentin Fischer
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Jingjing Zhu

GIGA Speakers

Sylvia Tielens
Joseph Jorssen
Sophie Bekisz
Nesrine Farhat
Ning An

Flash Talks

Tristan Drouet
Victor Grentzinger
Lola Langhendries
Fanny Lardinois
Mélanie Louras
Kim Mottard
Lobna Oueslati
Pauline Salpetier
Alexandre Szedleskai
Thomas Trevisan

avantor

MERCK



Hello & Welcome

We are pleased to welcome you to this year's scientific event. This abstract book reflects the excellence and diversity of the research conducted at ULiege, within the GIGA-center, from fundamental discoveries to clinical translation. We warmly thank our sponsors for their support, which makes this day possible, and we are especially grateful to our invited speakers for generously sharing their expertise and insights. We hope these contributions will inspire stimulating discussions, new collaborations, and a stronger sense of community.

The GIGA Direction,
Brigitte Malgrange
Laurent Nguyen
Clio Ribbens
Sandrina Evrard

INVITED SPEAKERS

MICHAEL HENEKA

Luxembourg Centre for Systems Biomedicine, University of Luxembourg

Prof. Michael Heneka and his group are involved in basic science and translational research with a focus on neurodegeneration and neuroinflammation. Major diseases of interest and research topics include Alzheimer's disease, amyotrophic lateral sclerosis, septic encephalopathy and multiple sclerosis. In clinical neurology, Prof. Heneka holds special expertise in neurodegenerative and autoimmune CNS disorders.

Amicroglia's fate in Alzheimer's disease

The accumulation of neurotoxic amyloid beta peptides along with neurofibrillary tangle formation are key pathological hallmarks of Alzheimer's disease. The brain has been considered as an immune-privileged organ, however, increasing evidence from translational, genetic, and pathological studies suggests that activation of distinct innate immune pathways are a third important disease hallmark which actively contributes to disease progression and chronicity.

Microglia play a pivotal role in this immune response and are activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors. This immune activation initially aims to resolve the

pathological challenge through TAM receptor ligation. Over time, however, it results in the chronic release of inflammatory mediators and diverts microglia cells from their physiological functions and tasks. Sustained NLRP3 inflammasome activation in causes a hyperinflammatory microglial cell death called pyroptosis which is the release of ASC specks. The latter contributes to seeding of pathology by enhancing the propensity of beta-amyloid peptides to aggregate. This mechanism may account for the spread of pathology within a brain region, but also from one brain area to another. Increased cell death in turn will cause proliferation of microglial cells generating a subpopulations. Interestingly these subpopulations show changes in the transcriptome when compared to non-proliferating cells and also restricted Ab clearance capacity.

FREDERIC KERFF

Integrative Biological Sciences (InBioS) , CIP, Université de Liège

Prof. Frédéric Kerff received a master's in physics at the university of Liège, followed by a Ph. D from the same University on the structural characterization of class D b-lactamases at the Center from Protein Engineering (CIP) under the direction of Prof. Paulette Charlier. He did then a posdoc at the Boston Biological Research Institute in the lab of Prof. Roberto Dominguez working on actin related proteins. He eventually returned to the CIP in Liège and obtained a Research Associate position (FRS-FNRS). Over the years his research has been related to a wide array of proteins related to the peptidoglycan metabolism in bacteria and the mechanisms of resistance to the b-lactam family of antibiotics.

The AI revolution in structural biology and what can AlphaFold3 do for you at ULiège

The determination of biomolecular structures has long been a cornerstone of structural biology, yet experimental approaches such as X-ray crystallography, NMR spectroscopy, and cryo-electron microscopy cannot keep pace with the rapidly growing number of known sequences. With more than 250 million protein sequences in UniProtKB and

only ~225,000 structures in the Protein Data Bank, artificial intelligence has become an essential tool to bridge this gap. The advent of AlphaFold2, developed by DeepMind, marked a breakthrough in protein structure prediction and revolutionized the field with unprecedented accuracy and confidence metrics. AlphaFold3, released in 2024, extends these capabilities by introducing a diffusion-based modeling approach and broadening the prediction scope to proteins, nucleic acids, ligands, and complexes. This enables modeling of diverse biomolecular interactions and provides tools to discriminate between alternative models through confidence scoring.

In this seminar, we review the history of AI-driven structure prediction, the principles underlying AlphaFold's success, and the key metrics to evaluate model reliability. We then discuss the potential of AlphaFold3 as well as other similar software both on the public webserver and through our local installation at the University of Liège, which enables high-throughput predictions, ligand docking, and flexible modeling scenarios.

VALENTIN FISHER

Evolution and Diversity Dynamics Lab, Geology, Université de Liège

Prof. Valentin Fischer is a geologist (ULiège, 2009) and paleontologist (Ph.D. Royal Belgian Institute of Natural Sciences and ULiège 2013). He completed a postdoctoral fellowship at Oxford as a Newton Internal Fellow before accepting a position as professor of paleontology at ULiège in 2015. He is also the curator of the animal and plant paleontology collections and heads the science outreach department at ULiège (Réjouissciences). His research focuses on fluctuations in the biodiversity of prehistoric marine predators.

How ancient climate change affected top predators

Amniotes secondarily adapted to aquatic life have been the dominant predators in marine ecosystems since the catastrophic mass extinction of the Permian-Triassic boundary, 252 million years ago. Over that extended time span, these top predators underwent dramatic changes in the composition of their assemblages, in parallel with profound modifications in climate, food resources, and ecosystem structures. These changes can be seen as past replications of a general phenomenon where environmental changes spiral up to force total extinction of predatory

clades. In this presentation, we will investigate how the extreme climate warming that happened \approx 100 million years ago, notably forcing sea levels 200m higher than today, resulted in a profound turnover amongst marine predators. The signature of these events are still evident in nowadays ecosystems.

OLIVIER MALAISE

Rheumatology, CHULiège & Laboratory of Rheumatology, GIGA

Prof. Olivier Malaise is a rheumatologist and has been interested in osteoarthritis since he finished his medical studies. Working on the one hand in the clinical rheumatology department of the University Hospital of Liège, where he meets many patients suffering from this pathology, he maintains a basic research activity at the GIGA where he tries to answer the questions he asks himself in front of his patients.

Laboratory advances in osteoarthritis: from synovial fibroblasts to JAK inhibition

Osteoarthritis is an incurable degenerative disease affecting the cartilage and whose treatment is based exclusively on physiotherapy, corticosteroid injections and, ultimately, prosthetic replacement surgery. This sentence, too often heard, contains as much inaccuracy as the words that compose it. Through this talk, we demonstrate that osteoarthritis is not only a disease of the cartilage, but also affects the synovial membrane. We study the effects of corticosteroids on the metabolic and senescent component of osteoarthritis and put into perspective the effects of mesenchymal stem cells and Jak-kinase inhibitors in this pathology.

JINGJING ZHU

Ludwig Institute & Immunity & Cancer, Institut de Duve, UCL

Jingjing Zhu obtained her PhD in Biomedicine from KU Leuven in 2013, where she developed high-specificity nanobodies targeting furin for anti-cancer therapy. She then pursued postdoctoral training in Prof. Benoît Van den Eynde's laboratory, uncovering major immunosuppressive mechanisms that shape cancer progression and limit therapy response, notably Fas ligand-induced apoptosis of tumor-infiltrating T cells (Nat Commun 2017; Cancer Immunol Immunother 2019). Since 2017, she has led her own research group at the de Duve Institute, advancing innovative strategies to enhance antitumor immunity. Her team created a CD8 T cell-based delivery platform for anti-PD-L1 nanobodies, achieving superior tumor control in preclinical models (Cancer Immunol Res 2022). In 2023, they identified $\alpha 2$ -adrenergic receptors as novel immunotherapy targets (Nature), a discovery recognized with the Baillet Latour Medical Award in 2024. As a group leader, she continues to translate fundamental discoveries into next-generation immunotherapies.

From neurotransmission to tumor rejection: exploring the $\alpha 2$ -adrenergic immune axis

We report that $\alpha 2$ -adrenergic receptor ($\alpha 2$ -AR) agonists show potent antitumor activity in multiple immunocompetent tumor models, including ICB-resistant settings, but not in immunodeficient hosts. Their effects were blocked by $\alpha 2$ -AR antagonists and absent in *Adra2a*-knockout mice, confirming on-target action in host cells. Treated tumors displayed greater T cell infiltration and myeloid suppressor cell apoptosis, with single-cell RNA sequencing revealing activation of immune pathways in macrophages and T cells. Functional studies showed that efficacy required $CD4^+$ and $CD8^+$ T lymphocytes as well as macrophages.

These findings position $\alpha 2$ -AR agonists—some already clinically available—as promising candidates to boost immunotherapy and overcome ICB resistance.



SYLVIA TIELENS

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Elongator controls the maturation tempo of brain ependymal cells

Conditional deletion of *Elp3* in the mouse forebrain leads to microcephaly at birth. In this study, we demonstrate that these mice also develop postnatal hydrocephalus, associated with an enlargement of the brain ventricles. In wild-type mice, ependymal motile cilia are properly aligned to facilitate the circulation of cerebrospinal fluid (CSF) within the ventricles. Our findings reveal that *Elp3* loss induces endoplasmic reticulum (ER) stress and upregulation of ATF4 expression in ependymal cell progenitors, which compromises Notch signaling and accelerates their maturation. This is accompanied by a disruption in the establishment of rotational and translational polarities of the motile cilia of maturing ependymal cells, resulting in disorganized cilia bundles. Collectively, these molecular abnormalities lead to the premature and abnormal development of ependymal cells, culminating in cilia beating dysfunction, impaired CSF clearance, and the development of hydrocephalus.

JOSEPH JORSSSEN

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Eosinophils: Ontogeny and mechanisms of lineage expansion in eosinophilic diseases.

Eosinophils are specialized granulocytes predominantly considered for their diagnostic value in type 2 immune disorders, but have also been attributed roles in immune homeostasis, microbial defense, metabolism, or anticancer protection. Despite the increasing use of biological therapies targeting eosinophils through their dependency on IL-5, the biological activities, ontogeny and mechanisms of lineage expansion of eosinophils are less resolved than those of other immune cells. We integrated single-cell proteomics and transcriptomics with a novel IL-5Ra reporter mouse model to comprehensively resolve eosinophil development. This approach reconciled human and murine eosinophilopoiesis and facilitated further study of the eosinophil lineage. We observed that the eosinophil lineage expands via a transit amplification mechanism enabled and promoted by IL-5 bioavailability. Eosinophil lineage transit amplification was characterized by increased cycling activity, prolonged proliferative capacity, and delayed

maturation of committed eosinophil progenitors. Conversely, deletion or neutralization of IL-5 attenuated eosinophil progenitor transit amplification without compromising maturation, challenging previous assumptions. Further comparison of residual eosinophils in IL-5-depleted murine or human hosts indicated that IL-5 depletion does not impair eosinophil maturation. Overall, this work provides valuable resources and insights into eosinophil ontogeny, the effects of precision therapeutics, and the regulation of eosinophil development in health and disease.

SOPHIE BEKISZ

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Practical insights into in vitro–in silico integration in lymphatic biology

Lymphangiogenesis, the formation of new lymphatic vessels from pre-existing ones, plays a central role in tissue homeostasis, immune responses, and various pathological conditions such as cancer and chronic inflammation. Its study has relied on in vitro models, including cultured lymphatic endothelial cells (LECs), and in vivo models, which capture the complexity of the microenvironment but remain costly and ethically constrained. More recently, the rise of in silico approaches, based on mathematical and computational modeling, together with advanced in vitro systems such as organoids and organ-on-chip technologies, has opened new avenues for investigating such a multifactorial process.

In this context, this work focuses on the integration of in silico models with 3D and microfluidic in vitro systems to study lymphatic sprouting dynamics across different spatio-temporal scales. The in silico models involved continuous ordinary differential equations, discrete agent-based simulations, and inference-based approaches. On the experimental

side, 3D spheroid assays were used in parallel with an in vitro microfluidic system, in which LECs can invade a collagen gel in response to a molecular gradient, potentially combined with interstitial and/or luminal flow.

Integrating these complementary methodologies provides deeper insights into lymphatic biology, enhances translational relevance, and supports the development of innovative therapeutic strategies while reducing dependence on animal models.

NESRINE FARHAT

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Linking extracellular vesicles derived-miRNAs to Cardiac Remodeling Mechanisms in Pediatric Congenital Heart Defects

Congenital heart disease (CHD) is the most frequent congenital malformation in newborns and remains a major healthcare burden. Chronic hemodynamic overload due to structural defects drives progressive myocardial remodeling, a key determinant of long-term dysfunction, yet its molecular mechanisms remain poorly defined. This study investigates infants and children with CHD presenting with volume overload, with or without right ventricular pressure overload, undergoing corrective cardiac surgery. We focus on circulating extracellular vesicle-derived microRNAs (miRNAs) and neutrophil extracellular traps (NETs), given their roles in regulating hypertrophy, fibrosis, apoptosis, and inflammation. Seventy-five patients under 18 years were enrolled: 25 with CHD and 50 controls. Blood samples were collected from all participants, and myocardial tissue was obtained from CHD patients at surgery. CHD subgroups were defined according to overload type: atrial septal defect (ASD), ventricular septal defect

(VSD) and tetralogy of Fallot (TOF). Circulating markers of myocardial stress, inflammation, and fibrosis were quantified in plasma, and immunohistological detection of NETs was performed in myocardial tissue across the CHD subgroups. Our analyses of miRNA expression profiles highlighted pathways related to processes that may contribute to cardiac remodeling, including cardiovascular development, regulation of fibrosis and apoptosis, cell–cell junctions, and endoplasmic reticulum stress. Analysis of selected cytokines and biomarkers revealed distinct profiles across the CHD subgroups. Significant differences were observed in the levels of ST2, NT-proBNP, IL-33, IL-8, and NuQ-NETs. The most pronounced alterations were found in the VSD and TOF subgroups.

