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Application deadline: March 24, 2024
Mahmoud Abdellatif
Division of Cardiology, Department of Internal Medicine, Medical University of Graz

Project Title: Energizing the failing heart

Background:
A significant proportion of heart failure patients, accounting for at least 50%, do not manifest a major deficit in left ventricular systolic function. Instead, they present with diastolic impairment, leading to what is known as preserved ejection fraction heart failure (HFpEF). This condition becomes more prevalent with age and currently stands as a major cause of hospitalization among the elderly. Despite its substantial socioeconomic burden, HFpEF has limited evidence-based therapies capable of enhancing patients' survival and quality of life.

Hypothesis and Objectives:
We hypothesize that HFpEF is an age-related disease driven by compromised cellular quality control due to impaired autophagy. Therefore, our objectives include investigating: (i) recently identified targets for the regulation of autophagy, known as autophagy checkpoints; (ii) the extent of impairment of these checkpoints during HFpEF development; and (iii) the potential for targeting these checkpoints as a therapeutic approach for HFpEF.

Approaches and methods:
The research will employ a comprehensive array of cutting-edge techniques, encompassing autophagic flux and mitochondrial assays, echocardiography, invasive hemodynamics, as well as multiomics and data integration. Both genetic and pharmacological interventions will be applied in vivo using relevant mouse models and human HFpEF tissue. The selected PhD candidate will collaborate closely with other fellows in the Cardiovascular Aging group, led by M. Abdellatif. Additionally, the PhD student will have the opportunity to conduct parts of the study abroad, collaborating with distinguished international research groups involved in this European project. These collaborations include the labs of Prof. Guido Kroemer (Paris), Prof. Maria Mitterbrunn (Madrid), and Prof. Rudolf de Boer (Rotterdam).

References:
Nerea Alonso Lopez

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz

**Project Title:** CXCR4 role in the anabolic response of teriparatide in bone

**Background:**
Osteoporosis is a common disease causing major public health and patient burdens. It is characterised by reduced bone formation and increased bone resorption; a process yet not fully understood. Patients with established osteoporosis are treated with teriparatide (TPTD), a highly effective anabolic agent. However, it is a costly treatment and the individual response is variable. Our preliminary data suggest that the genetic background plays a key role in the success of the treatment, appointing to a locus in chromosome 2, where the CXCR4 is located. In vitro experiments suggest that CXCR4 is involved in osteoblast migration and mineralisation in response to TPTD. Moreover, we found for the first time that TPTD could also exert a direct effect on osteoclasts via CXCR4.

**Hypothesis and Objectives:**
This grant proposal aims to investigate the role of CXCR4 in bone metabolism in response to TPTD and to identify novel pathways that could have therapeutic potential for patients with established osteoporosis.

**Approaches and methods:**
We will use a CXCR4 haploinsufficient mouse model (Cre+CXCR4lox/+ model and analyse changes in bone tissue by microCT, histomorphometry and 3-point bending test and serum biomarkers in response to TPTD. We will also investigate in vitro the role of CXCR4 in the osteoblast and osteoclast response to TPTD to identify cellular changes. At this stage, both cell types will be analysed separately and also in combination to assess not only individual response but also cross-talk changes. mRNAseq and proteomics will be performed to identify novel pathways involved in bone turnover that could be used in future as markers for osteoporosis appearance and/or as therapeutic targets to improve bone health.

**References:**


Katharina Artinger  
Division of Nephrology, Department of Internal Medicine, Medical University of Graz

Project Title: Atypical chemokine receptors in kidney disease

Background:
This project aims to evaluate the roles of two atypical chemokine receptors (ACKRs), ACKR1 and ACKR4, in murine kidney disease. Atypical chemokine receptors are involved in the regulation of immune cell movement through their interaction with chemokines, although they do not transmit conventional intracellular signals. So far, ACKR1 has been described on venular endothelial cells, erythroid cells and cerebellar neurons. Interestingly, the majority of individuals of West African ancestry carry a single nucleotide polymorphism of ACKR1 which disrupts transcription of ACKR1 in the erythroid lineage only, thus resulting in “erythroid silent” ACKR1 phenotype. It has been well-documented that people of West African ancestry have a higher incidence of kidney diseases resulting in more severe disease parameters and more unfavorable outcomes. In line with this, it was shown previously that ACKR1−/− mice develop more severe kidney disease as compared to control mice. So far, there was no knowledge on the renal expression pattern of ACKR1 in diseased kidneys. Therefore, one goal of this project is to evaluate whether expression of ACKR1 in the kidney changes in response to renal disease. Furthermore, this project is set to discern whether endothelial expression of ACKR1 or its erythroid cell expression would be responsible for the observed aggravated renal phenotype in ACKR1−/− mice. For this work, a well-established and reliable murine model of experimental glomerulonephritis (nephrotoxic serum nephritis, NTSN) will be used. The second part of the project will be centered around another atypical chemokine receptor, ACKR4. Our preliminary data using ACKR4eGFP reporter mice revealed a polar ACKR4 expression of only few glomerular cells, which until now has not been reported. ACKR4 scavenges chemokines like CCL21, a classical CCR7 ligand. The fact that CCR7 is implicated in the pathogenesis of NTSN and our preliminary data on the expression of ACKR4 in the kidney suggest a potential role for this atypical chemokine receptor in kidney disease. One main goal of the project will therefore be to define the cells expressing ACKR4 in the kidney in health and disease. The contribution of ACKR4 to the development of kidney disease will be evaluated using the murine model of NTSN. A central part of this project will also be to establish the role of ACKR4 as expressed in the kidney as compared to ACKR4 expressed lymphoid tissues.

Hypothesis and Objectives:
This project aims to map molecular and cellular pathways involving atypical chemokine receptors ACKR1 and ACKR4 and evaluate their pathogenic contributions to experimental murine glomerulonephritis. Regarding ACKR1, our preliminary experiments show that the loss of this receptor on either endothelial cells or bone marrow erythroblasts results in an aggravated NTSN phenotype. Building on this data, we plan to investigate the pathomechanisms and involvement of ACKR1
expressed by erythroblasts versus endothelium, contributing to leukocyte recruitment into the kidney. Furthermore, this project aims to establish how ACKR4 contributes to the development of NTSN including the potential involvement of the newly discovered ACKR4-expressing subset of glomerular cells.