NING AN

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tRNA wobble editing controls FSP1 expression and dictates ferroptosis sensitivity in lung cancer

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Reprogramming of mRNA translation is central to cancer development, mediating cancer cell adaptation and supporting tumor progression. tRNAs are highly modified molecules that are essential to correctly translate mRNAs into proteins. Recently, the importance of tRNA-modifying enzymes in promoting cancer development and therapy resistance through codon-specific translation reprogramming has been uncovered. tRNA-specific adenosine deaminase 2 (ADAT2) is an evolutionarily conserved enzyme that catalyses the conversion of adenosine to inosine at the wobble position of tRNAs (A34). Here, we found that ADAT2 is up-regulated in human lung cancers and is essential for the growth of lung cancer cells and xenograft tumors.

Importantly, genetic deletion of Adat2 in mice lungs strongly impairs tumor development in a KrasG12D/+ model of spontaneous lung cancer. Using a combination of proteomics, polysome profiling and ribosome sequencing, we demonstrate that ADAT2 depletion directly alters the translation of a specific subset of metabolic enzymes that are enriched in C-ending codons. As a result, ADAT2 loss in lung cancer cells leads to impaired glucose and glutamine metabolism, accumulation of reactive oxygen species and rewiring of lipid metabolism. In addition to global metabolic changes, we demonstrate that ADAT2 directly regulates the translation of the ferroptosis suppressor protein 1 (FSP1). Accordingly, ADAT2 knockdown decreases FSP1 expression, triggers ferroptosis activation and sensitizes lung cancer cells to GPX4 inhibition. Finally, we show that an ADAT2 translational signature is enriched in KEAP1-mutated lung tumors and correlates with poor outcome in lung cancer patients. Taken together, our data uncover the importance of tRNA wobble editing in controlling cellular homeostasis in lung cancer and highlight new metabolic vulnerabilities to be exploited for future therapies.



POSTER 1 MIR-183-5P-ENRICHED ENDOTHELIAL EXTRACELLULAR VESICLES: KEY PLAYERS IN PRE-METASTATIC NICHE FORMATION IN BREAST CANCER?

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Breast cancer (BC) remains one of the most prevalent cancers globally. Although primary tumors can often be treated successfully, metastatic BC remains incurable and is the primary cause of mortality. Previous work from our group discovered that endothelial cell-derived extracellular vesicles (EVs), enriched in miR-142-5p, miR-183-5p, and miR-222-3p—collectively termed “miR-TAM”—promote macrophage polarization towards a pro-tumorigenic M2-like phenotype, thereby enhancing tumor growth in murine BC models. However, the role of miR-TAM in metastasis, particularly in pre-metastatic niche (PMN) formation, has not been investigated. To address this gap, we conducted both in vitro and in vivo studies. We treated macrophage and fibroblast cell lines—key contributors to PMN development—with miR-TAM-enriched EVs. This treatment led to a marked upregulation of several pro-tumorigenic genes, including Csf3, Cxcl1, Col3a1, Il-1 β , and Ccl3. In parallel, we administered miR-TAM-enriched EVs peritumourally every

two days to 4T1 tumour-bearing mice. Strikingly, treated mice exhibited a significant reduction in pulmonary CD4 $^+$ and CD8 $^+$ T cell populations, indicating the establishment of an immunosuppressive microenvironment conducive to metastatic colonization.

In conclusion, our findings reveal a previously unrecognized role of miR-TAM-enriched endothelial EVs in promoting PMN formation and metastatic progression in breast cancer. These insights suggest that miR-TAM-enriched EVs may represent novel therapeutic targets to intercept metastasis in BC patients.

POSTER 2 UNMASKING SILENT OVARIAN DAMAGE: AGE AND DOSE MATTER AFTER CHEMOTHERAPY

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In female survivors of childhood cancer, chemotherapy-induced infertility is a major long-term concern. Ovarian tissue cryopreservation and transplantation offer a fertility preservation option, yet indication depends on the degree of ovarian damage. While the gonadotoxicity of high-dose chemotherapy is well established, the long-term impact of lower doses, particularly regarding age at exposure, remains poorly understood. This study aimed to characterize ovarian damage progression and fertility outcomes following high- or low-dose chemotherapy in a mouse model, according to the age at treatment. Peripubertal (4 weeks) and young adult (8 weeks) female C57BL/6 mice received six injections over two weeks of cyclophosphamide and busulfan at low or high doses. Ovarian reserve, estrous cyclicity, ovulatory capacity, and fertility were evaluated at 24 hours, 21 days, and 4 months post-treatment. High-dose chemotherapy caused premature ovarian insufficiency (POI) in both age groups. Ovarian reserve dropped within 24 hours and progressed to follicular depletion, disrupted

cyclicity, reduced ovulation, and impaired fertility by four months. Low-dose chemotherapy induced subtler, age-dependent effects. In young adult mice, ovarian reserve declined at 24 hours, while in peripubertal mice, it decreased significantly only at four months. Cyclicity and fertility were not affected in both groups. However, at four months, ovulatory response to superovulation was reduced, indicating the development of diminished ovarian reserve (DOR), a condition often observed clinically. These findings highlight the importance of long-term monitoring of ovarian function after chemotherapy. Even when fertility appears preserved, delayed onset of DOR may progress toward POI. This model accurately reflects clinical outcomes in childhood cancer survivors and provides a valuable tool to investigate mechanisms of chemotherapy-induced ovarian dysfunction.

POSTER 3 EXOSOMAL PVR: A REGULATOR OF LUNG CARCINOMA PROGRESSION VIA IMMUNE MODULATION

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Immunotherapy has transformed cancer treatment, but resistance remains a challenge. This study explores the role of extracellular vesicles (EVs) in immune escape. We developed a method to identify multiple immune checkpoint proteins (ICPs) on EV surfaces from single-liquid biopsies and focused on the PVR/CD155 axis. First, we examined the role of PVR in several lung cancer models. Then, we investigated the impact of EVs displaying PVR (EV-PVR) on immune escape. Plasma samples from healthy individuals (n=24) and cancer patients (n=25) undergoing immunotherapy were collected. A longitudinal study included responders (n=3) and non-responders (n=3). ICPs on EVs were identified by multiplex ELISA (MAGPIX, Luminex). Functional assays were performed in H1299 and LLC cells using siRNA and CRISPR-Cas9 to knock down PVR. To explore their role in a mouse lung cancer model, EV-PVRWT/KO were injected into tumor-bearing mice. Impact on the microenvironment was assessed by spectral flow cytometry. Murine bone marrow-

derived macrophages (BMDMs) were treated with EVs for 24 h, and M1/M2 markers and PD-L1 were quantified by immunofluorescence. EV-associated PVR was elevated in lung cancer samples, particularly in non-responders, suggesting resistance. ICP analysis in human and mouse cells revealed PVR enrichment in EVs. PVR knockdown reduced migration and proliferation in both cell types. In vivo, EV-PVR-KO slowed tumor growth, increased M1-like macrophages, and decreased PD-L1 expression compared to WT-EV. This phenotype was confirmed in vitro in BMDMs. These results suggest that EV-associated PVR contributes to immune escape by modulating macrophage polarization. Our findings position exosomal PVR as a mediator of immunotherapy resistance in lung cancer and present a minimally invasive strategy for ICP profiling via liquid biopsy.

POSTER 4 DECIPHERING HOW PRENATAL ALCOHOL EXPOSURE IMPAIRS SOMATOSENSORY CORTEX DEVELOPMENT AT SINGLE CELL LEVEL

Manon Charlet | m.charlet@uliege.be | Molecular Regulation of Neurogenesis

Charlet-Briart M, Van Hees L, Stoufflet J, Oskera L, Boutsen A, Bonafina A, Reyskens C, Lavergne A, Close R, Epifanova E, Helgueta S, Didone V, Ndong Penda R, Lakaye B, Tielens S, Struder M, Laguesse S & Nguyen L.

Prenatal alcohol exposure (PAE) damages the fetal brain, causing lifelong cognitive and behavioral issues. As a major public health concern, understanding its pathophysiology is essential. Alcohol disrupts cerebral cortex development by affecting neurogenesis, neuron survival, and neurotransmission, though the underlying cellular and molecular mechanisms remain poorly understood. We aim to discover how PAE exposure impairs somatosensory cortex development by analyzing migrating projection neurons and behavioral impact. Method: We use a mouse model of FASD where pregnant mice voluntarily consume high levels of alcohol, reaching blood concentrations similar to human binge drinking. To study alcohol-induced corticogenesis defects, we analyzed embryonic cortex development, focusing on glutamatergic projection neurons. We observed a moderate microcephaly phenotype in PAE pups and reported a significant delay in upper layer

neuron migration following PAE. By using time-lapse imaging, we demonstrated an alcohol-induced defect in the multipolar-bipolar transition as well as in the locomotion step of neuronal migration. We observed further postnatal defects such as abnormal morphology of upper-layer neurons, reduced callosal projections, and impaired tactile sensitivity. Furthermore, single-cell RNA-seq analysis identified several mRNA differentially expressed in migrating neurons populating the sensory cortex of PAE and water control embryos. We identified a specific target responsible for the alcohol-induced migration defects in addition to abnormal morphology and behavioral impairments. Using our voluntary alcohol model, we identified several effects of PAE on cortical development, including delayed projection neuron migration, abnormal morphology, and impaired tactile sensitivity.

POSTER 5 EOSINOPHILS ARE ESSENTIAL FOR OPTIMAL RESPONSE EFFICACY TO PD1 CHECKPOINT BLOCKING IMMUNOTHERAPY IN A PRECLINICAL MODEL OF TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is an aggressive and difficult-to-treat breast cancer (BC) subtype associated with a poor prognosis. TNBC is routinely treated with immune checkpoint inhibitors (ICIs), particularly nivolumab or pembrolizumab, an antibody targeting PD-1. However, only a subset of patients respond positively to this type of therapy. Studies have noted an increased eosinophil count in positive responders. Nevertheless, preclinical studies investigating eosinophils in the context of ICI-treated TNBC remain scarce. This study focuses on eosinophils in a murine 4T1 model. Eosinophils were depleted pharmacologically (Eos-Dep group), and tumor progression was compared to a control group with normal eosinophil counts. In two independent experiments, we observed a markedly reduced response to anti-PD-1 in the Eos-Dep group compared to the control. White blood cell monitoring revealed a rise in eosinophil count in control mice, particularly after anti-PD-1 administration, consistent with findings in patients who respond positively to nivolumab. We identified CD8+ and CD4+ T cells as players in

the underlying immune mechanism, along with increased granzyme B-mediated cell death and reduced Foxp3+ Treg infiltration in the control group tumors compared to the Eos-Dep group. Together, our results suggest an anti-tumorigenic role for eosinophils in TNBC treatment with anti-PD-1. We are addressing the same question at the level of secondary lung metastasis of breast cancer using E0771/C57BL6 syngeneic model.

POSTER 6 EXPLORING INNOVATIVE METHODS TO UNCOVER IMMUNE CHECKPOINT TARGETS IN AML

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Immune evasion remains a major challenge in acute myeloid leukemia (AML), contributing to relapse rates of up to 68% despite intensive chemotherapy and allogeneic hematopoietic cell transplantation. Immunotherapies targeting immune checkpoint molecules (ICMs) have shown highly variable efficacy in clinical trials, underscoring the need for more targeted approaches. This project aims to identify and characterize ICMs that allow AML cells surviving chemotherapy (persisters) to escape T-cell-mediated clearance, thereby promoting relapse. We hypothesize that persister cells express specific ICMs that shield them from T cell-elimination during remission. Our innovative three-phase approach begins by identifying ICM candidates through co-culturing 16 diverse AML cell lines with pre-activated allogeneic T cells, followed by bulk RNA-sequencing analysis and resistance score correlations to identify relevant ICM targets while avoiding stress-response artifacts. Preliminary findings reveal that AML resistance is associated with impaired T-cell proliferation, downregulation

of activation markers (CD25, CD69, LAG3, PD-1), and contact-dependent killing. In phase two, we will refine the ICM candidate list using longitudinal single-cell RNA-sequencing published data from AML patients' bone marrow at diagnosis and post-chemotherapy (days 14 and 30), along with survival analysis in 677 bulk RNA-sequencing patient datasets. Finally, the most promising membrane ICM candidates will be functionally validated via CRISPR knockout in AML cell lines, followed by co-culture assays. The therapeutic potential of the inhibition or silencing of our identified ICMs will be evaluated in humanized AML mouse models. This integrative approach bridges immunology, oncology, and genomics to develop novel immunotherapeutic strategies targeting the immune evasion mechanisms of AML persister cells.