**Approaches and methods:**
Mice will be preimmunized with rabbit IgG and three days thereafter, injected with rabbit anti-mouse glomerular basal membrane (GBM) serum leading to the induction of NTSN. Flow cytometry, confocal microscopy, scRNAseq, and spatial transcriptomics as well as conventional ELISA, histopathology and quantitative real-time PCR will be applied. Global ACKR1, ACKR4 deficient mice and ACKR4-eGFP reporter mice and their appropriate controls will be used in these experiments as required. The PhD student will learn all needed methods including animal experiments under the supervision of the PI and our technician.

**References:**


Project Title: The role of the circadian clock in pulmonary fibrosis

Background:
Pulmonary fibrosis is a chronic, progressive and often fatal lung disease characterized by excessive deposition of fibrotic connective tissue in the lungs. The onset of pulmonary fibrosis is usually initiated by the dysregulation of tissue repair mechanisms which can be induced by various causes, such as air pollution, cigarette smoke, antineoplastic drugs, and respiratory viral infections such as influenza A virus and SARS-CoV-2 infections. However, there is currently no curative treatment; the only approved drugs, nintedanib and pirfenidone, can only slow down the progression of pulmonary fibrosis (1). Thus, investigating new promising molecular pathways involved in fibrogenic responses is urgently needed.

Circadian regulation causes the activity of biological processes to vary over a 24-h cycle. Various studies to date demonstrate the fundamental interactions of circadian clock molecules, such as REV-ERBα or BMAL1, with lung inflammatory responses and the development of chronic lung diseases (2). However, the mechanism and role of the circadian clock in lung fibrogenesis is still poorly understood (3, 4).

Hypothesis and Objectives:
Circadian clock molecules are involved in the development of pulmonary fibrosis and represent a promising therapeutic target to halt the progression of the disease. Therefore, we aim to investigate the relationship between the circadian clock and fibrogenesis and to pharmacologically target the circadian clock in a comprehensive translational approach that includes established in vitro assays, in vivo models and analysis of patient samples.

Approaches and methods:
The recruited PhD candidate will be trained in and perform isolation of different immune cell populations from blood of healthy donors and fibrosis patients. Further, in vitro (co-)culture and functional assays for immune cells, fibrocytes, fibroblasts, and epithelial cells will be employed for this study (1st and 2nd year). The student will use established mouse models (e.g. intranasal LPS, bleomycin) and precision cut lung slices, generated from mouse or human lungs, to validate her/his findings in vivo/ex vivo (2nd and 3rd year). Expression and activation of the circadian clock or disease-specific markers will be monitored in cells and tissue by multi-color flow cytometry, Western blot, qPCR, immunohistochemistry, in situ hybridization, and immunofluorescence microscopy. Changes in glycolytic versus oxidative metabolism of the cells will be determined by Seahorse analysis. To target the circadian clock, siRNA silencing, selective synthetic agonists/antagonists, and
knockout/transgenic mouse strains will be used. Inflammatory mediators will be measured by mass spectrometry or multiplex ELISA.

References:


Isabell Dorn
Department for Blood Group Serology and Transfusion Medicine, Medical University of Graz

Project Titel: Genetic variants of the mechanosensitive ion channel Piezo1 in red blood cells - their role in human physiology and evolution

Background:
Tissue oxygenation by red blood cells (RBC) is one key factor defining fitness and survival under stress conditions. Therefore, genetic variants of proteins controlling RBC number and properties are crucial for natural selection.¹ The mechanosensitive ion channel Piezo1 is such a protein. Gain of function (GOF) mutations affecting the channel pore cause the hemolytic disorder hereditary xerocytosis (HX).² Another GOF mutation, located in the less investigated blade part, protects against severe malaria and is prevalent in 30% of the African populations in malaria-rich areas.³ The Piezo1 gene is one of 87 genes that differ between Neanderthals and modern humans. The Neanderthal variant of Piezo1 has a mutation, which according to simulations, also affects the blade part and is likely to modulate channel function.

Hypothesis and Objectives:
We aim to establish a link between RBC Piezo1 polymorphisms and tissue oxygenation. We hypothesize that present-day Piezo1 variants differ from the Neanderthal variant in their channel properties and alter tissue oxygenation. Therefore, Piezo1 may be subject to selection in ongoing human adaptive evolution. Research at the genome, protein, cellular and systemic levels complemented with mathematical modelling, will elucidate the link between Piezo1 polymorphisms, channel activity and tissue oxygenation in modern humans. It will be the first study of its kind to examine the physiological effects of an archaic versus a modern human protein variant on O₂ supply and tissue oxygenation. The results are expected to shed light on the fundamental mechanisms of human evolution and the role of Piezo1 in RBC physiology and pathologies.

Methodology:
1) PhD student Graz: Genome engineering of human induced pluripotent stem cells to produce iPSCs and finally RBCs expressing archaic Piezo1.⁴, ⁵ Archaic and modern human RBCs will be compared for their cellular properties. 2) Saarland University, Germany: Systemic effects of Piezo1 variants will be investigated in a mouse model with genetically/pharmacologically modified channel activity. The influence of Piezo1 on human tissue oxygenation will be analyzed in a cohort of HX patients in comparison to healthy controls. 3) University Zürich, Switzerland: Obtained results and databases will be used for mathematical modelling to reconstruct the demographic history of Piezo1 polymorphisms and to model potential environmental effects on present-day Piezo1 polymorphisms.
Primary researchers involved:

It is an interdisciplinary project with partners from Switzerland and Germany. A strong scientific exchange with secondments to the project partners is planned. I. Dorn and A. Reinisch (Medical University Graz, Austria) are experts in RBC differentiation from hiPSCs and CRISPR/Cas9 genome editing. L. Kaestner (Saarland University, Germany) is specialized in RBC ion-channel characterization, RBC biophysics and experienced with transgenic animal models. N. Bender and P. Eppenberger (University of Zürich, CH) will provide their knowledge of human evolution and mathematical modelling.