POSTER 7 TRNA EDITING BY THE ADENOSINE DEAMINASE ADAT2 REGULATES COLON HOMEOSTASIS AND CONTROLS COLON CANCER PROGRESSION

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The regulation of mRNA translation has emerged as a central mechanism driving the adaptation of cancer cells during progression and response to therapy. Transfer RNAs (tRNAs) are heavily modified molecules that actively regulate mRNA translation. In particular tRNA modifications at the wobble position (base 34) have an impact on translation kinetics and efficiency. Our laboratory has shown that wobble tRNA modifications promote cancer initiation, metastasis and treatment resistance. One key modification is the conversion of adenosine (A34) to inosine (I34) by the tRNA-specific adenosine deaminases ADAT2 and ADAT3, which allows tRNAs to pair with multiple codons, hence enhancing the decoding capacity of a cell. Despite their conservation through evolution, the A34 editing of ADAT2/3 in the context of cancer remains largely unexplored. In this project, we found that ADAT2 is up-regulated in human colon cancer biopsies and supports cell-autonomous proliferation of human colon cancer cells and murine colon cancer stem cells. Surprisingly, we observed that genetic deletion of

Adat2 significantly shortens mice survival in an Apc-driven mouse model of intestinal cancer. Shorter survival of VillinCre; Apc^{+/min}; Adat2^{-/-} mice is associated with increased tumorigenesis in the colon (but not in the small intestine) and enhanced inflammation in the gut. We further confirmed that Adat2 loss in intestinal epithelial cells is sufficient to trigger inflammation in the colon of wild-type mice, as evidenced by frequent presence of ulcerative areas, distortion of crypt architecture and increased lymphocytosis. Finally, using a combination of unbiased approaches, we demonstrate that Adat2-depleted epithelial cells display increased lipid catabolism and activation of pro-inflammatory signalling pathways. We are currently investigating the molecular mechanisms that regulate Adat2-dependent inflammatory response. Taken together, our data uncover the importance of tRNA wobble editing in controlling cellular homeostasis and will potentially lead to the development of therapeutic strategies targeting chronically-inflamed intestinal disease.

POSTER 8 STRUCTURE-ACTIVITY RELATIONSHIPS OF SIGNALING PROTEINS OF THE NON-CANONICAL NFKB PATHWAY IN IMMUNE RESPONSES

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The activation of the non-canonical NFκB pathway relies on two kinases, NIK and IKKα. Although the kinase activity of both proteins is required for the proteolytic cleavage of p100 in p52, how the molecular complex forms has remained elusive. We have used AlphaFold 3 to dissect the putative molecular interactions at the interface of NIK and IKKα. We identified at the C-terminal region of NIK a folded domain that contains two anti-parallel beta-sheet (1&2) followed by an alpha helix and two anti-parallel beta-sheet (3&4) followed by two very small putative anti-parallel beta-sheet (5&6). In particular, we scrutinized the beta-2 and the alpha helix and found specific amino acids that form ionic bond or hydrophobic interaction with particular amino acids within the helix-loop-helix domain (HLH) of IKKα. We have validated our predictive model of NIK/IKKα interaction by loss of function mutation in NIK and in IKKα using cellular functional assays. In addition, we analyzed a few VUS (Variant of Uncertain Significance) for NIK reported in the ClinVar database

and we could highlight new loss of function mutations of NIK for these patients.

POSTER 9 FUNCTIONAL STUDY OF RETINOIC ACID-ACTIVATED REGULATORY SEQUENCES AND THEIR ROLE IN THE EXPRESSION OF HOXBB GENES IN ZEBRAFISH

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Hox genes are essential for vertebrate embryonic development, particularly for body segmentation and anterior-posterior axis patterning. These genes are notably regulated by retinoic acid, a vitamin A derivative with crucial roles in health and disease. Studies in chick, mice, and zebrafish have shown that RA deficiency leads to malformations affecting several organs, such as the central nervous system, heart, forelimbs (pectoral fins), and pancreas. RA regulates gene expression through that the binding of receptors which recognize specific regulatory sequences (RAREs) near their target genes. Several RAREs located within murine Hox clusters have been shown to regulate Hox gene expression. RAR ChIP-seq experiments performed by our research groups have revealed the presence of several RAREs far downstream of the *hoxbb* locus, located in introns of the neighboring *skap1* gene. Analysis of mouse RAR ChIP-seq data⁸ indicated that similar RAREs are present in the same locations, some showing strong sequence conservation among vertebrates. To investigate their functional role, we first inserted these RAREs upstream the minimal *cfos*

promotor fused to GFP and tested their activity in vivo. GFP reporter expression was very similar to the *hoxbb* expression profile for three RAREs. Secondly, we removed the RAREs from zebrafish genome by generating various deletion. While a small deletion removing one RARE region had no significant effect on the *hoxbb* gene expression, larger deletions removing several RAREs resulted in a significant decrease of all *hoxbb* genes. Interestingly, the spatial expression profile was modified for some *hoxbb* genes, showing a posterior shift due to the RAREs deletion. These findings highlight the crucial role of distant RAREs in *hoxbb* gene regulation. Our data support the model in which Hox cluster activation is driven by distant regulatory regions located far on the 3' side of the clusters, potentially contributing to progressive chromatin opening.

POSTER 10 DETERMINING THE ROLE OF THE MITOCHONDRIAL PROTEIN OPA1 IN EXTRACELLULAR VESICLE BIOGENESIS

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FLASH TALK

Drouet T, Herkenne S

Mitochondria, traditionally recognized for their role in energy production, are now appreciated as key regulators of diverse cellular processes, including inter-organelle communication. In this study, we uncover a novel function for mitochondria in the regulation of extracellular vesicles (EVs) secretion through their interaction with the endosomal system. We demonstrate that mitochondria form physical contacts with endosomes and contribute to the maturation and fate of multivesicular bodies (MVBs), the EV precursors. Remarkably, loss of the inner mitochondrial membrane protein OPA1 leads to cytosolic accumulation of CD63⁺ MVBs and a significant reduction in EVs release, suggesting impaired MVB trafficking or fusion with the plasma membrane. These findings highlight a previously unrecognized role for OPA1 in maintaining proper endosomal dynamics required for EVs biogenesis. To our knowledge, this is the first evidence of a direct functional connection between mitochondria and the EV secretion pathway. Our ongoing work aims to decipher the molecular mechanisms underlying this novel mitochondria-endosome crosstalk and identify the key factors

involved, offering new insights into the regulation of intercellular communication by mitochondria.

POSTER 11 CHARACTERIZATION OF A NOVEL ROLE FOR FET FUSION-DERIVED ONCOGENIC TRANSCRIPTION FACTORS IN THE REGULATION OF MRNA STABILITY

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Gene fusions resulting from chromosomal translocations are important genomic alterations in cancer. Some genes are more often involved in this type of genetic abnormality, such as the FET (FUS, EWSR1, TAF15) gene family, which encode RNA-binding proteins (RBP). Gene fusions involving FET genes systematically fuse them to various transcription factor (TF) genes, promoting various sarcomas and leukemias. The resulting FET fusion proteins invariably includes the aminoterminal low-complexity disordered domain (NTD) of the FET partner fused to a carboxyterminal domain (CTD) derived from the TF partner including its DNA-binding domain. To date, the FET-derived oncogenic fusions are reported to act primarily as aberrant transcription factors driving cancer progression by actively rewiring gene expression programs. However, our lab uncovered a post-transcriptional role for EWSR1::FLI1, the main driver of Ewing sarcoma, in the regulation of mRNA stability. For this function, EWSR1::FLI1 is recruited to specific transcripts through interactions of its CTD with

RBPs. Then, EWSR1::FLI1 interferes with mRNA stability by recruiting cytoplasmic mRNA deadenylation machinery through its NTD.

This project aims to demonstrate that all FET fusions can promote mRNA decay, extending the model previously established for EWSR1::FLI1. Using RNA-seq and reporter assays, we demonstrated that other FET fusions also reprogram gene expression by modulating mRNA stability. We are currently exploring the common molecular mechanisms underlying this post-transcriptional gene regulation, focusing on protein interactions and the role of the 3'UTR of mRNA in FET fusion-mediated decay.

All in all, our current model proposes an additional role for oncogenic FET fusions in the regulation of mRNA stability through RBP interactions. Consequently, deciphering this novel post-transcriptional function could lead to the discovery of a new therapeutic vulnerability for cancers caused by these oncogenic fusions.

POSTER 12 THE MTORC2-AKT-GSK3-FBXW7 AXIS REGULATES ELP1 STABILITY AND U34 TRNA MODIFICATION IN LUNG CANCER

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*equally contributed

Lung cancer remains a leading cause of cancer-related mortality, with therapeutic resistance driven by tumor heterogeneity and adaptive survival mechanisms. tRNA modifications at the U34 position are emerging as key regulators of mRNA translation, influencing tumor progression and drug resistance. Our previous work identified mTORC2-PI3K signaling as a major modulator of U34 tRNA modifications, specifically regulating ELP1, the structural subunit of the Elongator complex, in melanoma. While therapy-resistant non-small cell lung cancer (NSCLC) exhibits mTORC2 overexpression, its impact on U34 tRNA modifications remains unexplored.

Here, we investigated U34-enzyme regulation in lung cancer cell lines (A549, H460, H23) and demonstrate that ELP1 stability is controlled by the mTORC2-AKT-GSK3-FBXW7 axis. Using immunoprecipitation assays, we show direct binding and ubiquitination of ELP1 by FBXW7, confirming its role in ELP1 degradation. Notably, this ubiquitination was abolished upon

GSK3 inhibition, further validating the role of the mTORC2-AKT-GSK3-FBXW7 axis in ELP1 turnover.

Proteomic analysis revealed that FBXW7 loss enhances translation of U34-sensitive codon-enriched genes, establishing a mechanistic link between mTORC2-FBXW7 signaling and codon-biased translation in lung cancer. Importantly, the FBXW7-ELP1 signature correlated with poor patient survival, highlighting its potential as a prognostic biomarker.

Gene Set Enrichment Analysis (GSEA) of the FBXW7-ELP1 proteome identified migration as a key pathway regulated by this axis. Functionally, ELP1 depletion blocked the increased migration of lung cancer cells induced by FBXW7 loss, demonstrating its critical role in FBXW7-mediated tumor cell motility.

Our results identify mTORC2-AKT-GSK3-FBXW7 as a key regulatory axis of ELP1 stability, linking U34 tRNA modifications to oncogenic translation and tumor cell migration in lung cancer.

POSTER 13 INHIBITION OF NNOS NEURONS IN THE VENTROMEDIAL HYPOTHALAMUS REDUCES MALE MOUSE AGGRESSION

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In rodents, nitric oxide synthase-expressing neurons (nNOS) in the ventromedial hypothalamus (VMH) have recently been linked to the regulation of female sexual behaviour, but their function in males remains largely unexplored. Given the VMH's central role in male aggression and its emerging role in modulating female sexual behaviour, we investigated the contribution of nNOS neurons to male social behaviours, focusing on aggression and mating behaviours. To assess the function of these neurons, we employed a chemogenetic approach in male nNOS::Cre mice, delivering a Cre-dependent AAV vector encoding the inhibitory DREADD hM4Di bilaterally into the VMH. This allowed for temporally and spatially precise, reversible inhibition of nNOS neurons via intraperitoneal administration of deschloroclozapine (DCZ, 0.1 mg/kg). To control for potential non-specific effects of DCZ, control animals received a Cre-dependent AAV encoding mCherry only. Animals were tested using standard paradigms for territorial aggression (resident-intruder test), sexual behaviour, and the three-chamber social approach task. Inhibition of VMH nNOS neurons

significantly altered territorial aggression: latency to the first attack increased, threat behaviours were reduced, and there was a trend towards fewer biting attacks compared to mCherry controls, suggesting decreased aggressive behaviour. While we observed a significant increase in the inter-intromission interval in the hM4Di group, other mating parameters remained unchanged suggesting that VMH nNOS neurons do not alter sexual behaviour in a physiologically meaningful way. Importantly, sociability tests revealed no significant interaction between treatment and AAV type, indicating that the observed effects on aggression and mating are unlikely to result from general social impairments. In conclusion, our results highlight a critical role for VMH nNOS neurons in driving male territorial aggression and suggest a more subtle role in sexual behaviour.

POSTER 14 WOBBLE TRNA MODIFICATION PROMOTES METABOLIC REPROGRAMMING OF TUMOR-ASSOCIATED NEUTROPHILS IN METASTATIC BREAST CANCER

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* The authors contributed equally to the study

During cancer progression, neutrophils undergo transcriptional reprogramming and acquire a Tumor-Associated Neutrophil (TAN) phenotype. TANs exhibit functional plasticity, exerting either pro-tumoral activities by promoting an immunosuppressive microenvironment and modulating angiogenesis, or anti-tumoral effects by inhibiting tumor cell proliferation and orchestrating effective immune responses. In metastatic breast cancer, early neutrophilia is linked to poor clinical outcome and contribute to metastasis by facilitating the formation of a pre-metastatic niche. Here, we show that neutrophils undergo metabolic reprogramming when infiltrating the primary breast tumor. Normally glycolysis-dependent, tumor-infiltrating neutrophils shift to mitochondrial metabolism. This shift is paired with increased expression of wobble uridine tRNA-modifying

(U34-TM) enzymes (ELP1-6, ALKBH8, CTU1/2) in neutrophils from breast tumor-bearing mice. We hypothesized that U34-TM, through regulation of codon-specific mRNA translation, supports metabolic reprogramming via translational control during cancer progression. To test this, we created a neutrophil-specific U34-TM loss-of-function model by crossing ELP3lox/lox mice with the Mrp8-Cre strain. ELP3 deletion impairs mitochondrial morphology in neutrophils and prevents the expression of mitochondrial proteins in tumor-infiltrated neutrophils. Strikingly, ELP3 deletion in neutrophils leads to a strong delay in primary tumor growth and prevents lung metastasis. Specifically, ELP3 KO neutrophils slow the transition from mammary intraepithelial neoplasia to adenocarcinoma. This correlates with reduced endothelial cells infiltration, which is presumably indicating a defect in angiogenesis. These findings reveal a key role for wobble tRNA modification and codon-specific mRNA translation in regulating neutrophil function in breast cancer development. Our ongoing work focuses on understanding how U34-TM modulates TANs function and impacts on the tumor micro-environment in breast cancer.