References:

Julia Feichtinger
Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Title: The immunomodulatory effects of the NR4A1/p53 axis in diffuse large B cell lymphoma

Background:
Diffuse large B cell lymphoma (DLBCL) is the predominant lymphoid malignancy in the western world. While immune checkpoint blockade therapy is in clinical practice for a number of cancer types, the response rates for B cell lymphomas remain disappointingly low. Importantly, immune evasive mechanisms are still poorly investigated in B cell lymphomas, which has impeded the progress in developing new therapeutic approaches targeting the lymphoma immune landscape. This in-silico project aims to address this gap by investigating the immunomodulatory effects of the NR4A1/p53 axis, two pivotal transcription factors and tumour suppressors.

Hypothesis and Objectives:
Our preliminary data strongly suggests that NR4A1 and p53 build a functional axis to orchestrate immune evasive mechanisms in DLBCL. We therefore hypothesize that the NR4A1/p53 status results in distinct expression patterns of immunomodulatory factors (such as coinhibitory immune checkpoint receptors/ligands, MHC and cytokines), which in turn influences the anti-tumour response. Furthermore, we anticipate that the NR4A1/p53 status holds significant clinical relevance in human DLBCL. This project is part of a consortium initiative with objectives that include the investigation of mouse models with Nr4a1 and/or p53 knockout and the exploration of treatment options. Additionally, the aim is to translate findings of the Nr4a1/p53 axis in mice into a clinical setting using the GRAZ lymphoma cohort.

Methodology:
The PhD will focus on the in-silico part of the consortium initiative by leveraging bioinformatics methods, data analysis and mining techniques. The analysis of transcriptomics data (qPROseq, (single-cell) RNA-seq) will serve as the foundation for investigating the expression patterns of immunomodulatory factors, anti-tumour response and tumour microenvironment. To explore the clinical relevance of the NR4A1/p53 axis and inter-patient heterogeneity, a comprehensively annotated DLBCL dataset will be processed and analyzed. This dataset includes multiple read-outs such as the NR4A1/p53 status, mutations, expression data of immunomodulatory factors, composition of tumour-infiltrating immune cells, ex vivo response rates and clinical parameters.
References:


Gernot Grabner (PI)
Dagmar Kratky (Co-PI)
Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Molecular Biology and Biochemistry), Medical University of Graz

Project Title: Deciphering the role of lipolysis in brain lipid and energy metabolism

Background:
Triacylglycerols (TAGs) are the major storage form of fatty acids in mammals and are deposited in specialized subcellular organelles called lipid droplets (LDs) [1]. Emerging evidence demonstrates the importance of TAG metabolism in normal brain physiology and LD accumulation in brain cells is a common hallmark in various brain pathologies [2]. TAG biosynthesis is an important mechanism for coping with cellular stress and LD formation is crucial for brain cell survival [3]. The process of TAG degradation, called lipolysis, is catalyzed by the consecutive activities of lipases [1]. Although accumulating evidence suggests an important role of TAG metabolism in brains [4], it remains unclear which lipases are responsible for degrading TAGs within different brain cells. How lipolysis affects downstream metabolic pathways as well as cellular and whole-brain (patho-)physiology remains unknown.

Hypothesis and Objectives:
Our preliminary results indicate an important role of a distinct lipase in TAG catabolism in brains of mice and humans. We hypothesize that TAG hydrolysis mediated by this lipase in neurons critically affects brain lipid metabolism, inflammation, energy substrate utilization and mouse behavior. The overall objective of this project is to understand how defective lipolysis in distinct brain cells affects cellular and whole brain physiology in mice.
In our specific aims we will address consequences of impaired lipolysis in neurons on
Aim 1: Lipid composition of isolated cells and mouse brains
Aim 2: Energy metabolism in isolated cells in vitro
Aim 3: Gene expression and inflammatory response in brains

Approaches and methods:
The Gottfried Schatz Research Center as host institution, provides an excellent environment for research in the fields of biochemistry, molecular and cell biology as well as lipid and energy metabolism. All proposed experiments are based on established methods at the host or collaborating institutes. The PhD candidate will isolate and cultivate primary cells from brains (neurons, astrocytes, microglia) and will perform lipid loading and lipid analysis experiments. This will involve sample preparation and analysis via LC-MS and GC-MS in collaboration with the Core Facility Mass
Spectrometry. Primary cells will be further analyzed via SeaHorse respiratometry using selective lipase inhibitors. The PhD candidate will characterize lipase-deficient mice which involves analysis of gene expression, lipid composition, and histology of brain sections. Thereby, effects of dietary interventions and proinflammatory stimuli will be investigated. The PhD candidate will have the opportunity to acquire a diverse set of methods to expand and enhance his/her scientific skills at the interface of lipid and energy metabolism and neuroscience.

References:
Project Title: Prone Adiposity: Programming of neonatal adipose tissue by maternal gestational diabetes

Background:
Influences in pregnancy form the susceptibility for non-communicable diseases later in life of the offspring (1). This ‘fetal programming’ occurs largely through epigenetic changes, such as altered DNA methylation patterns (2). Offspring exposed to gestational diabetes (GDM) have an increased risk for obesity and metabolic diseases associated with adipose tissue dysfunction, including type 2 diabetes or metabolic syndrome (3). Dysfunctional adipose tissue is characterized by altered paracrine activity, vascularisation, lipid/ganglioside composition and insulin resistance (4). Disturbances in adipocyte differentiation (adipogenesis) are suggested to play a key role in the development of adipose tissue dysfunction (5). Mesenchymal stem cells (MSCs) are the precursors of adipocytes, and the umbilical cord is a source of neonatal MSCs which can be isolated and differentiated to adipocytes in vitro.

Hypothesis and Objectives:
The intrauterine environment in GDM reprograms fetal MSCs. This affects adipogenesis and function of the differentiated adipocytes regarding paracrine activity, lipid composition and insulin resistance.

Approaches and methods:
Umbilical cord MSCs will be isolated after pregnancies with and without GDM. MSCs will be cultured and differentiated to adipocytes. Both cell types will be studied for their epigenetic differences, paracrine and pro-angiogenic activity, lipid and ganglioside composition and response to insulin. Data will be related to clinical characteristics of mothers and neonates.