POSTER 15 IMPACT OF ESTROGENIC AND MENOPAUSE TREATMENTS ON THE MICROENVIRONMENT OF ER-NEGATIVE CANCERS

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Women can be exposed to estrogenic treatments in different ways, including menopausal hormone therapy (MHT). Even if these treatments are important to improve the quality of life of women impacted by menopause, MHT has been linked to an increased risk of venous thromboembolism and breast cancer. Moreover, estrogen impacts a wide range of cell types and tissues, since estrogen receptors (ER) are expressed in many cell types. Our team already demonstrated that estradiol (E2) promotes the growth of ER-negative tumors, such as melanoma or lung cancer, by modulating the tumor microenvironment, primarily by increasing angiogenesis and lymphangiogenesis. Given the growing interest in immunotherapy, we extended our research to characterize the impact of different MHT on the immune component of ER-negative tumors. In this study, we compared the impact of 2 different MHTs to E2, used as a reference treatment. In ER-negative tumors, E2 enhances angiogenesis and vascular maturation, thereby reducing hypoxia and necrosis. In addition,

E2 displays immunosuppressive properties that contribute to tumor growth in vivo. However, the MHT we tested differentially modulated these parameters, showing the persistence of immune cells in the tumor microenvironment of ER-negative tumors. In conclusion, E2 treatment promotes tumor growth in ER-negative cancers by increasing the tumor angiogenesis and inducing an immunosuppressive microenvironment. Depending on the nature of MHT, the impact on the tumor environment is variable, suggesting that the use of such treatments should be carefully considered in the management of women with ER-negative cancers.

POSTER 16 PRE-CLINICAL MANIFESTATIONS OF PARKINSON'S DISEASE: LOCUS COERULEUS, SLEEP, AND COGNITION

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Parkinson's disease (PD) is the second most prevalent type of neurodegeneration, characterized by both motor and non-motor symptoms. Recent evidence highlights the relevance of the Locus Coeruleus (LC), located in the brainstem, as a key brain structure for this disease, exhibiting signs of neurodegeneration years prior to clinical manifestation of the symptoms. This brain site regulates sleep-wake cycles, and its structural and functional abnormalities most likely contribute to PD-related sleep disturbances. Additionally, the LC has proven critical for cognitive decline, which is another non-motor symptom of the disease. By further investigating the role of the LC in PD and its contribution to sleep disturbances and cognition, the main aims of my doctoral project are to determine how sleep and cognitive functioning relate with LC structure and function in healthy participants with known genetic risk for the disease and to explore if these variables exhibit different associations depending on the age-range considered. For this purpose, data will be collected on one-hundred healthy adults aged 20 to 70y using Ultra-High-Field (UHF) 7 Tesla

Magnetic Resonance Imaging (MRI). The whole project comprises three complementary work packages. Here, we will present the first preliminary results that will relate LC structure to REM sleep quality and cognition in at least 75 healthy individuals aged 18 to 75y. These first results will determine some of the core link between sleep, cognition and the LC, and will pave the way for future investigations related to PD risk and PD prodromal stages. the Mrp8-Cre strain. ELP3 deletion impairs mitochondrial morphology in neutrophils and prevents the expression of mitochondrial proteins in tumor-infiltrated neutrophils. Strikingly, ELP3 deletion in neutrophils leads to a strong delay in primary tumor growth and prevents lung metastasis. Specifically, ELP3 KO neutrophils slow the transition from mammary intraepithelial neoplasia to adenocarcinoma. This correlates with reduced endothelial cells infiltration, which is presumably indicating a defect in angiogenesis. These findings reveal a key role for wobble tRNA modification and codon-specific mRNA translation in regulating neutrophil function in breast cancer development. Our ongoing work focuses on understanding how U34-TM modulates TANs function and impacts on the tumor micro-environment in breast cancer.

POSTER 17 DECIPHERING THE MOLECULAR PROPERTIES OF THE PUTATIVE RNA-BINDING PROTEIN TFIP11 IN REGULATING ALTERNATIVE SPLICING

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Genco A, Obeid A.M, Galvan B, Uriarte M, Flasse L, Pendeville H, Mottet D can govern splicing decisions that ultimately impact the alternative splicing of a set of essential genes.

RNA-binding proteins (RBPs) are a class of proteins that regulate the metabolism of RNAs throughout their lifecycle. Many RBPs can be cell-, tissue- or condition specific and are capable of regulating various molecular processes such as alternative splicing of messenger RNAs which is essential for the proper expression of multiple proteins. These proteins possess domains essential for interaction with the target RNA called RNA binding domains. Typically a RBP has multiple RBDs. Here, we are studying the TFIP11 protein, a spliceosomal protein that possesses two putative RBDs; a G-patch domain at its N-terminal region and a dsRBD at its C-terminal region. The laboratory has already demonstrated the importance of TFIP11 in regulating spliceosome assembly and activation but little is known about the functionality of its RNA-binding modules, nor about the molecular mechanisms governing its role in regulating alternative splicing. Our current study focuses on the importance of TFIP11 domains for the formation of splicing complexes, as well as their importance for protein-RNA interaction, as these protein/protein and protein/RNA interactions

POSTER 18 CONTRIBUTION OF MULTIMODAL QUANTITATIVE BIOMARKERS IN THE PRECLINICAL DETECTION OF EARLY NEURODEGENERATION OF FRONTOTEMPORAL DEMENTIA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS.

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50% of patients with amyotrophic lateral sclerosis (ALS) present with cognitive and behavioral impairments (ci, bi, cbi) and 15% meet the criteria for frontotemporal dementia (FTD) [= ALS+]. This study aims to establish early multimodal biomarkers from brain microstructural alterations in ALS+. ALS subgroups (ALS-pure motor, -ci, -bi, -cbi, -FTD, N=20 each) and 100 healthy controls matched for age, sex and education will be examined according to a 3T quantitative MRI MultiParametric Mapping (MPM) protocol (R1, R2*, MTsat, PD, QSM) allowing advanced characterization of microstructural tissue properties and their changes with pathology. Diffusion imaging (NODDI) will enable the quantitative estimation of axonal diameter and neurite densities. Each modality will be analyzed using GLM (SPM), with age, sex, and disease duration as covariates and participants as random effect. Group differences (ALS+ vs ALS-pure motor and controls) will be assessed using a two-tailed F test linear contrast. For modalities showing a significant effect, one-tailed t tests will be performed to identify specific group differences. Statistical inferences will be conducted at

p<0.05, after correction of multiple comparison. A multivariate analysis of variance (MANOVA) model will be specified using the design matrices of the six univariate models in the MSPM toolbox, a newly developed toolbox working under SPM as a multivariate extension of univariate GLM. 18F-FDG cerebral PET will confirm dysfunction in altered regions. Histopathological and proteomic analyses on brain tissue, blood and sweat will explore underlying mechanisms and be integrated with MRI findings for a multimodal assessment of neurodegeneration. We hypothesize that neuronal loss and inflammation in the frontal cortex of ALS+ will be reflected by altered R1, R2, QSM, and MT values compared to ALS-pure motor and controls, associated with concordant hypometabolism and supported by biological evidence of degenerative mechanisms.

POSTER 19 A PROTOCOL FOR THE CONSTRUCTION OF A MULTIMODAL BRAIN DATASET OF HEALTHY CONTROL SUBJECT

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Gillet A, Palmeri D, Bernard C, van der Lande GJM, Aubinet C, Cardone P, Núñez P, Alnagger NLN, Annen J, Bicego A, Cecconi B, Fritz P, Gosseries O, Grégoire C, Lejeune N, Louras M, Maquet N, Marie N, Martens G, Martial C, Meys M, Regnier A, Seyfzadeh F, Szymkowicz E, Tshibanda L, Vanhauzenhuysse A, Vitello M, Thibaut A, Withofs N, Hustinx R, Sala A.

While behavioral assessment remains the gold standard for the diagnosis of Disorders of Consciousness (DoC), the use of advanced neuroimaging such as positron emission tomography (PET), magnetic resonance imaging (MRI) and electrophysiology techniques is recommended whenever possible, as they can reveal residual consciousness in behaviorally unresponsive patients, with strong implications for patients' prognosis and treatment. While control databases serve as a critical reference in the diagnostic work-up, the field of DoC still faces a significant gap in this respect, due to the lack of multimodal normative datasets in healthy individuals. We aim to develop a high-quality reference database through the acquisition of a multimodal database from a representative cohort of 62 healthy adults, aged 18 to 79. Here, we present an experimental protocol that includes

a 30-minute MRI scan with structural and functional sequences, a 12-minute eyes-closed EEG, and a 60-minute dynamic fluorodeoxyglucose ($[^{18}\text{F}]\text{FDG}$) PET acquisition. After a preliminary screening, participants undergo final eligibility tests and additional behavioral assessments on the day of the acquisitions. To reduce the risk of drop-out, the acquisitions take place in a single day. Since the 17th of July 2023, 52 participants were recruited for inclusion. However, two were excluded due to high scores on the Beck Depression Inventory or the Beck Anxiety Inventory, two others were excluded due to abnormal results in neuroimaging exams, and three more due to excessive movement during the PET scan. This resulted in a final dataset of 45 participants. The database is 80.65% complete, and recruitment is expected to end in the summer of 2025. The progress made so far demonstrates the feasibility of such a protocol. This reference dataset will provide high-quality multimodal data for the instrumental assessment of patients with a DoC and other neurological conditions, enabling applications in diagnosis and prognosis.

POSTER 20 ROLE OF EOSINOPHILS IN CLEAR-CELL RENAL CELL CARCINOMA

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Eosinophils (EOS), mainly known for their role in allergic and parasitic responses, have recently emerged as potential players in cancer immunity. This work investigated their impact in localized clear-cell renal cell carcinoma (ccRCC), focusing on both circulating (B-EOS) and tissue-infiltrating eosinophils (T-EOS). We retrospectively analysed clinical data from 169 patients with localized ccRCC. T-EOS infiltration was quantified by Major Basic Protein staining in tumour samples from 92 patients, segmented into tumour centre, invasive margin, and peritumoral region. Lower baseline relative (REC) and absolute B-EOS counts were significantly associated with increased risk of distant relapse ($p = 0.015$ and 0.022 , respectively) and shorter distant progression-free survival (dPFS; $p = 0.006$ and 0.017), but not with cancer-specific survival ($p = 0.97$ and 0.71). A novel index combining lymphocyte and eosinophil counts relative to neutrophils—the eosinophil-lymphocyte/neutrophil ratio—outperformed the neutrophil-to-lymphocyte ratio in predicting

dPFS ($p = 0.0019$ vs. 0.015). T-EOS were predominantly located at the tumour periphery, especially within the invasive margin ($p = 0.013$), and to a lesser extent in the peritumoral region ($p = 0.058$), compared to the tumour centre. T-EOS levels modestly correlated with baseline REC ($\rho = 0.31$, 0.27 , and 0.23 in the margin, centre, and peritumoral tissue, respectively; $p < 0.05$). Higher T-EOS infiltration in the invasive margin was associated with a trend toward longer dPFS (HR = 0.75 , 95% CI: 0.54 – 1.03 ; $p = 0.073$). These findings suggest that higher blood and tissue eosinophil levels may help restrain tumour progression in ccRCC. Further studies should explore the mechanisms of eosinophil-mediated tumour control and their potential as prognostic biomarkers.

POSTER 21 LIFTING THE VEIL ON CHALLENGING MEDICALLY RELEVANT GENES

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FLASH TALK

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While the cost of DNA sequencing has never been cheaper, a number of genetic diseases remain difficult to diagnose. Nearly 400 medically relevant genes are still challenging to characterize due to the complex nature of their sequence. This complexity can arise from a variety of factors, such as the existence of pseudogene, large Short Tandem Repeat region or Variable Number Tandem Repeat region. As such, the access to reliable and cost-effective genetic tests is limited. To resolve this issue, we decided to focus on improving the characterization of the following genes by using long-read sequencing: PKD1/PKD2, responsible for Autosomal Dominant Polycystic Kidney Disease (ADPKD), and FLG, involved in Atopic Dermatitis. For PKD genes, we amplified their sequence by long-range PCR before sequencing the products by Oxford Nanopore Sequencing. We were able to retrieve all variants previously confirmed by Sanger sequencing on 34 samples with ADPKD. For FLG, while investigating the 23 publicly available PacBio HiFi data of the 1000 Genome project, we identified new undescribed alleles in African samples. To determine

if these variations are population specific, we analyzed 1111 additional public samples with long-read data. We discovered 6 novel alleles mostly from Sub-Saharan populations. Our next goal is to design cost efficient techniques to improve the sequencing of these challenging medically relevant genes in a clinical setting.

POSTER 22 THE S20G SUBSTITUTION IN HUMAN ISLET AMYLOID POLYPEPTIDE (HIAPP) EXACERBATES NLRP3 INFLAMMASOME ACTIVATION BY HIAPP IN MACROPHAGES

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FLASH TALK

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Amyloid deposition and inflammation are key features of islet pathology in type 2 diabetes and contribute to beta-cell loss. Aggregation of human islet amyloid polypeptide (hiAPP), the unique peptide constituent of amyloid deposits in islets, activates the NLRP3 inflammasome and elicits interleukin-1 beta (IL-1 β) production in islet macrophages. Since the serine-to-glycine substitution at position 20 (S20G) in hiAPP leads to faster aggregation and renders hiAPP more toxic, we sought to determine whether S20G also exacerbates the hiAPP pro-inflammatory effect and increases IL-1 β production. PMA-differentiated THP-1 cells were treated for 6 and 24 hours with increasing concentrations (0-20 μ M) of synthetic hiAPP or synthetic S20G-hiAPP mutant. At the end of each treatment period, NLRP3 and IL-1 β mRNA levels were quantified by qRT-PCR. NLRP3 inflammasome activation was determined by measuring caspase-1 activity (via bioluminescent Caspase-Glo 1 inflammasome assay) and secretion of active IL-1 β (via ELISA) in

the media. Exposure of macrophages to both hiAPP and S20G-hiAPP significantly upregulated NLRP3, IL-1 β gene expression in a time- and dose-dependent manner. Caspase-1 activity and IL-1 β protein levels also increased significantly in a time- and dose-dependent manner with exposure to hiAPP and S20G-hiAPP. Further, upregulation of inflammatory genes was significantly higher in macrophages treated with S20G-hiAPP vs. hiAPP (at 20 μ M for 6 hours at 10 μ M for 24 hours). Similarly, caspase-1 activity and IL-1 β protein levels were higher after exposure to S20G-hiAPP when compared to hiAPP. Key results were confirmed in primary human Monocytes-Derived Macrophages. In conclusion, the S20G-hiAPP mutant enhances the pro-inflammatory potential of hiAPP and exacerbates NLRP3 inflammasome activation by hiAPP in macrophages. Thus, this pro-inflammatory effect could contribute to the earlier development of type 2 diabetes in individuals carrying this gene mutation.

POSTER 23 METHYLGLYOXAL: A METABOLIC WEAKNESS IN THERAPY-RESISTANT COLORECTAL CANCER STEM CELLS

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FLASH TALK

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Colorectal cancer is the second leading cause of cancer-related death worldwide. High recurrence rates and therapeutic resistance remain major clinical challenges, largely driven by cancer stem cells (CSCs)—a subpopulation capable of self-renewal, therapy resistance, and metastatic spread. CSCs in colorectal cancer are marked by high aldehyde dehydrogenase (ALDH) activity, metabolic plasticity, and enhanced glycolytic flux. A key glycolysis by-product is methylglyoxal (MG), a reactive and cytotoxic dicarbonyl metabolite. MG causes glycation stress, affecting proteins, lipids, and nucleic acids, and is primarily detoxified by the glyoxalase system (GLO1/GLO2). Our work explores MG as a driver of CSC survival and chemoresistance, via its impact on Wnt signaling—a pathway essential for CSC maintenance and linked to resistance to 5-fluorouracil (5-FU). Using ALDH-based sorting and a STAR reporter system labeling intestinal CSCs, we isolated and characterized CSC-enriched populations from colorectal cancer cell lines (HT-29, SW480, SW260). These CSCs show

elevated glycolysis, MG accumulation, and strong Wnt pathway activation. Transcriptomic analysis after stable GLO1 knockdown reveals upregulation of Wnt effectors (CTNNB1, LEF1, TCF4), suggesting MG stress enhances Wnt-dependent transcription and contributes to 5-FU resistance. We are currently assessing MG scavengers such as carnosine and aminoguanidine as potential therapeutic adjuvants. Preliminary results show that combining them with 5-FU significantly reduces CSC viability and restores drug sensitivity. These findings are being validated in vivo using CRC xenografts and patient-derived organoid models. Altogether, our study identifies MG as a potential metabolic vulnerability in CRC-CSCs. Targeting MG stress could offer an effective strategy to overcome therapy resistance and improve outcomes in colorectal cancer.

POSTER 24 INVESTIGATING THE CELLULAR AND MOLECULAR EFFECTS OF THE SUBVENTRICULAR ZONE ON HOSTED GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is the most common and lethal primary brain tumor in adults. The subventricular zone (SVZ) is a unique brain region located along the wall of the lateral ventricles, hosting the largest population of neural stem cells. GBMs in contact with the SVZ are associated with a poorer prognosis and a more invasive phenotype. In xenograft models, GBM cells migrate towards the SVZ, where they are more protected against ionizing radiation. In the SVZ, GBM cells are also in contact with the cerebrospinal fluid (CSF), which was shown to enhance treatment resistance. In this context, we aim to investigate the interactions between the SVZ environment including the CSF, and the GBM cells nested in this zone. Bulk RNA sequencing was performed on four patient-derived GBM cultures plated in CSF or in control medium in vitro for 24 hours. Patient-derived GBM cells were also orthotopically injected into immunodeficient mice. Once the tumors were implanted, the transcriptome of GBM cells from the tumor mass (TM) vs. the SVZ was analyzed by single-cell RNA sequencing. Several differentially

expressed genes in the CSF compared to control medium in vitro and in the SVZ compared to the TM in vivo were highlighted. Downstream gene set enrichment analysis and Ingenuity Pathway Analysis showed an activation of some pathways in both experiments such as the TNF α signaling via NF κ B, p53 pathway, hypoxia and an increase in invasive functions. Boyden chamber and 3D invasion assays confirmed an increased invasion of GBM cells in the CSF compared to control medium, also associated with an increased adhesion. GBM cells are therefore influenced both by the CSF in vitro and by the SVZ environment in vivo. Several pathways are notably activated, suggesting that they may play an important role in SVZ-associated tumor persistence. Invasive capacities are also activated in the CSF. The results will be validated on patients' samples and more deeply investigated.

POSTER 25 TRANSCUTANEOUS AURICULAR VAGUS NERVE STIMULATION IN PATIENTS WITH PERSISTENT POST-CONCUSSION SYMPTOMS: A PROTOCOL FOR A RANDOMIZED DOUBLE-BLIND SHAM-CONTROLLED TRIAL

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FLASH TALK

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Persistent post-concussive symptoms (PPCS) can affect 20-50% of patients following a concussion and can persist for months or even years when not appropriately managed. These symptoms encompass a wide range of somatic (e.g., headaches, dizziness), cognitive (e.g., memory or attention deficits), emotional (e.g., anxiety, depression), and sleep-related disturbances. Despite the significant impact on patients' quality of life, effective treatments remain limited. Transcutaneous auricular vagus nerve stimulation (taVNS), a non-invasive technique targeting the auricular branch of the vagus nerve, has demonstrated potential in modulating brain function and alleviating symptoms comparable to those experienced by patients with PPCS. This randomized double-blind sham-controlled clinical trial investigates the (neuro-) physiological and clinical effects of taVNS in a cohort of 48 adults (based on an a priori sample size) aged 18 to 65 who sustained a concussion and experience PPCS between 1 and 12 months prior to enrollment. Participants will be

randomized to receive either active (1-3 mA, depending on patients' pain threshold) or sham taVNS (0.1 mA). The intervention comprises 15 sessions over two weeks, including two in-lab sessions (S1, S15) and 13 supervised home-based sessions (S2-S14). All outcome measures will be assessed at S1 and S15. Our primary outcome measure is PPCS severity. Secondary outcomes will include self-reported symptoms, quality of life and cognitive performance, respectively assessed through validated questionnaires and neuropsychological tests. Finally, electroencephalogram and electrocardiogram recordings will also allow to evaluate (neuro-) physiological changes as potential correlates of symptom improvement. At one-month follow-up, the same questionnaires will be collected to monitor symptom progression. This protocol aims to generate robust evidence on taVNS as a therapeutic option for PPCS and to elucidate the underlying neurophysiological mechanisms.

POSTER 26 HYPOTHALAMIC KISSPEPTIN NEURONS MODULATE SEXUAL BEHAVIOURS

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Kisspeptin, a major neuropeptide regulating reproductive hormone secretion is predominantly found in two hypothalamic populations: the anteroventral periventricular (AVPV) nucleus and the arcuate (ARC) nucleus. Previously, we have shown that AVPV kisspeptin neurons play a key role in regulating female sexual behaviour. Recent work has highlighted the potential role of ARC kisspeptin neurons in facilitating the LH surge however, whether these neurons also modulate sexual behaviours remains unknown. To further determine the role of hypothalamic kisspeptin neurons in regulating sexual behaviours we employed Designer Receptor Exclusively Activated by Designer Drugs (DREADD) technology to selectively silence kisspeptin neurons in the AVPV or ARC nucleus and measured the effect on sexual behaviour. We injected cre-dependent adeno-associated viral constructs into the AVPV and ARC nucleus of kiss-cre male and female mice to induce the expression of either: the inhibitory DREADD hm4DG_i or as a control the fluorescent protein mCherry only, into the AVPV or ARC of Kiss-Cre male and female mice. Male mice were left intact

and females were ovariectomised, E2-replaced and hormonally primed to be in behavioural estrus. Thirty minutes before behavioural testing mice received subcutaneous injections of either saline or the chemical actuator deschloroclozapine (DCZ, 0.1 mg/kg) to activate inhibitory DREADDs and consequently silence kisspeptin neurons. We found that inhibition of AVPV kisspeptin neurons disrupted mate partner preference in both sexes but only sexual behaviour in females. Conversely, inhibition of ARC kisspeptin neurons did not affect mate preference or sexual behaviour in females. In conclusion, these results confirm previous findings that AVPV kisspeptin neurons are an important component of the neural circuitry underlying female sexual behaviour and suggest a novel role in mediating partner preference in males.

POSTER 27 INHIBITING METHYLGLYOXAL STRESS TO RESTORE ANTITUMOR IMMUNITY AND REDUCE METASTATIC POTENTIAL IN TRIPLE-NEGATIVE BREAST CANCER

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Cancer cells often favor aerobic glycolysis, leading to the accumulation of methylglyoxal (MG), a reactive by-product that glycates proteins. MG is detoxified by glyoxalase 1 (GLO1), which converts it into D-lactate. We previously showed that MG accumulation suppresses tumor-inhibitory pathways and promotes tumor growth and metastasis in breast, colon, and pancreatic cancers. However, its role in shaping the immune microenvironment remains unclear. Myeloid-derived suppressor cells (MDSCs), which inhibit anti-tumor T cell responses, are major contributors to immune evasion and resistance to immunotherapy. Here, we explored the impact of MG stress on MDSC recruitment in murine breast cancer models. Treatment with carnosine, an MG scavenger, reduced MDSC infiltration in MMTV-PyMT tumors. Using 4T1 and 67NR cells with stable GLO1 knockdown, we found that MG stress increases MDSC accumulation in primary tumors and lungs. Strikingly, GLO1 depletion

in the non-metastatic 67NR model induced lung metastases and was associated with elevated MDSC levels. Combining carnosine with anti-PD1 immunotherapy in the 4T1 metastatic model led to a marked reduction in MDSC infiltration, a strong anti-tumor response, and a near-complete suppression of metastases. These findings indicate that MG stress promotes immunosuppression and metastatic dissemination by promoting MDSC accumulation. To uncover the underlying mechanisms, we are analyzing cytokine profiles in GLO1-deficient cells and integrating TCGA data from TNBC patients to explore associations between MG signatures, MDSC markers, and immunosuppressive pathways. Our results highlight MG stress as a novel metabolic driver of immune escape and metastasis in TNBC, supporting its targeting as a promising therapeutic strategy.

POSTER 28 DEVELOPMENT AND VALIDATION OF A NEW PTK7-TARGETED AAV-MEDIATED SUICIDE-GENE THERAPY FOR GLIOBLASTOMA

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FLASH TALK

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Glioblastoma (GBM, IDH WT, grade 4) is a dreadful primary brain tumor. Despite the current standard-of-care, patient survival remains dramatically poor. This highlights the urgent need for new therapeutic strategies. In this context, we developed a novel therapy targeting Protein Tyrosine Kinase 7 (PTK7). By combining an anti-PTK7 Nanobody® (VHH) with a 'suicide-gene therapy' (SGT), we aim to design a highly-specific targeted therapy that holds promising therapeutic potential.

First, we assessed PTK7 expression by immunofluorescence and flow cytometry on patient tissue samples and patient-derived GBM stem-like cell (GSC) cultures. PTK7 was highly expressed in GBM samples compared to non-tumoral brain tissues, and highly expressed in GSCs, even upon therapy-mimicking stress conditions.