References:
Philipp Jost
Division of Oncology, Department of Internal Medicine, Medical University of Graz

Project Title: Deciphering the role of necroptosis-induced inflammation in lung adenocarcinomas

Background:
Despite the success of immunotherapy in lung cancer (Herbst et al, Nature 2018), most patients experience relapse within months to years. This acquired resistance to immunotherapy illustrates our failure to comprehend the complex biology of the tumor microenvironment, the immune system, and its interaction with tumor cells. Importantly, the lack of biomarkers that can predict which patient will respond to immunotherapy and who will relapse, remains a critical roadblock for physicians for effective lung cancer treatment. Hence, understanding inflammatory processes in cancer cells and how they impact on the immune environment will critically contribute to our understanding of predictive and prognostic biomarkers in lung adenocarcinoma and beyond. Necroptosis represents a molecularly defined signaling pathway that ultimately results in cancer cell death and may amplify anti-tumor immunity of cancer therapy due to its highly inflammatory nature (reviewed in Linkermann and Green, NEJM, 2014). Hence, we need to understand how tumor cells repress necroptosis, what the effects of failed necroptosis are on tumor cell survival and how necroptosis, or the lack thereof, shapes the tumor-immune environment.

Hypothesis and Objectives:
We hypothesize that lung adenocarcinoma represses cancer cell-intrinsic necroptosis and associated inflammation to prevent immune cell infiltration and thereby secure its sustained tumor cell survival and tumor progression. Following aims will be addressed by this project.
Aim 1: Elucidate the role of necroptosis-associated transcriptional and immune cell profiles in human lung adenocarcinomas.
Aim 2: Dissect the mechanism of alterations of necroptotic genes.
Aim 3: Investigate functional consequences of necrosome signaling using clinically relevant endogenous murine model systems.

Approaches and methods:
To dissect transcriptional and immune cell profiles of both human and murine lung adenocarcinoma, the student will employ computational methods, patient samples and clinically relevant CRISPR-Cas9-based mouse model systems of lung adenocarcinoma harbouring KrasG12D and loxP-flanked Tp53. Our mouse models allow us to functionally dissect the contribution of individual necroptotic genes to lung cancer development, maintenance and tumor-immune interaction. All proposed mouse models have
been approved by the Austrian Federal Ministry of Education, Science, and Research (Vienna, Austria) approved (2021-0.588.538).

References:
Background:
Rare cancers are rare, but patients suffering from rare cancers are common. A large part of global molecular pathology research is focused on big tumor entities like colon, lung and skin tumors. However, a substantial percentage of patients suffer from a variety of rare tumor types of different etiology and biology. Our research group focuses on rare tumor types which present unique challenges in diagnosis and treatment. Currently our work is mainly focused on urogenital cancers, mostly of the cervix, vulva and penis. Human papilloma virus (HPV)-induced invasive cervical squamous cell cancer (SCC) develops via the precursor lesion high-grade squamous intraepithelial lesion (HSIL). We are investigating the events leading to progression of the precursor lesions thin and thick HSIL to microinvasive and fully invasive SCC. Thin HSIL has a prevalence of 22.5% of non-high-risk HPV genotypes compared to less than 3% in thick HSIL and invasive SCC. Genetic mutations of driver genes are not apparent in precursor lesions but seem to play an important role in later stages and we have described a very high prevalence of TP53 and CDKN2a mutations in SCC of the cervix and related penile cancers. More recently we also published reports on the classification and molecular subtyping of intraepithelial neoplasia and the mutational landscapes in penile cancer and squamous cell cancers of the cervix.

Our lab is structured into a core diagnostic laboratory providing molecular pathology-based diagnostics in southern Austria and a research and development lab which strives to transition innovative and novel analysis methodologies from the basic research setting into clinical diagnostics. The prospective PhD student will be embedded in the research and development laboratory and work in close collaboration with our other PhD and diploma students.

Hypothesis and Objectives:
- Genetic analysis can lead to correct diagnosis and elucidation of the etiology in rare cancers.
- Genetic changes are the hallmark of progression from precursor lesion to invasive cancer.
- TP53 and CDKN2a mutations play an important role in carcinogenesis of rare cancers
- Structural DNA damage is contributing to the evolution of rare cancers

Methodology:
We have access to cohorts of penile, vulvar and cervical cancers which have been characterized by histology, immunohistochemistry and focused NGS panel analysis. We want to expand on this genetic analysis using exome sequencing based on a new NovaSeq 6000 instrument recently made available to our lab. Genetic analysis combined with multi-color and multi-cycle immunohistochemistry will
inspire hypotheses on the interplay of genetic mutations of driver and tumor suppressor genes with structural DNA damage and protein expression in these progressive stages of cancer development. We want to further establish a tissue culture-based system to test the oncogenicity of different genetic changes in-vitro and possibly also in-vivo in murine xenotransplantation models. Introduction of genetic mutations will be performed with the CRISPR/Cas system by recapitulating oncogenic mutations known from the genetic screenings, or by modifying the DNA repair system leading to structural DNA changes.

The successful PhD candidate will use cutting edge sequencing technology and bioinformatics to characterize and correlate mutation and gene expression profiles as well as genomic copy number changes in retrospective cohorts of rare cancers. Subsequently we will focus on the generation and characterization of meaningful tissue culture models to study the progression of these cancer types from precursor lesions to invasive cancer.