In parallel, anti-PTK7 VHH were produced and validated using bio-layer interferometry and flow cytometry. These sequences were then combined with a recombinant AAV, and a suicide-gene delivery. The resulting anti-PTK7 AAV-SGT was tested for specific

GBM cell death induction, using live cell imaging. To further confirm the specificity of this targeted SGT, we generated a PTK7-KO GSC culture, by CRISPR-Cas9 technique, and used it as a control in all experiments.

The combination of anti-PTK7VHH with AAV-SGT effectively and specifically targeted and killed GBM cells following drug-induced activation of the SGT, leading to a concentration-dependent cytotoxicity. Notably, this cytotoxic effect was PTK7-dependent, as PTK7-KO cells remained unaffected by the targeted therapy. Ongoing in vivo studies aim to validate this strategy.

To conclude, PTK7 was identified as a GBM-specific cell surface protein that could serve as a new target for therapy. We successfully validated VHH antibodies against PTK7 that show good affinity and specificity for GBM cells. We also generated promising preliminary data showing that AAV-mediated SGT, using PTK7 as an entry signal, induces GBM cell death.

POSTER 29 DECIPHERING THE ROLE OF THE PUTATIVE RNA-BINDING PROTEIN TFIP11 IN REGULATING TISSUE-SPECIFIC ALTERNATIVE SPLICING

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RNA-Binding proteins (RBPs) comprise a large class of proteins that regulate the metabolism of RNA transcripts throughout their life cycle. Many regulatory RBPs function in a cell-, tissue-, or condition-specific manner and are capable of regulating variety of molecular processes such as alternative splicing of messenger RNAs that is critical for appropriate protein expression in the corresponding tissues. The proper activity of RBPs is essential for human physiology, and their dysregulation has been implicated in diseases including neurodegeneration, autoimmune disorders, and cancer. RNA-Binding Domains (RBDs) in RBPs are the functional units responsible for RNA binding. Multiple RBDs are often present in a single RBP and these modular domains can coordinate and enhance binding to RNA in a sequence and/or structure-specific manner. Intrinsically Disordered Regions (IDRs) often behave as linkers connecting RBDs within a single RBP but these regions also have the ability to mediate interactions with RNA directly. Tuftelin-Interacting Protein 11 (TFIP11) is a spliceosomal protein that harbors two

putative RBDs : an N-terminal G-patch domain and a C-terminal double-stranded RNA-Binding Domain (dsRBD), both flanked by IDRs. While we have previously shown that TFIP11 plays critical roles in spliceosome assembly and activation, the RNA-binding functionality of its individual domains and their roles in splicing regulation remained uncharacterized. In this study, we employed a multidisciplinary strategy combining in cellulo, in vitro, in silico, and in vivo approaches to investigate the RNA-binding properties of TFIP11's RBDs and IDRs, and to define their functional relevance in alternative splicing regulation. Our findings provide novel mechanistic insights into TFIP11's role as a splicing regulator and support its classification as a previously unrecognized RNA-Binding Protein with broad implications for RNA biology and disease.

POSTER 30 WOBBLE TRNA MODIFICATION SHAPES TUMOR ASSOCIATED NEUTROPHILS IN METASTATIC BREAST CANCER

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FLASH TALK

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During cancer progression, neutrophils undergo transcriptional reprogramming and acquire a Tumor-Associated Neutrophil (TAN) phenotype. TANs exhibit functional plasticity, exerting either pro-tumoral activities by promoting tumor growth and metastasis, or anti-tumoral effects by inhibiting epithelial cell proliferation and modulating angiogenesis. In metastatic breast cancer, early neutrophilia is linked to poor outcomes. Neutrophils contribute to metastasis by facilitating the formation of a pre-metastatic niche, particularly in the lungs. Using low-throughput proteomics, we showed that neutrophils undergo metabolic reprogramming when infiltrating the primary tumor. Normally glycolysis-dependent and using glycogen stores under low glucose, tumor-infiltrating neutrophils shift to mitochondrial metabolism. Neutrophils from cancer patients show increased oxidative phosphorylation (OXPHOS) and a more immature phenotype. This shift

is paired with increased expression of tRNA-modifying (U34-TM) enzymes, which affect the wobble position in tRNAs. We found that U34-TM enzymes are upregulated in neutrophils from breast tumor-bearing mice compared to tumor-free controls. We hypothesized that U34-TM supports metabolic reprogramming via translational control during cancer progression. To test this, we created a neutrophil-specific U34-TM loss-of-function model by crossing *Elp3lox/lox* mice with the *Mrp8-Cre* strain. *Elp3* deletion reduced neutrophil numbers in the lungs and spleens of tumor-bearing mice and significantly lowered metastatic burden in PyMT mice. Unexpectedly, it also led to fewer and smaller primary tumors. Additionally, *Elp3* loss changed mitochondrial morphology and altered the metabolic state of TANs. These findings reveal a key role for U34-TM in regulating neutrophil function in breast cancer. Our work focuses on understanding the immune regulatory networks behind the anti-tumoral response and the translational changes in neutrophils within the tumor.

POSTER 31 MTORC2-DEPENDENT REPROGRAMMING OF LIPID METABOLISM IS A METABOLIC VULNERABILITY IN LUNG CANCER

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Lung cancer is a genetically heterogeneous disease characterized by a multitude of tumor-promoting alterations. This heterogeneity renders lung tumors resistant to current treatment strategies. Importantly, genomic amplification of RICTOR, the defining component of mTOR complex 2 (mTORC2), frequently occurs in lung cancer. However, despite high therapeutic potential, targeting mTORC2 activity remains challenging. In this study, we show that elevated mTORC2 signaling in patients with lung adenocarcinoma is associated with poor overall survival and high frequency of TP53 mutation. Furthermore, proteomic characterization of patient biopsies reveal that RICTORhi tumors exhibit a prominent hypoxia phenotype and undergo extensive metabolic rewiring. In order to model this molecular subtype, we have generated a new mouse model of lung cancer by overexpressing Rictor in the lungs of KrasG12D/+Tp53-/- mice (KPR and KP models respectively). In comparison

to the KP model, KPR mice display increased tumor burden and have shortened survival. In line with the patient data, KPR tumors are hypoxic and metabolically reprogrammed towards increased glucose and lipid utilization. Mechanistically, we identified the transcription factor HIF-1 β as a potential mTORC2 target and demonstrate that RICTOR controls HIF-1 β stability through an mTORC2-PKC signaling axis, independently of AKT activity. Using a combination of proteomics, metabolomics and lipidomics, we further demonstrate that HIF-1 β supports lung tumor growth by promoting mTORC2-dependent sphingolipid metabolism. Finally, we show that RICTOR and HIF-1 β are frequently co-expressed in lung cancer biopsies and that this association correlates with poor outcome in lung cancer patients. Taken together, our results support the rationale of targeting mTORC2-dependent lipid metabolism in lung cancer and highlight HIF-1 β as a clinically relevant target for the development of future anticancer therapies.

POSTER 32 DECIPHERING THE MOLECULAR MECHANISMS RELATED TO PROTEASOME INHIBITOR RESISTANCE IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is the second most common blood cancer, with 50000 new cases diagnosed annually in Europe. The median survival of patients is about 5 years, ranging from six months to ten years. Proteasome inhibitors (PI) such as Bortezomib (BTZ) and Carfilzomib (CFZ) are commonly used as first-line therapies. Unfortunately, patients inevitably develop resistance to these treatments.

transcripts of FOXM1 are alternatively spliced in PI-resistant cells, which is an attractive target for controlling drug-resistant and difficult-to-treat cancers.

Aberrant RNA splicing is known to be a crucial mechanism in the emergence of cancer resistance to chemotherapy. To understand the molecular basis of MM resistance, we performed an RNA-seq experiment using BTZ-resistant and CFZ-resistant AMO1 cells compared to sensitive AMO1 cells and analyzed differentially expressed genes as well as altered splicing events. Among thousands of dysregulated genes, we are interested in the upregulation of some genes implicated in the WNT/ β -catenin pathway. Indeed, this pathway is known to be a central player in cancer development and cell proliferation. Regarding splicing alteration, we found that exon skipping is the main affected event. Interestingly, we found that

POSTER 33 SYNERGISTIC EFFECTS OF MUSIC AND NEUROMODULATION IN REHABILITATION OF PATIENTS WITH DISORDERS OF CONSCIOUSNESS: PROTOCOL FOR A RANDOMISED, SHAM-CONTROLLED, SINGLE-SESSION STUDY

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Following a severe acquired brain injury some patients may remain in a disorder of consciousness (DoC), a clinical spectrum encompassing coma, unresponsive wakefulness syndrome, and minimally conscious state. Despite medical advances, effective treatments remain limited, necessitating innovative approaches. Non-invasive brain stimulation approaches have been investigated but their effects remain moderate. Music is another promising approach, as it may influence consciousness and responsiveness by modulating neural oscillations in the brain's auditory-motor network. However, clinical outcomes are still modest. It is hypothesised that enhancing the auditory-motor network oscillatory dynamics could boost the effect of music therapy. Transcranial Alternating Current Stimulation (tACS) offers a mean to modulate such dynamics. To explore this hypothesis, a randomised, sham-controlled, single-session study is designed to investigate the synergistic effects of tACS applied synchronously to music, on neural, behavioural, and

physiological outcomes. Specifically, the study examines how this combined intervention affects coupling between auditory and motor brain networks. The study will include 35 DoC patients (aged 18+) due to acquired brain injury. Each will complete four sessions, separated by a washout period of one week, in a pseudo-randomised order: 1) music + tACS, 2) tACS only, 3) music + sham tACS, and 4) control (audiobook + sham tACS). Preferred music at 100 bpm will be used, while tACS will be applied over the primary motor cortex of the dominant hand at 2mA peak-to-peak, and synchronised to the rhythm of the music. Outcome measures, including EEG, Coma Recovery Scale-revised, heart rate, and video recordings, will be collected before and after stimulation. The result of this project is to further unravel the mechanism of auditory-motor coupling on the recovery of patients with DoC and bring forward a novel therapeutic strategy for such a population.

POSTER 34 USING BREAK CHOICE OPTIONS TO REGULATE COGNITIVE FATIGUE INDUCTION? A PRELIMINARY STUDY

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Fatigue increases effort discounting which in turn reduces the willingness to persist cognitive tasks. As breaks were shown to promote fatigue recovery, offering the choice (persist vs. rest) under "fatigue" may offer a promising avenue to study its regulation and recovery. Our goal was to validate a new procedure assessing fatigue dynamic using choices during demanding cognitive tasks. We expected participants would take more breaks with the first break being taken earlier in the more fatiguing task. 83 young adults (20.71 ± 2.94 y.o.; 74% women) were divided into two groups that completed either the easy ($N_1 = 41$) or hard ($N_2 = 42$) version of the TLDB task. They performed 4-min. bouts of task to acquire points and were told the task would end at 16 pts. After each task block, subjective fatigue was assessed using VAS and participants were asked to choose between persisting (+2 pts. in case of success) or taking a 1-min. break (-2 pts.). Several task parameters were extracted: Subjective Fatigue (SF), Decision Latency (DL), Number of Breaks (NB), and First Break Onset (FBO). Statistical analyses consisted of linear mixed models fitted on SF

and DL seeking for block, group and interaction effects. NB and FBO were compared between groups using independent t-tests. We observed a significant SF increase with time ($t(81.25) = 10.94, p < .001$), with higher fatigue in the hard task ($t(82) = 3.34, p < .01$) but no interaction effect, as well as a quadratic effect of time on DL ($t(61.57) = -4.91, p < .001$) following a u-shaped curve. NB ($W(82) = 852, p = 0.747$) and FBO ($t(22) = -1.04, p = 0.311$) did not differ between groups. Overall, the results show that, despite being fatigued overtime, and as suggested by increasing decision latency during the second half of the task, participants would rather persist in the current activity, probably to avoid being punished (i.e., losing points). Importantly, our results also highlight methodological considerations for future studies.

POSTER 35 MTORC2-DEPENDENT REMODELLING OF ALVEOLAR MACROPHAGES SUPPORTS IMMUNE EVASION IN LUNG CANCER

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Lung cancer is one of the deadliest cancers and is marked by high histological and genetic heterogeneity. Importantly, mTORC2 hyperactivation is frequently observed in lung tumor biopsies, as evidenced by high levels of RICTOR protein expression and correlates with poor clinical outcomes. To model the impact of mTORC2 activation on lung cancer progression, we generated a novel genetically engineered mouse model of lung cancer in which we combined the lung-specific overexpression of RICTOR with the expression of the oncogenic KRASG12D mutation and the loss of the TP53 tumor suppressor gene (KPR model). Compared to the KP model (KRASG12D; TP53fl/fl), KPR mice exhibited accelerated disease progression and shortened survival. Integrative multi-omic profiling revealed profound tumor-intrinsic and microenvironmental changes in KPR tumors. Proteomic analyses revealed that mTORC2 activation in lungs was associated with an important rewiring of lipid metabolism. Moreover, Single-cell RNA sequencing and CITE-seq

immunophenotyping uncovered extensive immune remodeling, including reduced effector T cells function and a reprogrammed alveolar macrophage landscape in KPR tumors. These changes included a depletion of M1-like macrophages and the emergence of a metabolically rewired macrophage subset characterized by the expression of Arginase 1. Similar dysregulation of metabolic and immune pathways were also observed in human lung tumor biopsies with high RICTOR expression, reinforcing the clinical relevance of our findings. By specifically targeting this newly identified macrophage population in KPR tumors, we aim to better understand the influence of epithelial mTORC2 activation on the reshaping of the lung microenvironment and to improve the efficacy of immunotherapy. Together, our data reveal epithelial mTORC2 activation as a key orchestrator of tumor progression and microenvironmental dysregulation in lung cancer, offering new perspectives for targeted intervention.