References:


Michael Khalil  
Department of Neurology, Medical University of Graz

Project Title: Temporal dynamics of blood biomarkers in relation to clinical and radiological disease worsening in multiple sclerosis

Background:  
Multiple sclerosis (MS) is a chronic autoimmune mediated disease that is characterized by episodes of focal inflammation in the brain and spinal cord that affects both the white and grey matter.\(^1\) From a traditional point of few, the disease typically starts with a relapsing-remitting MS (RRMS) phase and after approximately 10 to 20 years, a progressive clinical course develops in many of the persons affected, eventually leading to impaired mobility and cognition.\(^1\) A growing body of evidence however suggests that the clinical course is rather considered as a continuum with contributions from concurrent pathophysiological processes that vary across individuals and over time.\(^2\) The evolution of a progressive course seems to be related to a shift from predominantly localised acute injury to widespread inflammation and neurodegeneration.\(^2\) Early diagnosis and treatment of disease progression in patients with MS (pwMS) is a critical unmet need to provide optimal care.\(^3\) The value of magnetic resonance imaging (MRI) in pwMS for diagnostic, prognostic, and monitoring purposes is well established.\(^4\) International consortia aim to translate novel research findings of MRI into clinical practice to improve the use of MRI in the clinical management of individuals with MS.\(^4\) Apart from MRI, body fluid biomarkers and particularly blood based biomarkers hold the potential to assist in disentangling the complex interplay between neuronal, glial, and immune cells that ultimately cause irreversible disease progression.\(^3\) In the last years, tremendous progress has been made in studying blood biomarkers in particular for neuro-axonal injury (serum neurofilament light chain - sNfL)\(^5\)\(^-\)\(^7\) and astroglial markers (glial fibrillary acidic protein - GFAP)\(^8\) that have been related to disease activity and progression in MS.\(^6\)\(^-\)\(^8\) However, so far only scarce information is available on how temporal changes of these biomarkers are related to clinical and radiological disease worsening in pwMS.

Hypothesis and Objectives:  
Recently it was shown that sNfL elevates in advance of clinical worsening and thus may indicate to a potential time frame of ongoing dynamic central nervous system pathology that precedes the diagnosis of clinical disease worsening in MS.\(^4\) Apart from NfL, some evidence also exists for blood GFAP to reflect, and potentially also predict worsening of disability in pwMS.\(^8\)\(^,\)\(^9\) However, it is still unclear how longitudinal changes of both blood biomarkers (sNfL and sGFAP) are related to clinical and radiological disease worsening in MS.

Methodology:  
The PhD student will focus on investigating sNfL and sGFAP proteins in serial samples of pwMS and relate them to longitudinal clinical and MRI data obtained from a 3T scanner. The student will learn
to use the ultrasensitive single molecule array (Simoa) platform using the HD-X analyzer for quantification of blood biomarkers.

The student will further have the possibility to learn the routine diagnostic cerebrospinal fluid (CSF)/serum work up, including determination of CSF white cell count, total protein, lactate, albumin CSF/serum quotient, calculation of immunoglobulin G, A and M indices and kappa free light chains and the determination of oligoclonal bands by isoelectric focusing followed by immunoblotting, as well as isolation of DNA from whole blood and peripheral blood mononuclear cells.

The student will learn how to handle larger clinical data sets and merge them with biochemical and comprehensive MRI data prior to statistical analyses. All MRI analyses and data post-processing procedures will be performed in collaboration with our Neuroimaging Unit. Statistical analysis will be performed in close collaboration with a biostatistician.

References:
Background:
Cardiovascular diseases (CVDs) are the leading cause of death worldwide [1]. Low-density lipoprotein (LDL) is a key-player in the human cholesterol transport system and is intimately linked to the development of CVDs [2]. LDL is a 22 nm complex nanoassembly, consisting of phospholipids, a large monomeric protein, triglycerides, and its cargo: free- and esterified cholesterol [3, 4]. The endothelial uptake of LDL particles by specific receptors critically depends on LDLs sole protein moiety apolipoprotein B-100 (apo B-100). Structural deviations within LDL and apo B-100 are correlated with an enhanced risk for the development and progression of atherosclerosis [5]. Therefore, it is of high medical relevance to understand the structural, dynamical and molecular features of LDL and apo B-100.

Hypothesis and Objectives:
Given the immense advances in cryo-EM technology, as well as in algorithm development, we hypothesize that it is possible to achieve a well resolved cryo-EM 3D map of apo B-100. Insights into apo B-100’s atomic structure will help to decipher disease-relevant functional protein domains. The identification of new molecular targets might offer new, effective and specific treatment options to regulate LDL levels and improve cardiovascular health.

Specific objectives of this PhD thesis are:
- To isolate, purify and stabilize apo B-100 in a lipid-free detergent-solubilized form from normolipidemic, triglyceride-rich and oxidized LDL.
- To screen for and select environmental conditions where purified apo B-100 is evenly distributed on an EM grid
- To collect a cryo-EM dataset eligible for single-particle analysis and to obtain a high-resolution 3D model of apo B-100
- To complement cryo-EM studies with small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulation studies

Methodology:
The PhD candidate will isolate LDL from human plasma by sequential ultracentrifugation, followed by detergent-solubilization and chromatography to isolate lipid-free detergent-stabilized apo B-100 [6]. To assess the chemical composition and the quality of the sample colorimetric assays, as well as SDS-PAGE, negative-staining electron microscopy, circular-dichroism and infrared spectroscopy are applied on a routinely basis. Cryo-EM data collection and MD simulation studies will be done in close...
collaboration with our partner institutes (ILL, IBS) in Grenoble, France. Cryo-EM single-particle analysis will be done using CryoSparc and Relion. The PhD candidate will gain broad expertise in structural biology and biophysics, will be provided access to state-of-the-art infrastructure, and will be part of a highly multidisciplinary group integrated in an outstanding international network.

References:


Gunther Marsche

Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation (Pharmacology), Medical University of Graz

Project Title: Pioneering Inflammation Resolution with Engineered High-Density Lipoproteins

Background:
Lipoproteins are intricate nanoscale structures that are pivotal in cellular function, lipid metabolism, and disease progression. These complex assemblies, composed primarily of phospholipids and apolipoproteins, form a protective shell encasing a hydrophobic core containing triglycerides and cholesterol esters. Among these, high-density lipoproteins (HDL), primarily composed of apolipoprotein A1 (apoA-I) and phospholipids, are crucial mediators of reverse cholesterol transport. Beyond their lipid transport role, HDL actively interacts with and regulates the functions of immune cells and endothelial cells [1]. This dual functionality of HDL offers a promising avenue for the development of novel therapeutic strategies. In the quest for alternatives to the use of full-length apoA-I as the basis for HDL, short synthetic peptides - termed apoA-I mimetics - have emerged as potential candidates [2]. These apoA-I mimetics share similar amphipathic α-helical structures with apoA-I, suggesting their potential to replicate the beneficial effects of HDL.

Hypothesis and Objectives:
ApoA-I mimetics are molecules designed to mimic the beneficial effects of apolipoprotein A-I, the main protein found in HDL. ApoA-I mimetics are being studied as potential treatments for a variety of diseases, including atherosclerosis, lung disease, inflammatory bowel disease, sepsis, and cancer. One of the main goals of this research project is to understand how the structure and size of synthetic HDL (containing apoA-I mimetics) affect their biological activity. The anti-inflammatory capacity of synthetic HDL on activated blood cells, such as monocytes, neutrophils, and platelets, will be investigated, and their ability to neutralize endotoxin and modulate endothelial function. The effectiveness of apoA-I mimetics in animal models of chemotaxis and lung inflammation will be studied. This will enable us to design and engineer synthetic HDLs with optimized biological properties for therapeutic purposes.

Methodology:
The student will learn techniques such as preparing synthetic HDL using apoA-I mimetic peptides and phospholipids, isolating HDL from serum, performing native gel electrophoresis, and performing Western blotting. In addition, practical skills will be provided to isolate leukocytes and platelets from peripheral blood, perform flow cytometry and multiplex ELISA, and study the functional responses of...
neutrophils and endothelial cells. This includes studying shape change, integrin upregulation, chemotaxis, Ca2+ signaling, and in vivo chemotaxis and lung inflammation.

References:
Gerit Moser  
Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Title: LAMB3-DAG1 axis in early placental development

Background:  
Uterine glands fulfil major functions during implantation and placentation in early pregnancy by supporting histiotrophic prior to hemotrophic nutrition of the embryo. They provide glucose, lipids, glycoproteins and growth factors that stimulate rapid proliferation and differentiation of the villous trophoblast and thereby the developing placenta. The connection between maternal and fetal compartment is accomplished by invasion of fetal trophoblast cells (EVTs) invading the maternal endometrium, they also invade and thereby connect the uterine glands with the placenta and thereby guarantee the constant supply of the developing embryo with glandular secretions (so-called endoglandular trophoblasts). Only recently, tissue-clearing-based 3D imaging has unravelled the fundamental morphology of the human endometrial glands. Other than hitherto assumed, endometrial glands are not only tubular; about two thirds of the endometrial glands are branched and form a plexus network in the basal layer and expand horizontally along the myometrium Thus, many more glands and their secretions are in constant contact with the developing placenta than previously thought, which further strengthens their putative fundamental role in implantation and early pregnancy.

Hypothesis and Objectives:  
It is likely, that the role of the uterine glands in general and their invasion by EVTs in particular for successful implantation and subsequent maintenance of a healthy pregnancy has been underestimated so far. Factors that may dictate this pattern of invasion and thus may influence reproductive success are currently unknown.  
Preliminary bioinformatics analysis enabled identification of a first promising receptor ligand pair between uterine epithelial cells and EVTs, namely Laminin-Subunit Beta 3 (LAMB3) and Dystroglycan (DAG1). The localisation of LAMB3-DAG1 within first trimester decidua tissue reflects a potential role for endoglandular trophoblast invasion. This project aims to analyse the role of LAMB3-DAG1 in endoglandular trophoblast invasion and evaluate its contribution to fertility complications such as recurrent missed abortion (RM) and implantation failure (RIF).

Methodology:  
- Isolate glandular epithelial cells from placental tissues and endometrial biopsies  
- Optimize and apply novel in vitro model systems for endoglandular trophoblast invasion and for long-term culture of glandular epithelial cells, including a model for in vitro harvest of glandular secretions (organoid- and monolayer culture) for functional assessment of LAMB3-DAG1 axis
- Evaluate progress of cell growth and effects of treatments by standard techniques in our laboratory (tissue processing for histology, immunostaining, Western Blot, qPCR, etc)

- Evaluate LAMB3-DAG1 expression levels in larger cohorts (first trimester placentas, endometrial biopsies from RM/RIF patients) and in specific cell culture experiments with various methods such as software-assisted analysis of immunostainings, padlock probe technique, qPCR and Western Blot, invasion assays

References:


Gerit Moser
Gottfried Schatz Research Center for Cell Signaling, Metabolism & Aging (Cell Biology, Histology and Embryology), Medical University of Graz

Project Title: Modeling reproductive failure in humans

Background:
In a successful pregnancy, the blastocyst attaches to and implants into the endometrium - the lining of the uterus. Altered receptivity and function of the endometrium may lead to fertility issues. Such issues are more common than one might think, more than 10% of all couples worldwide are affected at least once in their lifetime. In particular, the endometrial epithelial glands and their secretions are important for fertility. These glands fulfil major functions in reproduction by supporting nutrition of the early conceptus.

Investigating human reproduction is a particular challenge. Apart from ethical concerns, processes such as implantation and subsequent placentation differ tremendously between animals and humans. Endometrial organoids offer an elegant in vitro method for investigation, besides avoiding the use of animal models. These organoids expand long-term, are genetically stable and differentiate depending on the respective treatment with reproductive hormones. By modeling the endometrium in physiological and pathological condition, organoids can be used to identify biomarkers, evaluate treatment options and predict response.

Hypothesis and Objectives:
We hypothesize that (1) common treatment options for reproductive failure alter genotype and secretome of uterine glandular epithelium and (2) these alterations of the glandular epithelium in turn influence reproductive success.
Aim of this is to establish patient individual endometrial organoids from women with fertility issues, evaluate common clinical treatments options, predict response on an individual level, and thereby move a step towards personalized treatment options in future.

Methodology:
- Isolate glandular epithelial cells from placental tissues and endometrial biopsies
- Optimize respective long-term cultures (organoids and monolayer) and treatment
- Evaluate progress of cell growth and effects of treatments by standard techniques in our laboratory (tissue processing for histology, immunostaining, Western Blot, qPCR, etc)
- Isolate and prepare samples for tissue dissociation for single cell RNAseq for genotyping of glandular epithelium (bioinformatics analysis will be performed by Dr. Julia Feichtinger).
- Evaluate identified targets in larger cohorts (endometrial biopsies from patients with fertility issues) and in specific cell culture experiments with various methods such as software-assisted analysis of immunostainings, padlock probe technique, qPCR and Western Blot, invasion assays.