POSTER 36 UPR RELATED GENES IN HEALTH AND MICROCEPHALY ASSOCIATED DISORDERS

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The cerebral cortex is the largest part of the mammalian brain, controlling high-order functions like movement, sensory perception, and cognition. During corticogenesis, several classes of glutamatergic projection neurons and inhibitory GABAergic interneurons are generated sequentially from distinct progenitor cells in the forebrain germinal zones. Cortical development requires high protein synthesis to support progenitor proliferation and neurogenesis. Proper protein quality control is critical to maintain proteostasis, with the unfolded protein response (UPR) pathway playing a key role. UPR ensures protein synthesis does not exceed the endoplasmic reticulum (ER) capacity by detecting unfolded or misfolded proteins. My laboratory showed that disruption of ER homeostasis during development activates the PERK branch of UPR, favoring direct neurogenesis (one neuron plus one progenitor) over indirect neurogenesis (two intermediate progenitors) and increasing apoptosis of newborn neurons, leading to microcephaly. Here, I focus on EIF2S3 and TMEM167,

two UPR-related genes. Importantly, mutations in these genes have been associated with microcephaly and monogenic forms of diabetes. Therefore, my project aims to elucidate how patient-associated mutations of these genes lead to microcephaly associated disorders. First, I have characterized the physiological role of those two genes in the morphogenesis of the mouse cortex via in utero electroporation. My preliminary data suggest that loss of EIF2S3 affects cell fate and promote cell death. On the contrary, TMEM167 might be more involved in cell cycle regulation and cell migration dynamics.

POSTER 37 EXPLORING THE ROLE OF CELLULAR ORIGIN IN PANCREATIC CANCER PROGRESSION: A ZEBRAFISH AND SINGLE-CELL RNA SEQUENCING APPROACH

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Déom E, Goossens C, Lolos C, Kessels M, Manfroid I, Peers B, Voz M identifying new therapeutic targets.

Pancreatic cancer is among the deadliest cancers due to limited diagnostic tools and the lack of targeted therapies. While murine models suggest that both acinar and ductal cells can give rise to pancreatic cancer, the impact of cellular origin on tumor progression remains largely unknown. Furthermore, the proportion of tumors of ductal versus acinar origin in humans is still unknown, which is crucial information for developing origin-specific treatments. To address these questions, we will compare the transcriptional trajectories of tumor cells originating from both acinar and ductal cells, from initiation to metastasis, using our zebrafish models and single-cell RNA sequencing (scRNAseq). This will allow us to define specific signatures for each tumor origin, aiding in determining the proportion of these tumors in humans. Additionally, by comparing our findings with mammalian models, we aim to identify factors, pathways, and processes conserved across species, which is a strong indication of an important role in the disease. Finally, we will determine the role of selected conserved factors through loss-of-function experiments, potentially

POSTER 38 HUMAN T-CELL LEUKEMIA VIRUS TYPE-I HBZ RNA AFFECTS EZH2/HOTAIR COMPLEX

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Although the pleiotropic functions of the HBZ protein are well established, the discovery that a mutant HBZ transcript lacking a translation start codon can still promote T cell proliferation highlights the oncogenic potential of the HBZ ribonucleic acid (RNA) itself. The predominant nuclear localization of the HBZ RNA suggests regulatory roles, particularly in the epigenetic modulation of gene expression. Consistent with this, we previously demonstrated that HBZ RNA inhibits sense transcription of HTLV-1 by disrupting the basal transcription complex at the 5'LTR (Gazon et al., 2020, Blood Adv. 4:5574). To further elucidate the molecular mechanisms underlying this regulation, we aimed to identify interacting partners of the HBZ RNA and characterize their role in epigenetic processes. Candidate RNA-binding proteins were first predicted in silico using RPIseq, and interactions were experimentally validated by RNA immunoprecipitation (RIP). Transcriptional activity in patient-derived samples was assessed by RT-qPCR, epigenetic changes were analyzed by immunoblotting, and functional consequences were

evaluated using luciferase reporter assays. Our results demonstrate that HBZ RNA directly interacts with EZH2, the H3K27 methyltransferase component of the Polycomb repressive complex 2 (PRC2), thereby interfering with EZH2's association with the HOTAIR long non-coding RNA. This interaction alters global H3K27-trimethylation levels and correlates with the overexpression of HBZ, EZH2, and HOTAIR in cells from adult T-cell leukemia/lymphoma (ATLL) patients. Together, these findings reveal that HBZ RNA disrupts the EZH2-HOTAIR complex, leading to epigenetic alterations that may contribute to its oncogenic potential.

POSTER 39 CHARACTERIZATION OF CEMIP EXPRESSION PATTERNS, INCLUDING LOCALIZATION AND INTENSITY, IN SKIN BIOPSIES FROM PATIENTS WITH SYSTEMIC SCLEROSIS

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FLASH TALK

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CEMIP (Cell Migration-Inducing Protein) is a protein belonging to the hyaluronidase family. It has been studied mainly in oncology, where it is involved in epithelial-mesenchymal transition, tumour growth, angiogenesis and metastasis. In rheumatic diseases, it appears to play a pro-inflammatory and pro-fibrotic role, particularly in osteoarthritis. Systemic sclerosis (SSc) is a rare autoimmune disease characterized by fibrosis of the skin and internal organs. The pathophysiology of SSc remains poorly understood. Given the discovery of the pro-fibrotic and pro-inflammatory roles of CEMIP, it seems relevant to study its involvement in SSc. The aim of this study was to investigate CEMIP expression in the skin of SSc patients. Immunohistochemical analyses were performed on skin biopsies from six SSc patients and four healthy controls. CEMIP expression was measured quantitatively using QuPath software and semi-quantitatively by whole slide microscopy. Both approaches showed significant overexpression of CEMIP in SSc skin compared with controls (SSc: 27.25%, healthy: 5.03%; $p < 0.01$). The

increase was marked in the epidermis (keratinocytes) (SSc: 73.65%, healthy: 6.56%; $p < 0.01$) and in the dermis, particularly in endothelial cells and fibroblasts (SSc: 5.41%, healthy: 0.65%; $p < 0.01$). Semi-quantitative analysis confirmed these results for keratinocytes and endothelial cells, but not for fibroblasts. These results are currently being confirmed by multiplex immunofluorescence (CEMIP, CD31 for endothelial cells, CD45 for immune cells and α SMA for myofibroblasts) on a second cohort (30 SSc and 15 healthy controls). This work shows that CEMIP is significantly over-expressed in the skin of SSc patients, particularly in endothelial cells and keratinocytes, suggesting a potential role in the pathogenesis of SSc. To ensure the robustness of these findings, the next step will be to validate them using multiplex immunofluorescence with specific cell markers.

POSTER 40 HOW DOES ZOLPIDEM IMPACT THE BRAIN AND THE BEHAVIOR? A DOUBLE-BLIND PLACEBO-CONTROLLED RANDOMIZED CLINICAL TRIAL

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Zolpidem is a non-benzodiazepine sedative approved for the treatment of insomnia. However, it exerts paradoxical awakening effects in a fraction of the general population (~16%). To characterize these sedative and awakening effects, this study investigated neuropsychological, electrophysiological, and phenomenological effects of zolpidem in neurotypical individuals. METHODS: This pilot cross-over double-blind placebo-controlled randomized clinical trial (RCT) was performed in two sessions during daytime with a one-week washout period. During each session, baseline electroencephalography (EEG) and electrocardiography (ECG) were recorded. Then, zolpidem (10 mg) or placebo (mannitol) was administered in a randomized order with EEG and ECG being recorded continuously for 45 minutes. One hour after placebo and zolpidem intake, post-intervention neuropsychological assessment was conducted, and participants reported their phenomenological experiences. RESULTS: Preliminary results in two neurotypical individuals (25 and 28yo; female) responding non-

paradoxically to zolpidem showed impaired executive functions with lower number of correct responses, more changes in decisions, as well as longer thinking and execution time under zolpidem effect, compared to placebo. Phenomenological reports indicated that zolpidem altered conscious perception as evidenced by visual distortion of reality in one participant. Preliminary EEG results were indicative of variations in power spectrum and connectivity measures after zolpidem vs. placebo intake. ECG measures illustrated decreases in time-domain heart rate variability after zolpidem intake. CONCLUSION: This pilot study provides preliminary evidence on how zolpidem affects cognitive functions and neurophysiological activity of the brain. It also confirms the feasibility of a large-scale RCT in paradoxical and non-paradoxical responders to zolpidem.

POSTER 41 IFN γ PREDISPOSES ACUTE MYELOID LEUKEMIA TO CHEMORESISTANCE AND IMMUNE EVASION

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Acute myeloid leukemia (AML) patients frequently relapse following frontline therapies such as high-intensity chemotherapy and hypomethylating agents. As in other malignancies, this relapse is thought to be driven by persisters (cancer cells that survive chemotherapy). We recently discovered that AML blasts present MHC peptides recognized by T cells at diagnosis, leading to activation and cytokine secretion. While such responses may aid leukemia clearance, we hypothesized that pro-inflammatory cytokines, particularly IFN γ , secreted by immune cells might contribute to therapy resistance and promote persister survival. To investigate this, we conducted large-scale transcriptomic analyses comparing blasts obtained at diagnosis from patients who either responded or failed to respond to cytarabine + anthracycline therapy. Our analyses revealed an upregulation of IFN γ signaling signatures in patients resistant to therapy. Similar signatures were observed in patients resistant to the hypomethylating agent 5-azacytidine. Consequently, patients expressing high IFN γ signaling scores at diagnosis exhibited poorer

survival outcomes. Functionally, treating multiple AML cell lines with IFN γ in vitro significantly increased the number of persisters following cytarabine exposure. These IFN γ -treated persisters also re-expanded more rapidly in drug-free conditions, demonstrating enhanced relapse potential and suggesting that IFN γ signaling may accelerate disease recurrence in patients. Moreover, IFN γ conferred resistance to T-cell-mediated cytotoxicity, while blockade of the IFN γ -JAK-STAT axis sensitized persisters to cytarabine. Further investigation into the role of IFN γ signaling in modulating chemotherapy resistance in AML is crucial for improving treatment strategies and patient outcomes.

POSTER 42 ADAMTS2 IN CANCER PROGRESSION: FRIEND OR FOE?

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ADAMTS2 is a metalloproteinase involved in the cleavage of fibrillar procollagens and the regulation of lymphangiogenesis (by proteolytic maturation of pro-VEGF-C into fully active VEGF-C). Unpublished results from our laboratory also indicate that ADAMTS2 represses the growth of primary tumors while, conversely, it stimulates the dissemination of metastases. Our aim was therefore to identify the mechanisms explaining this dual role in cancer progression. The evolutions of spontaneous (MMTV-PyMT mice) and induced (subcutaneous injection of cancer cells) tumors were evaluated in ADAMTS2-KO mice and in their respective WT controls. Primary tumors, draining lymph nodes and lungs (preferred organs for metastasis) were then characterized at different levels to identify the specific role(s) of ADAMTS2. The faster growth of primary mammary tumors in the absence of ADAMTS2 does not result from (i) increased proliferation or altered phenotype of cancer cells, (ii) tumor vascularisation or (iii) collagen accumulation. In contrast, our current data indicate an involvement of ADAMTS2 in the

regulation of macrophage polarization. The underlying mechanism has not yet been elucidated and is the subject of ongoing research. As a working hypothesis, shedding of cell surface receptors by ADAMTS2 could affect macrophage polarization and, therefore, tumor growth. We also noticed a significantly lower size of tumor draining lymph nodes in ADAMTS2-KO mice as compared to the WT. We are currently trying to determine whether this effect relates from defects in pro-VEGF-C cleavage and alteration of the VEGF-Rs signaling pathways. The immune cell populations present in the lungs under physiological conditions were also studied, as they could be linked to differences in the formation of lung metastases. Increased numbers of alveolar and interstitial macrophages were notably observed in ADAMTS2-KO mice. These data need to be confirmed and their significance for metastasis formation demonstrated.

POSTER 43 TARGETING U34 TRNA THIOLATION TO OVERCOME RESISTANCE IN LUMINAL BREAST CANCER THERAPY

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Breast cancer remains the most frequently diagnosed malignancy worldwide and a leading cause of cancer-related mortality. Metastatic progression accounts for approximately 90% of breast cancer deaths, often driven by acquired resistance to therapeutic interventions. Emerging evidence highlights the regulation of mRNA translation as a key driver of cancer progression, metastasis, and therapy resistance. Transfer RNAs (tRNAs), which undergo extensive post-transcriptional modifications, play a crucial role in modulating translation efficiency. In particular, modifications at the wobble position (nucleotide 34) influence translation kinetics and codon decoding fidelity. Our laboratory has demonstrated that wobble tRNA modifications contribute to cancer initiation, metastatic progression, and resistance to therapy. In this study, we investigated the mechanisms underlying resistance in luminal breast cancer (LBC) to the standard-of-care endocrine therapy (fulvestrant) in combination with the CDK4/6 inhibitor palbociclib. Using a

CRISPR-Cas9 loss-of-function screen targeting 174 mRNA translation regulators, we identified key enzymes essential for the survival of resistant LBC cells. Notably, we found that U34 thiolation (mcm5s2U34), catalyzed by the Molybdenum Cofactor Synthesis 3 (MOCS3) and the Cytosolic Thiouridylase Subunit 1 (CTU1) enzyme, is critical for the survival of resistant T47-D and CAMA-1 cells *in vitro*. To further elucidate the role of tRNA-dependent translation reprogramming in resistance, we are employing integrative approaches including proteomics, polysome profiling, and RNA sequencing to analyze mRNA translation dynamics and codon usage shifts. Our findings reveal that tRNA wobble modifications are pivotal in the adaptation of cancer cells to combined endocrine and targeted therapy. This work underscores the potential of targeting tRNA thiolation enzymes as a novel therapeutic strategy to combat resistance and prevent metastatic progression in breast cancer patients.

POSTER 44 SUCCINATE RECEPTOR AS AN EMERGING TARGET IN RENAL ISCHEMIA/REPERFUSION INSULT

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FLASH TALK

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Succinate, a key intermediate of the tricarboxylic acid cycle, accumulates under various metabolic stresses such as ischemia, inflammation, or Warburg metabolism. Efficient mitochondrial and cellular transporters enable its rapid release into the extracellular space, where succinate acts as a signaling molecule through SUCNR1 (Succinate Receptor 1), a G-protein-coupled receptor coupled to both Gi and Gq pathways. SUCNR1 is expressed across numerous cell types, particularly macrophages. Notably, high extracellular succinate levels drive macrophages toward a pro-inflammatory M1 phenotype via Gi signaling, while lower levels promote anti-inflammatory M2 polarization through Gq. The succinate-SUCNR1 axis is involved in the regulation of renin secretion, platelet activation, erythro- and megakaryopoiesis, adipocyte lipolysis, skeletal myocyte remodeling, and retinal angiogenesis. Its pathological implication has been demonstrated or presumed in a range of conditions including hypertension, diabetic retinopathy, autoimmune arthritis, hepatic fibrosis, hypertrophic cardiomyopathy, cancer progression,

and ischemia/reperfusion (I/R) injury.

In experimental I/R models, SUCNR1 activation appears to exert dual effects: beneficial ones, such as stimulating post-ischemic angiogenesis in brain tissues, and deleterious effects by increasing the M1/M2 macrophage ratio, as observed in hepatic injury. Renal I/R injury, recognized as the leading cause of acute kidney injury (AKI), is associated with over two million deaths worldwide annually, representing a major clinical challenge.

To investigate the role of SUCNR1 in renal I/R lesions, we employed a murine surgical model with pharmacological modulation: a potent synthetic agonist (cis-epoxysuccinate) and a selective negative allosteric modulator (compound 4c). This study aims to elucidate SUCNR1's impact on macrophage polarization and overall renal impact in renal I/R injury, and to assess its potential as a therapeutic target.

POSTER 45 TF/FVIIA-MEDIATED MICA SHEDDING: IMPACT ON TUMOR CELL RESISTANCE TO NK CELL CYTOTOXICITY

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FLASH TALK

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Tissue Factor (TF) is a membrane protein overexpressed by certain cancer cells and has emerged as a central player linking coagulation and cancer. TF is mainly known as the primary initiator of the coagulation cascade, serving as the transmembrane receptor for the first proteolytic factor of the coagulation cascade, FVIIa. Adding to coagulation-dependent pro-metastatic functions documented by us and many others, TF has also been shown to promote tumor progression through hemostasis-independent mechanisms such as the activation of the Protease-Activated Receptor 2 (PAR2), inducing its signaling and promoting tumor growth and angiogenesis. At the basis of this project, MHC Class I polypeptide-related sequence A (MICA) was identified as a substrate of the TF/FVIIa proteolytic complex, opening the possibility that TF/FVIIa could modulate Natural Killer (NK) cells/tumor cells interactions. MICA is a membrane protein expressed by cells undergoing cellular stress, such as malignantly transformed cells, and is one of the major activating ligands of the NKG2D receptor expressed by NK cells, which leads to their

activation in the circulatory system. A well-known mechanism of immune evasion employed by tumor cells is the proteolytic shedding of MICA by canonical sheddases of the ADAM/MMP families. Our team is exploring whether TF/FVIIa-mediated MICA shedding is a similar yet distinct mechanism to ADAM/MMP shedding, being likely activated in different body locations. Western blot analysis of breast tumor cell line MDA-231, lung tumor cell line Calu1 and cervical tumor cell line C4-I conditioned media showed a TF/FVIIa-dependent release of a ~30 kDa soluble MICA fragment, distinct from the ~70-50 kDa fragments released by canonical sheddases. In vitro cytotoxicity co-culture assays are ongoing to determine the relevance of this shedding regarding NK cell cytotoxic function modulation.

POSTER 46 MULTIDIMENSIONAL TUNING OF VMH nNOS NEURONS TO SOCIAL STIMULI IN MICE

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The ventromedial hypothalamus (VMH) mediates sexual and aggressive social behaviors. We have recently shown that VMH nitric oxide synthase expressing neurons (nNOS), are important for both male and female innate social behaviors. Here, we aimed to fully characterize the responses of VMH nNOS neurons in male and female mice using fiber photometric in vivo calcium imaging. We recorded VMH nNOS activity in mice exposed to same- or opposite-sex conspecifics contained in a wire cup, allowing olfactory investigation without behavioral interaction. In both sexes, we observed time-locked calcium responses at the onset of investigation. Estrus and diestrus females showed robust responses to male (AUC: 9- and 8-fold increases, respectively) and female stimuli (8.6- and 6.3-fold), with no significant difference between the stimulus sex ($p = 0.11$). Males also responded strongly to both male and female stimuli (7- and 5.9-fold increases). Since conspecific interaction involves multiple sensory and contextual cues—such as novelty, olfaction, and motion of the stimulus, we next examined how VMH nNOS neurons respond to each of these aspects. As

for olfaction, we found that VMH nNOS neurons from males and females respond more strongly to social odors than to a non-social odor (amyl acetate). Indeed, responses to male or female urine exceeded amyl acetate responses by 2.5 to 4.3-fold ($p_{adj} < 0.05$) in both males and females. To isolate the contribution of novelty to the responses observed during social investigation, we introduced an empty wire cup into a familiar environment. VMH nNOS neurons showed robust activation at the onset of exploration, with 6.3- and 4.9-fold increases in females (estrus and diestrus) and a 3.4-fold increase in males (all $p < 0.05$). Altogether, our findings suggest that VMH nNOS neurons play a key role in integrating multimodal social cues by encoding both sensory and contextual aspects of conspecific investigation.

POSTER 47 COULD PULSED ESTROGEN-BASED THERAPIES ENHANCE THE SENSITIVITY OF ER+ BREAST CANCER TO ENDOCRINE THERAPIES AND PREVENT RESISTANCE?

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While endocrine therapies have been successful in reducing estrogen receptor (ER)- positive breast cancer recurrence and mortality in early-stage patients, about 20% still face recurrence due to resistance to these therapies in metastatic disease. It is crucial to identify new strategies to prevent endocrine resistance. Our team generated preliminary results showing that pulsed estrogen treatment modulates the expression of ER in vitro and in vivo in endocrine-sensitive breast cancer cells. We hypothesized that pulsed estrogen treatment could influence the sensitivity of ER-positive breast cancer to endocrine therapy and thus could prevent or overcome resistance. We aimed to characterize ER expression in parental and resistant cells after intermittent exposure and to evaluate the efficacy of endocrine therapies. Our results show modulation in ER expression not only in parental cells but also in Palbociclib/Fulvestrant-resistant cells treated with pulsed estrogen administrations. Interestingly, we observed a modulation of the sensitivity of parental cells to tamoxifen in vitro and in vivo. We observe the involvement of the

mTORC1 pathway in the upregulation of ER. Now, we aim to determine whether there is a link between ER upregulation, the mTORC1 pathway, and cell sensitivity to Tamoxifen following pulsed estrogen treatment. In conclusion, pulsed estrogen treatment appears to enhance receptor expression in both parental and resistant cancer cells, potentially improving their responsiveness to hormonal therapies. The next step is to investigate the effect of this pulsed treatment on the sensitivity of resistant cells to hormonal therapies, as Tamoxifen, Fulvestrant, and Elacestrant, by in vitro and in vivo experiments.

POSTER 48 INVESTIGATING MRKH SYNDROME USING NR6A1A/B MUTANT ZEBRAFISH MODELS

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The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a rare disorder affecting approximately 1 in 4500 women, characterized by uterine and vaginal aplasia, often associated with kidney and skeletal defects. While TBX6 and GREB1L mutations cause similar anomalies, many MRKH patients lack mutations in these genes, suggesting the involvement of other genes. Exome sequencing identified two highly pathogenic variants in NR6A1, encoding an orphan nuclear receptor. AlphaFold predictions suggest these mutations impair DNA binding or destabilize its structure. Gel shift assays revealed that mutant NR6A1 proteins bind DNA less efficiently than the wild-type protein. To explore the link between these variants and the disease, a zebrafish model was generated by inactivating the orthologous genes nr6a1a and nr6a1b. Phenotypic analysis of the nr6a1a mutants revealed reduced body and trunk size, fewer thoracic vertebrae, and absence of the anal fin. In these mutants, the mesonephros displayed altered morphology and reduced size, while pronephric segments and cloacal regions were disorganized. Double nr6a1a^{-/-};nr6a1b^{-/-} mutants

were more severely affected, showing unilateral or bilateral pectoral fin loss, posterior pharyngeal arch defects, and early lethality (12–15 dpf). Gene expression analysis through in situ hybridization revealed altered expression of posterior Hox genes, which are crucial to genital tract and kidney development. These findings suggest that NR6A1 mutations contribute to MRKH syndrome, partly through Hox dysregulation. To further investigate the disease mechanisms, RNA-seq was performed on WT and mutant zebrafish embryos at the 20-somite stage, confirming the upregulation of posterior Hox genes and the dysregulation of many other genes. In situ hybridization (ISH) experiments are ongoing to validate the alteration of these genes. Future work will use ChIP-seq to map loci bound by nr6a1a and explore its transcriptional network.

POSTER 49 GENOME-WIDE CHARACTERIZATION OF ABERRANTLY EXPRESSED TUMOR-SPECIFIC ANTIGENS THROUGH PROTEOGENOMICS

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Cytotoxic CD8+ T cells are essential for anticancer immunity. Typically, recognition of tumor cells is mediated through the interaction between their T-cell receptor and class I MHC-associated peptides (MAPs). While mutated MAPs (aka neoantigens) are often considered the main source of tumor recognition, the contribution of non-mutated aberrantly-expressed antigens to the set of MAPs (i.e. the immunopeptidome), and therefore to tumor recognition, is still misunderstood. In particular, the contribution of non-canonical genomic regions such as introns or endogenous retroelements to the cancer immunopeptidome is unknown. In the present study, we are developing a proteogenomic (i.e. genomic-informed proteomics) approach to identify all genomic regions able to generate tumor-specific antigens (TSAs). Practically, we are first generating an annotation-free and genome-wide index of MAPs being lowly expressed in a vast array of RNA-seq data of normal tissues. These tissues include more than 50 organs in addition to medullary thymic epithelial cells and dendritic cells. MAPs being lowly expressed in these tissues will then be queried in RNA-seq data of eight different cancers and those having a low expression in normal tissues and high expression in cancer will be elected as TSAs. Their amino acid sequence will then be used to perform mass-spectrometry (MS) identification of TSAs presented by cancer samples. Finally, we will use computational approaches to correlate the expression of MS-verified TSAs with the infiltration of T cells in cancer RNA-seq data to determine which TSAs contributes to tumor recognition. The top candidates will be validated via functional in vitro assays. Overall, our study will enable the establishment of a database of TSAs which could help better understand the mechanisms of tumor recognition by T cells.

POSTER 50 LARGE-SCALE TRANS-EQTL MAPPING ACROSS 27 CELL TYPES IN THE CEDAR-II COHORT IDENTIFIES AND REPLICATES 50+ NOVEL ASSOCIATIONS

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for 27 different cell types. We used 200 controls as “primary” dataset and 100 controls and 100 cases as “replicational” dataset. As a result, we found more than 50 trans-eQTLs across all cell types which we successfully replicated on our “replicational” dataset. Further research is needed to better understand the possible molecular mechanisms underlying the associations found.

Identifying eQTL variants is essential in modern human genetics for understanding mechanisms of many complex diseases such as IBD and finding possible drug targets for treatment. Most of the studies looking into cis-eQTL: where SNP affected genes in the nearest region of the genome. However, these primary events trigger a cascade of secondary molecular events that ultimately cause the disease. These secondary “disease pathways” are equally important to understand, as they encompass equally relevant drug targets. Testifying of the power of genetics, these secondary pathways can likewise be studied by so-called trans-eQTL studies, meaning that one now looks at the effect of risk variants on the expression levels of distant genes. In this study we used CEDAR-II (for Correlated Expression and Disease Association Research) cohort for identifying common trans-eQTL variants. This cohort contains genotype information about 400 individuals (300 controls and 100 cases) and gene expression levels

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