References:


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Project Title: Novel approaches for β-cell protection - On the path to cure type 1 diabetes

Background:
Globally, type 1 diabetes mellitus (T1DM) incidence is increasing by about 2-3% per year with about 8.4 million cases worldwide in 2021 (1). T1DM is characterized by a complete loss of endogenous insulin production, leading to the need of life-long insulin substitution. However, treatment with exogenous insulin does not stop disease progression, causing significant health complications that affect quality of life and reduce life expectancy (2). So far, disease-modifying therapeutic approaches have focused on single immune-modulating compounds to protect β-cell function with varying long-term effects (3). Recently, non-immunomodulators that directly protect and restore β-cell health and stimulate insulin secretion, have come into focus as a potential novel therapeutic concept for curing T1DM (4).

Hypothesis and Objectives:
We hypothesize that direct protection and/or restoration of β-cell health will preserve insulin secretory ability and suppress immune cell-mediated destruction of β-cells, ultimately leading to novel treatments of T1DM in humans. For this PhD project, we will utilize established β-cell lines to investigate potential β-cell protective compounds that directly modulate cellular processes within β-cells to improve insulin secretion and metabolic health including ER stress and inflammation.

Methodology:
To investigate the therapeutic approach of protective compounds on β-cells (and thus potential novel anti-diabetic drugs), the PhD candidate will use state of the art molecular-biological techniques including generation of β-cells expressing intracellular biosensors, 2D- and 3D-cell culture as well as primary cell culture, flow cytometry, immunohistochemistry, and a next generation drug screening platform. The student will cooperate with different research groups and institutions.

References:
Project Title: Metabolic reprogramming in glioblastoma in response to drug therapy

Background:
Glioblastoma is a highly aggressive and rapidly growing brain tumor with limited effective treatment options. Treating glioblastoma is complex because the tumor has the capacity to reprogram its metabolism to increase energy production and support rapid growth and proliferation\(^1,2\). Hence, current efforts to treat glioblastoma focus on understanding the intricate metabolic reprogramming that takes place in the tumor. In this project, the PhD candidate will investigate mechanisms of efficacy and resistance to treatment with a special focus on metabolic reprogramming in tumor tissue and biomarkers in tissue and plasma. The results will add mechanistical insights to the findings of a currently on-going clinical trial.

Hypothesis and Objectives:
We hypothesize that drug treatment modifies the tumor metabolome rendering it sensitive or resistant to therapy. We aim to identify biomarkers and metabolic vulnerabilities that can be further exploited in therapy and industry.

Methodology:
Patient derived cells (PDCs) will be isolated from brain tumor biopsies of patients with glioblastoma. The efficacy of a panel of drugs will be tested in a drug screening platform\(^3,4\). Intracellular tumor metabolism will be examined in biopsies, PDCs and cell lines using mass spectrometry (Q-exactive and LCMS-8060). Cell organelles will be isolated by differential centrifugation to investigate the subcellular localization of top identified metabolites\(^5\). Tumor tissue and plasma biomarkers will be identified using mass spectrometry and standard techniques such as RT-qPCR, western blot and immunohistochemistry. The candidate should be interested in cell biology and metabolomics. Previous experience in mass spectrometry and bioinformatics is not required but is a plus.
References:
Project Title: An epigenetic-metabolic crosstalk reveals vulnerabilities of acquired drug resistance in glioblastoma

Background:
The main cause for disease relapse in patients with metastatic cancer is acquired drug resistance after an initially response to therapeutic intervention. While nearly half of disease recurrence occurs by acquiring additional genetic alterations, evidence suggests an epigenetically- and metabolic- regulated and reversible drug-tolerance state prior to permanent resistance. The impact of reversible cellular reprogramming has been shown in many solid cancers including melanoma or ovarian cancer, the molecular mechanisms, however, facilitating the shift of a dormant to a proliferative state remain underinvestigated. We recently identified O-linked N-acetylglucosamine transferase (OGT) driving the transition of cancer cells from dormancy to proliferation. Current treatment options for glioblastoma have limited effectiveness, with a median survival of 18 months. Standard therapies include surgical resection, radiation therapy, and chemotherapy with temozolomide. Epigenetic and metabolic changes play a crucial role in regulating metabolic behavior of glioblastoma cells and both promote or inhibit activity of metabolic pathways. This project aims to determine epigenetic and metabolic features in dormant and proliferative drug tolerant patient-derived glioblastoma cells.

Hypothesis and Objectives:
Drivers of a drug tolerant and proliferative persister state remain to be underinvestigated in glioblastoma. Determining epigenetic and molecular markers as vulnerabilities of drug resistance in glioblastoma will identify novel markers and potential druggable targets. Investigations will allow to test intermittent treatment schedules for a re-challenge response of patients. In-vitro testing of immortalized patient-derived glioblastoma cell lines for identification of top transcription factor...
binding motifs based on histone modifications (e.g. H3K4me3) and subsequent OGT involvement, combined with metabolome analysis of cell lines and their secretory products in the media supernatant to determine potential targets of key regulator sites. In vivo xenograft model\cite{9} to determine treatment efficacy of known treatment drugs with and without intermittent drug treatment with a constant monitoring using cerebral open flow microperfusion (cOFM) ISF sampling directly from inside the tumor to monitoring tumor and in-vivo drug resistance development. Immunohistochemistry of identified targets and markers in glioblastoma tissue.

**Methodology:**
- Induction of drug tolerant cells by continuous treatment of respective cell lines with indicated drug dosage followed by colony isolation.
- Metabolic analyses of cells and supernatant\cite{10} at set time-points,
- ATAC sequencing, ChIP sequencing or CUT&RUN and ChIP-qPCR analysis,
- Immunofluorescence and live cell imaging of organoids
- Xenograft mouse model with implanted cOFM probe to ascertain metabolic changes in the tumor during growth and potential in-vivo changes during resistance development.
- Immunohistochemistry in glioblastoma tissue.

**References:**
Beate Rinner  
Division of Biomedical Research, Core Facility Alternative Biomodels and Preclinical Imaging, Medical University of Graz

**Project Title:** Replace Mice Xenografts by Microfluidic Cancerogenesis Chips - ReplaceMICE

**Background:**  
With personalized cancer treatment the detection of malignantly transformed cells by tumorigenicity testing is essential. For testing the tumorigenicity of patient derived tumor cells the use of in vivo animal models - especially xenograft mouse models - is currently standard. However, animal models suffer of several drawbacks: (i) the interspecies physiological and genetic differences, (ii) the incomparability or lack of an active immune system, (iii) the ethical concerns and also (iv) the high cost. This project promotes an alternative to animal testing that overcomes these restrictions. To replace animal testing we promote a human *in vitro* model on a bioelectronic sensor chip. Using only animal-free materials this human model will help to overcome all the previous cited problems. With a immune-competent, vascularized human tissue model on a microfluidic chip we will realize animal-free tumorigenicity testing.

**Approach:**  
Using interconnected microchambers we will reproduce the sequence of tissues of the skin and will generate a human epidermis/dermis-equivalent on a chip. The four adjoining microfluidic chambers are a medium compartment for nutrient supply and drug supplementation, an epidermis compartment with a coculture of human keratinocytes and melanocytes, a dermis compartment with human fibroblasts and immune cells (CD4, CD8-T-cells, macrophages, monocytes), an adjoining vascular compartment with the walls lined with microvascular endothelial cells. The chambers are connected via fenestrated, micropermeable barrier sidewalls that enable the exchange of substances or signaling molecules. With this in vitro model on a chip, we will investigate the tumorigenic capacity of different tumor celllines. Starting with a healthy human model we will introduce human tumor cells via the medium chamber or the vascular chamber. As example, human melanoma cells will be seeded on the dermis compartment. Using microelectrode arrays on the chip we will continuously monitor the tumor promotion in real time by non-destructive bioimpedance sensing. For the first time the animal-free replacement of the mouse model for human tumorigenicity testing is realized. This tumorigenicity-chip features 100% human cells and features three innovations: (i) a well-defined dermal-vascular barrier (ii) the inclusion of human immune cells and (iii) the real-time bioimpedance measurement of tumor progression. With 3 defined tissue layers this animal-free model closely resembles the in vivo situation. The continuous bioelectronic monitoring of tumor progression is advantageous to achieve a personalized medicine without animal testing.
Primary researchers involved:
This proposal joins the expertise of a multidisciplinary team made of a Biomodel-specialist (B. Rinner, Meduni Graz), an immunologist (D. Wilflingseder, Meduni Innsbruck), a cell-chip specialists (P. Ertl, M. Frauenlob, TU Wien), a biomedical & microelectronics engineer (H. Wanzenboeck, TU Wien) and a cancer-on chip biologist (S. Prado-Lopez, TU Wien).

References:
Project Title: Bioactive Lipids: Key Players in Shaping Placental Function in Pregnancies with Adverse Metabolic Profiles

Background:
Pregnancy requires remarkable adaptations of the maternal metabolic system to ensure the availability of nutrients for proper fetal development. These include changes in the maternal lipid metabolism to meet the changing energy demands, but a wide range of lipids also act as signaling molecules, so-called bioactive lipids (e.g., sphingolipids and ceramides). Several pregnancy complications, such as preeclampsia, gestational diabetes mellitus, or obesity, are associated with an adverse metabolic profile and abnormal changes in the maternal lipid profile, or dyslipidemia (1). Importantly, such pregnancy complications are linked to adverse fetal outcomes, such as increased risk of cardiovascular disease (2).

The placenta acts as a mediator between the maternal and fetal metabolism, and bioactive lipids also play an important role in supporting the function of the placenta itself – a process tightly regulated by specific enzymes. In particular, the balance between sphingosine-1-phosphate (S1P) and ceramides is an important regulator of essential pathways in most placental cells, including the placental vasculature (3, 4). However, placental sphingolipid signaling and its relation to the maternal metabolic profile in normal and complicated pregnancies still remains poorly understood.

Hypothesis and Objectives:
Previous and preliminary data from our group show that in preeclampsia, the placental sphingolipid profile, the expression of relevant sphingolipid receptors, and the activity of key regulating enzymes are altered (5). We hypothesise that such alterations are linked to an adverse maternal metabolic state beyond preeclampsia and adversely affect placental function and vascular reactivity and function. We aim to identify relevant maternal metabolic markers, relate them to impaired placental sphingolipid signatures, and identify the key enzymes and regulatory pathways involved. We will also investigate the functional impact of these findings on placental vascular function, with a particular focus on S1P, its de novo synthesis, and the potential involvement of disturbed “inside-out” signaling by S1P within the placental endothelium (6). Overall, this study will help to identify women at risk of placental sphingolipid dysfunction, who may particularly benefit from interventions to improve placental (vascular) function and thus long-term outcomes for their offspring.
Methodology:
A study cohort consisting of samples of term placental tissue and maternal plasma from normal and metabolically compromised pregnancies will be used and extended over the course of the project. Targeted lipidomics and profiling by NMR-based metabolomics will complement existing lipidomics datasets to identify relevant aberrations in maternal plasma and placental tissue. Placental enzyme activity of the sphingosine and ceramide de novo and catabolic pathways will be investigated using activity-based protein profiling. This will be complemented by using specific enzyme inhibitors in vitro, e.g., in placental explants, and measuring their secretome to dissect the relevant pathways in different metabolic settings. The effect on the placental vasculature will be studied by myography of the placental chorionic arteries. These experiments will be informed by the metabolomics part of the study. In addition to comparing overall vascular reactivity, this will also enable us to investigate the impact of the (patho)physiological placental environment on vascular function and signaling. These experiments will be complemented by cell-based assays using primary placental endothelial cells under flow and in endothelial barrier integrity assays (electric cell-substrate impedance sensing). This will allow us to investigate the functional consequences of changes within the sphingolipid profile, with a special focus on the role of autocrine/paracrine S1P signaling.

References: