Life Science PhD Meeting 2024

Innsbruck, April 2024

Abstract Book

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This meeting is organized by:

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Abstract book designed by Lydia Riepler and Andreas Aufschnaiter

Mission Statement

The Life Science PhD Meeting provides a platform for the whole Life Science community, from undergraduate students up to PIs, to share their knowledge, experience and critical thinking. Furthermore we want to encourage all students to present their research to train this important skill for international conferences.

We are proud to present excellent scientific work from numerous fields, which is only possible due to the huge variety of scientific interests of the groups represented in the meeting. Therefore the organizing committee would like to take the opportunity to thank the research programs making it possible to organize this meeting for all the Life Scientists in Innsbruck:

- MCBD
- CBD
- Clinical PhD program
- SPIN
- ARDRE
- CavX
- HOROS
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Angios is a biotechnology company, specializing in vascular research to improve patients' lives all over the world. It was established in 2021 as a spin off from the laboratory of Professor Josef Penninger at the Institute of Molecular Biotechnology (IMBA) in Vienna and utilizes a unique Blood Vessel Organoid (BVO) model to accurately recapitulate human vasculature. The patented BVO technology allows for the generation of interconnected blood vessels from human induced pluripotent stem cells. The protocol originally developed at IMBA and further optimized at Angios is currently being used to establish the BVO model as a unique cell-based therapy for treatment of chronic wounds and drug screening. Together with the long-standing collaboration partner Abcellera Biologics, Angios has developed novel monoclonal antibodies that target vascular complications found in diabetic and cancer patients. Angios is backed up by Land Tyrol through the Health Hub Tyrol and by private investors and has also successfully secured funding from the HORIZON European grant support. Our team of international scientists is passionate about research into innovative approaches to find cures for vascular diseases and improve patient's lives.

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Selected short talks

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Ferroptotic susceptibility is determined by the molecular composition of mitochondrial membrane lipids

In ferroptosis, an iron and lipid peroxidation dependent type of cell death, mitochondria play a multifaceted role beyond their established function as a source of pro-ferroptotic reactive oxygen species (ROS). While it is widely recognized, that mitochondrial membrane potential, mitochondrial fission and fusion, and mitophagy contribute to ferroptosis alongside ROS production, our investigations have unveiled unexpected complexities. In the context of metabolic disorders that disrupt mitochondrial membrane lipids - particularly cardiolipins, the signature lipids of mitochondria - we observed an unexpected behaviour: affected cells were protected against ferroptosis, even under conditions of elevated mitochondrial ROS and a fissioned morphological state. In search for a mechanistic explanation, we proved the ferroptotic phenotype to be readily modulated by iron-availability and the lipid environment, without these factors explaining the actual protective mechanism. The function of the mitochondrial electron transfer chain and central carbon metabolism activity were also found to be influencing but not causative factors. Instead, our results revealed that the observed defect in ferroptosis execution can be linked to the pathologically altered cardiolipin composition, which prevents an efficient oligomerisation of mitochondrial membrane localized transporter systems in response to oxidative stress. This novel insight into the interplay between mitochondria signalling and ferroptosis execution expands our understanding of how lipid-metabolic enzymes can intricately modulate cell death pathways across cellular compartments.

Y. Wohlfarter 1; J. Hagenbuchner 2; A. Winter 1; J. Koch 1; G. Ömer 1; M. Seifert 3; V. Juric 1; J. Schwärzler 4; H. Talasz 5; U. Horzum 6; M. Hess 7; H. Farhan 6; G. Weiss 3; T. Adolph 4; J. Zschocke 1; M. A. Keller 1

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Discovering the resistance mechanisms in pancreatic neuroendocrine tumors to Everolimus treatment

The kinase network converging on mTOR (mechanistic target of rapamycin) is at the center of metabolic control in eukaryotic cells and organisms. Embedded in a multiply intertwined, highly dynamic network, mTOR governs the cellular response to nutrients, growth factors and stress and promotes cellular growth and survival. mTOR dysregulation is linked to many diseases related to metabolism and ageing, including cancer. Everolimus, an inhibitor of mTOR complex 1, is currently used in the clinics to treat pancreatic neuroendocrine tumor (panNET) patients. Even though most patients benefit from the treatment, a significant number of patients still develop disease progression. Towards disease mechanism-driven personalized therapies, we develop systems approaches to predict metabolic control by kinase networks. Through detailed modelling, experimental validation, and genome scale analyses of patient data we unraveled mTOR crosstalk with ancillary signaling networks and identified a new resistance mechanism to drug therapies targeting mTOR in distinct cancer entities with high PI3K/mTOR activity. With MS measurements we can monitor the metabolic changes in patient serum that are indicative for Everolimus resistance. We propose and prioritize combinatorial drug therapies to improve therapy response to mTOR inhibitors in panNETs.

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The effects of amino acids as biocompatible ligands on the stability and cytotoxic activity of platinum-based anticancer agents

The behavior of platinum coordination complexes in aqueous solutions and their interaction with macromolecules are crucial aspects in their development as potential anticancer agents. The solubility and stability of the complex in aqueous solutions determine the bioavailability of the compound. Additionally, sulfur-containing molecules have a high affinity for platinum, resulting in the sequestration of platinum-based anticancer drugs. The aim of our study was to enhance the stability of Zeise-like complexes by using amino acids as biocompatible chelating ligands. We synthesized four derivatives of Zeise's salt and characterized their structure. The stability in aqueous solution was evaluated using capillary electrophoresis experiments with a newly developed MEKC protocol. The interaction with sulfur-donor molecules was studied through NMR spectroscopy and MS experiments, and mechanisms were proposed for each complex studied. Finally, the complexes were tested in vitro for cytotoxic activity against different cancer cell lines, using cisplatin as a reference substance. The data suggests that the use of amino acids as chelating ligands improves the stability of platinum complexes against nucleophilic substitutions in general. However, the extent of the stabilization effect greatly depends on the inner coordination sphere. Furthermore, the concomitant functionalization with an amino acid and a modified acetylsalicylic ligand results in a noteworthy enhancement of cytotoxic activity. This improvement is comparable to that of the reference substance, cisplatin, in all tested cell lines. However, based on preliminary investigations, the mode of action of these complexes is likely different from that of cisplatin, potentially resulting in fewer and less severe side effects.

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Irradiation reactivates human endogenous retroviruses promoting development of calcific aortic valve disease

Calcific Aortic Valve Disease (CAVD) is increasingly prevalent in industrialized countries, paralleling a rise in life expectancy. Notably, patients subjected to thoracic irradiation therapy, such as for cancer treatment, exhibit a heightened risk of developing CAVD at a younger age. This study explores the underlying mechanisms of CAVD using a human aortic valve cell culture model subjected to ionizing irradiation (IR). Our findings demonstrate that IR triggers a cascade of cellular changes, including the upregulation of inflammatory processes, cellular senescence, and subsequent calcification. A critical discovery is the reactivation of human endogenous retroviruses (HERVs), which are typically dormant in non-irradiated, healthy cells. We establish that this re-expression is sufficient to initiate calcification even in unirradiated cells. Furthermore, silencing HERV elements in irradiated cells markedly reduces pro-inflammatory and senescent markers, underlining their pivotal role in CAVD pathology. Another significant finding is the IR-induced upregulation of double-stranded RNA species, known ligands for the Toll-Like Receptor 3 (TLR3). Disruption of TLR3 signaling in knockout models attenuates the inflammatory and senescent responses, alongside a decrease in calcification post-irradiation. These results suggest a central role for TLR3 in the pathogenesis of CAVD following irradiation, presenting a novel avenue for therapeutic intervention. Our study not only elucidates the molecular pathways involved in radiation-induced CAVD but also highlights potential targets for future therapeutic strategies.

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Elucidating the role of HSD17B13 in the progression of fatty liver disease

The hallmark of fatty liver disease is the accumulation of triglycerides in hepatocytes, also termed steatosis. This condition affects around 25% of the general population. Progression of fatty liver disease (FLD) leads to inflammatory processes, as well as scaring of the hepatic tissue (fibrosis), which can result in a dysfunctional liver and the need for transplantation. Currently, there is no distinct medical treatment available. Thus, elucidating the mechanisms behind the progression of the disease is of great medical relevance. Amongst others, the enzyme HSD17B13 has been determined as risk factor for the progression of FLD. This is of interest, since a distinct polymorphism in this gene has been described as a protective variant, rendering it a promising target for medical treatment. HSD17B13 is a hepatocyte specific hydroxysteroid dehydrogenase, which localizes to lipid droplets and is upregulated in patients with progressed FLD. Since the exact function of this enzyme in the fatty acid metabolism of hepatocyte´s is unresolved, we aim to shed light on its exact physiological role. This involves the contribution to the pathology of FLD, with a special focus on the protective variant. So far, we confirmed the ability of all tested HSD17B13 variants to localize to lipid droplets in-vitro, but also attenuated expression levels of a splicing and the protective variant, which potentially suggests protein instability. Additionally, we currently determining a possible link of HSD17B13 and the activation of hepatic stellate cells, which are the main driver of fibrosis but lack the expression of HSD17B13.

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Combining in-silico workflows to predict resistance mutations in kinases with different modes of action

Drug resistant mutations such as found in the pharmacological targets of kinase inhibitors severely limit the efficacy of these targeted cancer therapies and therefore the discovery of next generation compounds is required. These mutations are usually only identified after they occur in patients and therefore no effective treatment alternatives are immediately available. We aim to identify these resistance mutations already before they emerge in patients by combining two in-silico workflows based on two different resistance mechanisms. The first workflow investigates resistance mutations in the binding site of the inhibitors, which confer drug resistance by directly disrupting interactions with the inhibitors. To analyze mutations with an allosteric mode of action outside of the binding pocket, we evaluated the impact of mutations on the stability of conformational states to identify those, that stabilize active states relative to inactive protein conformations. Additionally, for both workflows mutational signatures were included considering the probability of certain amino acid mutations to be generated in various cancer types. We theoretically validated the results with mutation data from patient samples and were able to demonstrate an improved performance with the workflow combination compared to the individual workflows. The early identification of resistance mutations to approved cancer drugs opens new opportunities for the early discovery of drugs against these predicted mutations.

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TfR-1 as a potential therapeutic target in severe SARS-CoV2 infection by mitigating macrophage activation syndrome

The COVID-19 pandemic has raised questions about the role of iron metabolism in severe cases, linking it to hyperferritinemic syndrome and severe conditions like macrophage activation syndrome and septic shock. Thus, in this study we aimed to investigate changes in macrophage iron metabolism in a human primary system, avoiding animal-derived components where possible. We analyzed key regulators of iron metabolism - TfR, FPN1, Hepcidin, Ferritin, IL-6, IL-1b and IL-10. We examined gene expression, virus neutralization, protein expression, and localization. For this, M1 macrophages from healthy donors were exposed to SARS-CoV-2 variants of concern (VoC). After 4–24 hours, we performed neutralization assays and analyzed cytokines and iron-regulating proteins. Virus copy numbers were determined and virus localization, TfR and FPN-1 levels and were illustrated by confocal microscopy. SARS-CoV-2 VoCs exhibited different cytokine and iron-regulating protein profiles. While Omicron sub-variants BA.5 and XBB1.5 showed low levels of inflammatory signal and Iron regulation, the VoC Delta induced both significantly higher and thus exhibited an altered iron regulation. Within a primary, human macrophage model, we demonstrated that SARS-CoV-2 variants influence pro- and anti-inflammatory signals, crucial for cellular iron balance. Blocking the transferrin receptor (TfR) reverses this effect, offering therapeutic potential.

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Centrosome independent function of the PIDDosome in the development of NAFLD

The high prevalence of obesity in our society poses a significant challenge to our health system, as it contributes to the development of various diseases, including the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). Intriguingly, numerous studies have implicated caspase-2, a member of the caspase family, to be involved in the development of NAFLD. Caspase-2 has been found to cleave site 1 protease (S1P), subsequently activating sterol response element binding proteins (SREBPs) 1 and 2. Once activated, these transcription factors stimulate cholesterol and lipid synthesis by promoting the transcription of several important enzymes of the biosynthesis pathway. Despite multiple studies suggesting a link between ER stress and caspase-2 activation, none have provided a definitive mechanistic explanation. Therefore, we used a mouse model of fatty liver disease induced by a high-fat diet to investigate the potential involvement of the PIDDosome and supernumerary centrosomes as activators of caspase-2 in the development of NAFLD. We saw a clear protection of PIDDosome deficient mice from NAFLD under high fat diet, which was independent of extra centrosomes. This data suggests that metabolic induced ER stress cannot activate caspase-2 directly but rather induces activation of the PIDDosome and thereby caspase-2.

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Impact of glutamate metabolism and its inhibition on melanoma development and myeloid cells

Tumor immunity is negatively regulated by metabolites in the tumor tissue. Metabolic reprogramming impacts the activation and maturation of dendritic cells (DC). We work on the transgenic melanoma mouse model tg(Grm1)EPv, spontaneously developing melanoma due to an overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism drives tumor formation, but might also affect immune cell function. We study the metabolic changes in progressing tg(Grm1)EPv melanoma and possible effects on DC and T cell responses. Metabolic screening of the tg(Grm1)EPv mice at different disease stages with LC-MS technology revealed a shift towards glutamate and glutamine and a decrease in ATP. The lactate to pyruvate ratio increased during tumor progression. These changes might indicate a disruption of the respiratory chain and metabolic changes in the tumor microenvironment that are advantageous for the tumor cells and unfavorable for DC. We performed analyses of myeloid subsets in tumors and draining lymph nodes during tumor progression with multicolor flow cytometry. While cDC2 and macrophages decrease, neutrophils and monocytes increase in the tumor. An investigation of DC precursors in the bone marrow and in vitro DC differentiation assays showed no difference between tumor-bearing tg(Grm1)EPv and C57BL/6 mice. Current investigations focus on alterations caused by glutamate pathway inhibition in vitro and in vivo. The acquired knowledge can benefit the design of novel therapeutic strategies for cancer patients involving potential modification of tumor glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might improve response rates in cancer patients.

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The role of complement in dendritic cell activation during SARS-CoV-2 infection

Background: Early and effective SARS-CoV-2 sensing by the innate immune system is crucial for host defence. Dendritic cells (DCs) play a pivotal role in initiating antiviral immunity and together with the complement system are essential immune components in the fight against SARS-CoV-2. Methods: In this study, we investigated complement opsonization of SARS-CoV-2 wild type (WT) as well as other variants of concern and the interaction with DCs. We assessed the viral loads concerning DC binding and internalization as well as their mechanism via confocal microscopy. Furthermore, we investigated differences in DC maturation following stimulation with complement-opsonized SARS-CoV-2 and evaluated cytokine secretion to gain more insights into potential mechanisms of dysregulation. Results: Notably, we observed C3 deposition when SARS-CoV-2 was opsonized with normal human sera, in contrast to other body fluids such as human saliva or mucus. By comparing non-opsonized SARS-CoV-2 with different opsonisation patterns, complement (C), complement-immunoglobulin (CIg) and immunoglobulin (Ig), we observed a significantly higher DC binding and internalization, when the virus was complement-opsonized. By immunofluorescence, we corroborated the enhanced uptake/internalization of complement-opsonized SARS-CoV-2, by co-localization of C3 fragments and complement receptor 4 (CR4) with viral particles. Moreover, the complement-opsonized WT isolate induced activation in DCs, while this effect was not observed for the other variants of concern (VOCs) tested (Delta, Omicron variants). Conclusion: Taken all results together, complement opsonization of SARS-CoV-2 clearly influences the uptake and processing of the virus by DCs, resulting in a better antigen presentation, which is crucial for the stimulation of the adaptive immunity.

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Induction of local human papilloma virus-specific T cell responses upon intravaginal immunization

Human papilloma virus (HPV) infections are a major health burden worldwide. Infections with high-risk HPVs can induce various oral and anogenital cancers, being responsible for >95% of cervix carcinomas. Currently approved vaccines act in a preventive, but not in a therapeutic manner. We aim to elicit a local immune response in the mucosa of the mouse genital tract, as we hypothesize that this could be advantageous for the success of a therapeutic HPV vaccine targeting persistent HPV infections prior to malignant conversion.

Using the viral vector VSV-GP encoding HPV16 E2, E6 and E7, we demonstrated robust HPV-specific T cell responses upon systemic immunization in blood and regression of HPV16-expressing subcutaneous TC-1 tumors in a mouse model. We compared systemic and intravaginal immunization routes hypothesizing that local vaccination could be beneficial for induction of T cell responses in the vaginal mucosa, the primary HPV entry site. Both immunization routes induced HPV-specific T cell responses in the female mouse genital tract as well as in the spleen. Notably, local application induced HPV-specific tissue resident memory T cells in the genital tract, which are associated with the efficacy of therapeutic HPV vaccines. However, we also observed vector-specific T cell responses, potentially limiting the efficacy of homologous boosting regimens. To address this, we are currently analyzing heterologous prime/boost combinations.

Taken together, VSV-GP is a promising candidate as therapeutic HPV vaccine combining a favorable safety profile with a robust induction of HPV-specific T cell responses at an entry side of HPV.

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NORMOTHERMIC LIVER MACHINE PERFUSION OF EXPLANTED LIVERS WITH HEPATOCELLULAR CARCINOMA: A NOVEL PLATFORM FOR ONCOLOGIC ASSESSMENT AND TREATMENT

Liver cancer, particularly hepatocellular carcinoma (HCC), ranks as the fourth leading cause of cancer-related deaths. Targeted therapies, like sorafenib, and immunotherapy, such as atezolizumab in combination with bevacizumab, have shown promise in treating advanced HCC. However, their widespread use is hindered by the absence of reliable biomarkers for patient selection. To address these limitations, our project aims to establish an ex-vivo HCC cancer model using normothermic machine perfusion on HCC carrying hepatectomy specimens obtained from liver transplantation. To comprehensively evaluate the tumor, its microenvironment as well as the adjacent liver tissues, we will conduct longitudinal assessments using a multi-dimensional approach. This will include serial biopsies for hematoxylin and eosin staining as well as immunohistochemistry, spatial transcriptomics, high-resolution respirometry for bioenergetics evaluation as well as live cell confocal microscopy and hyperspectral imaging to examine viability characteristics in both the tumor and adjacent liver tissue. In addition, precision cut liver slices will serve as an in vitro platform for vector evolution using an adeno-associated virus (AAV) library. This facilitates the selection of AAV vectors with liver specificity for screening potential drug candidates and oncologic treatment regimens. This innovative approach, reflecting human physiology within perfused organs, overcomes limitations of other models, promising to offer unique insights into tumor biology. The model may advance personalized treatment strategies, potentially reducing the reliance on animal experiments. Ultimately, it holds the potential to contribute to ex-situ tumor therapy and organ repair, bringing us closer to implementing such advanced interventions into clinical practice.

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Discovery of subtype-selective L-type Cav1.3 voltage-gated calcium channel blockers

Introduction: High voltage-activated L-type Cav1.3-channels have recently emerged as attractive drug targets for the therapy of treatment-resistant hypertension, spasticity after spinal cord injury and neuroprotection in Parkinson's disease. So far, only non-selective L-type channel inhibitors are available, with even slight selectivity for Cav1.2. We therefore used homology modeling and virtual screening in combination with patch-clamp studies on recombinant human Cav1.3 and Cav1.2 channels to identify Cav1.3-selective inhibitors

Methods: Human Cav1.2 and Cav1.3 homology models were generated based on the available cryo-EM data of the rbCav1.1-nifedipine (PDB 6JP5) complex for structure-based virtual screening of commercially available molecules. Candidate molecules were tested using whole-cell patch-clamp studies (holding potential -89 mV, 0.1 Hz, 100 ms test pulses to 10-20 mV) in tsA-201 cells stably expressing human Cav1.2 or Cav1.3 (+ β3 and α2δ-1 subunits). Candidate compounds were further pursued if they inhibited at least 30% of Cav1.3 current at 10 µM concentration.

Results and discussion: From the pre-selected and tested 60 compounds, 7 showed activity on Cav1.3s with different potencies (IC50: 1.26 – 26.1 µM). 2 compounds showed only weak (~20%) block of Cav1.2 even at the highest concentration (50 µM). This corresponded to an at least 15-fold selectivity towards Cav1.3. This finding motivates the further analysis of structure-activity relationship of these compounds to improve both potency and selectivity. Such compounds would provide important novel pharmacological tools for elucidating the therapeutic potential of Cav1.3 channel inhibition in preclinical studies.

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Inducible 3D modelling of human brain ageing recapitulates hallmarks of ageing and identifies distinct transcriptomic signatures

Ageing is a critical factor for functional decline in the human brain, however, limited access to patient material has hindered the study of the aged brain. While models derived from induced pluripotent stem cells (iPSCs) offer a valuable tool to study human diseases, iPSC-type reprogramming causes the loss of age-related signatures and limits the ability to recapitulate in vitro late-onset conditions, such as brain ageing. Here we report an engineered human iPSCs line overexpressing Progerin in a Doxycycline (DOX)-inducible manner and its subsequent differentiation into neural lineages to study neuronal ageing. Transgenic iPSCs produced morphologically homogeneous cortical organoids for up to three months in culture without apparent changes in cellular composition. We found that Progerin overexpression in organoids induces key ageing hallmarks, including a 40% loss of H3K9me3-marked heterochromatin, a two-fold increase of double-strand DNA breaks as judged by γH2Ax, and increased senescence-associated β-galactosidase. Transcriptome-wide analysis via bulk RNA sequencing revealed strong transcriptomic changes in organoids upon 60 days of Progerin overexpression, with primary differences already arising after 30 days. In particular, we found 1,366 dysregulated genes in aged organoids, mirroring alterations reported in post-mortem tissue. These include, among others, dysregulation of previously described age-associated pathways, such as DNA repair and histone modifications, but also senescence, mitochondrial alterations, and impaired macroautophagy. Our results suggest that Progerin overexpression is a proxy for inducing ageing in neuronal lineages, allowing for the dissection of early ageing events. We anticipate that our system holds promise for future studies on neural ageing and neurodegeneration.

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Essential role of functional Interleukin 6 signal transducer in primary sensory neurons for regulating gut microbiota composition in mice.

Mounting evidence suggests that IL-6 significantly shapes microbiota composition and gut-barrier function. However, the exact pathways and mechanisms are still elusive. Interleukin 6 (IL-6), its receptor (IL-6R), with the IL-6 signal transducer (gp130/IL-6ST), are known regulators of innate immunity but also affect neuron morphology and function. We investigated the involvement of functional IL-6 signaling in the bilateral communication between primary afferent neurons and gut microbiota through the evaluation of gut motility, fecal composition, and barrier function. A transgenic mouse model with a conditional depletion of gp130 in neurons expressing the nociceptor specific ion channel NaV1.8 (SNS-gp130-/-) and littermate gp130fl/fl controls of both sexes were subjected to measurements of gut motility. Feces were collected, and subject to 16S sequencing to identify microbiota strains. Efferent neuron functions were assessed in vitro with colon preparation stimulation with depolarizing concentrations of KCl followed by ELISA quantification of calcitonin gene related peptide (CGRP). Colonic mucus thickness was observed using histological methods. IL6ST depletion in primary sensory neurons did not cause significant changes in gut motility. However, 16S analysis revealed genotype and sex-specific changes in specific fecal microbiota composition between the genotypes. Colonic mucus thickness was decreased in SNS-gp130-/- mice and this was accompanied by a reduced CGRP release. Our data indicate that peripheral sensory neurons affect microbiota composition, highlighting their role in communication through messengers requiring functional IL-6ST signaling pathways. The reduction in CGRP release and mucus thickness suggests that neuropeptides may qualify as relevant messengers of neurons regulating gut microbiota and gut barrier function.

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Paralemmin-3 - an essential constituent of the submembrane cytoskeleton of auditory hair cells

Dysfunction of the electromotile outer hair cells (OHCs) in the mammalian inner ear results in stark reduction in frequency fine-tuning and hearing sensitivity, which is a major cause of sensorineural hearing loss. The molecular basis of OHC somatic electromotility is the motor protein prestin – a transmembrane volume motor that allows voltage-dependent longitudinal length changes of the OHC lateral membrane – and the cortical lattice, which consists of highly organized cytoskeleton that is integral in translating forces into changes in cell shape. Here, we identified paralemmin-3 (Palm3) as a novel protein found along the lateral walls of OHCs and sensory inner hair cells (IHCs) that is indispensable for hair cell biology and hearing. On a functional level, auditory brainstem recordings of Palm3-KO mice revealed early-onset and progressive hearing impairment with attenuated distortion product otoacoustic emissions, suggesting corrupted cochlear amplification and a functional deficit in the peripheral auditory pathway. Morphologically, confocal analysis of acutely-dissected organs of Corti revealed progressive and extensive loss of OHCs in Palm3-KOs that was apparent as early as 2 weeks of age, in addition to a significant reduction in OHC length. In IHCs, absence of Palm3 mildly decreased synapse counts within the IHC basolateral region, as well as the abundance of the characteristically distributed large-conductance (BK) K+ channel clusters in the IHC neck region. In summary, Palm3 is a protein found in the submembrane cytoskeleton of cochlear hair cells that seems to have a consequential role in both OHCs and IHCs.

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Beta-Amyloid Enhances Vessel Formation in Organotypic Brain Slices Connected to Microcontact Prints

In Alzheimer's disease, the blood–brain barrier breakdown, blood vessel damage and re-organization are early events. Deposits of the small toxic peptide beta-amyloid (Aβ) cause the formation of extracellular plaques and accumulate in vessels disrupting the blood flow but may also play a role in blood clotting. In the present study, we aim to explore the impact of Aβ on the migration of endothelial cells and subsequent vessel formation. We use organotypic brain slices of postnatal day 10 wildtype mice (C57BL/6) and connect them to small microcontact prints (μCPs) of collagen. Our data show that laminin-positive endothelial cells migrate onto collagen μCPs, but without any vessel formation after 4 weeks. When the μCPs are loaded with human Aβ40, (aggregated) human Aβ42 and mouse Aβ42 peptides, the number and migration distance of endothelial cells are significantly reduced, but with a more pronounced subsequent vessel formation. The vessel formation is verified by zonula occludens (ZO)-1 and -2 stainings and confocal microscopy. In addition, the vessel formation is accompanied by a stronger GFAP-positive astroglial formation. Finally, we show that vessels can grow towards convergence when two opposed slices are connected via microcontact-printed lanes. In conclusion, our data show that Aβ promotes vessel formation, and organotypic brain slices connected to collagen μCPs provide a potent tool to study vessel formation.

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The regulatory role of the VIP/VPAC1/2 receptor system in the central amygdala on stress and anxiety responses in mice

Vasoactive intestinal polypeptide (VIP) is a neuropeptide found with its receptors (VPAC1 and VPAC2) in the central nervous system, where they are highly abundant in areas of the stress and anxiety circuit, such as amygdala. However, the exact role of VIP in stress and anxiety function is not fully known. Consequently, our main focus was to study the effects of intracerebral VIP/VPAC receptor agonists/antagonists administration on anxiety- and stress-related behaviors of C57Bl6/J mice. Thus, VIP, VIP(6-28) (a VIPAC1r and VPAC2r antagonist), KS-133 (a VPAC2r selective antagonist) or vehicle was administered bilaterally into the central amygdala (CeA), an area with high VIP receptor expression and tested in different behavioral tests for the assessment of stress- and anxiety-related behavior. We found that VIP-injected animals show a reduction of time spent in aversive zones of the elevated plus-maze indicating an anxiogenic-like effect and a decrease in active coping or escape-oriented behavior in the forced swim test, compared to vehicle-injected controls. Conversely, intra-CeA administration of the VPAC1/2 receptor antagonist VIP(6-28) induced an anxiolytic-like effect indicated by an increased time and frequency of entries into the aversive area of the light-dark test. Moreover, VIP(6-28) augmented active coping or escape-oriented behavior in the forced swim test, which could be replicated after intra-CeA administration of the specific VPAC2r antagonist KS-133. Overall, our findings show that the VIP/VPAC1/2 receptor system in the CeA is implicated in modulating stress and anxiety responses and suggest VIP receptors antagonists as potential therapeutic agents for the treatment of stress-related psychiatric disorders.

(FWF–P33534-B)

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ß-catenin1 knockdown inhibits regeneration in the flatworm Macrostomum lignano

ß-catenin is a crucial effector of the canonical Wnt pathway. The Wnt/ß-catenin molecules mediate key metazoan cellular processes like differentiation and proliferation. Precisely, Wnt/ß-catenin regulates head/tail specification in planarian flatworms, where inhibition of ß-catenin1 leads to a multi-headed individual. However, little is known about the role of ß-catenin in other flatworms. Here, we investigate the possible role of ß-catenin on regeneration following amputation in Macrostomum lignano. To achieve this, we mined macrostomum lignano genome for all the homologs of ß-catenin using Schmidtea mediterranea and Dendrocoelum lacteum sequence as bait. We performed phylogenetic inference and expression in vivo where we utilized the double-stranded interference RNA (dsRNAi) for the knockdown. Through a rooted tree, we found three paralogs. Following the taxonomy set in planarians, we assigned Mligßcat1, Mligßcat2 and Mligßcat3 names respectively. Mligßcat1 and Smeßcat1 are monophyletic, while Mligßcat2and Mligßcat3 are sister groups that emerged due to gene duplication. Additionally, neither cell differentiation nor proliferation was affected by knocking down of Mligßcat2and Mligßcat3, yet knocking down Mligßcat1 inhibited tail regeneration, suggesting that it has a role in determining the posterior axis. Moreover, the observed reduction in Brdu-labeled cells under Mligßcat1 dsRNAi treatment corroborates the involvement of the Wnt/β-catenin signaling pathway in cell proliferation and differentiation during tail regeneration. In conclusion, our findings provide additional insights into A-P axis determination by ß-catenin and regulatory mechanisms during the regeneration of Macrostomum lignano.

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A deep learning algorithm for fully automated myocardial infarct quantification from clinical CMR Scans

Late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) imaging is considered the in vivo reference standard for assessing infarct size and microvascular obstruction in ST-elevation myocardial infarction (STEMI) patients. However, the exact quantification of those markers of myocardial infarct severity remains challenging and very time-consuming. As LGE distribution patterns can be quite complex and hard to delineate from the blood pool or epicardial fat, automatic segmentation of LGE CMR images is challenging. In this work we present a deep learning-based method that allows to calculate the extent of myocardial infarction in a fully automated way. This is done without any human intervention, i.e. the volumes are calculated directly form the raw CMR images as they occur in everyday clinical practice. To test the accuracy of the proposed method we perform extensive experiments on an in-house data set consisting of over 1000 patients. We show that our fully automated pipeline is able to produce volume measurements, which match very closely with measurements performed by medical experts. Also, we find that the automatically measured infarct sizes correlate strongly with clinical parameters such as cardiac troponin and creatine kinase levels. Further, a qualitative study reveals that the automatized method can compete with the very time-consuming manual measurements even outperforming humans in some areas.

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Shape-aware deep learning framework for automatic extraction of head-neck arteries on computer tomography angriography images

Automated blood vessel extraction from 3D medical images plays a vital role in diagnosing vascular diseases. While many existing methods rely on convolutional neural networks (CNNs), they can encounter challenges in maintaining the continuity of extracted vessels, particularly when segmenting these slender tubular structures within 3D images. Our method includes the idea that preserving vessel continuity necessitates considering the global geometry. The approach involves employing a CNN for a preliminary localization of the arteries in the highly downsampled volume which is then used to extract seed points. This information is the input for another CNN - specialized in capturing the local appearance of the artery - to perform the segmentation. To recover the whole artery, we use a centerline based artery tracking algorithm to move the patch to be segmented along the artery until we reach the end of the vascular structure. The final segmentation mask is calculated by a new centerline- aware thresholding method. The approach results in a strong reduction of high- resolution patches seen by a neural network and copes with the high class imbalance as mainly patches with arteries are processed. The centerline-aware thresholding enforces the physical connectivity of the arteries.

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[177Lu]Lu-DOTA-MGS5 a novel candidate for the treatment of cholecystokinin-2 receptor expressing neoplasms

Cholecystokinin-2 receptors (CCK2R) are overexpressed in medullary thyroid carcinoma and some other malignancies. We recently reported on the development of DOTA-DGlu-Ala-Tyr-Gly-Trp-(N-Me)Nle-Asp-1-Nal-NH2 (DOTA-MGS5), a novel minigastrin analogue that exhibits improved stability and enhanced tumour targeting properties. Therefore, for patients with CCK2R-expressing neoplasms, targeting CCK2R with DOTA-MGS5 represents a potential new strategy.

The specificity of [177Lu]Lu-DOTA-MGS5 for CCK2R receptors was studied in CHO cells transfected with CCK1R or CCK2R. The cell uptake of [177Lu]Lu-DOTA-MGS5 was studied in the A431-CCK2R/mock cells. In addition, confocal microscopy studies were performed using MGS5 conjugated with the fluorescent dye ATTO-488. To investigate the therapeutic effect, colonogenic assay was performed on A431-CCK2R cells. The radiation dose response after incubation of cells with 250kBq-4MBq/ml [177Lu]Lu-DOTA-MGS5 were determined by colonies fixation and staining.

A cellular uptake of 8.1±0.7% was found in CHO-CCK2R cells for [177Lu]Lu-DOTA-MGS5, 2h after incubation. For the same time point, value **<**0.1% was found in CHO-CCK1R cells, confirming the specificity of [177Lu]Lu-DOTA-MGS5 for the CCK2R. A431-CCK2R cells show an increasing cell uptake over time with values of 22.64±6.2% at 30minutes and 68.04±3.0% at 4h after incubation. Cell internalisation of <0.5% in A431-mock cells was observed at all-time points studied. Fluorescence microscopy confirmed rapid receptor-specific translocation of ATTO488-MGS5 from the cell membrane to the intracellular compartment. In the preliminary colonogenic study, a significant reduction in the percentage of survival fraction of A431-CCK2R cells treated with [177Lu]Lu-DOTA-MGS5 was achieved compared to the control group.

The preclinical studies conducted support the therapeutic potential of [177Lu]Lu-DOTA-MGS5 in patients with CCK2R-expressing tumours.

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A novel dual-modality imaging agent targeting FAP based on Fusarinine C scaffold

Background

Dual-modality probes, combining PET with fluorescence imaging (FI) capabilities in the same molecule, can be used for preoperative imaging and intraoperative guidance and therefore can improve surgery outcomes. We herein present a PET/FI agent targeting the fibroblast activation protein (FAP): [68Ga]Ga-ZW800FFAPI. In this probe, the ZW800 fluorophore and two units of a FAPI-04 derivative were individually coupled to the siderophore Fusarinine C acting as core scaffold. An analogue compound without fluorophore was prepared as control.

Materials and methods

The selected fluorophore and two units of FAPI-04, respectively, were introduced on the modified siderophore by amide coupling and click reaction. Analogously, an acetylated ligand lacking the fluorophore was prepared as control. 68Ga-labelling of the precursors (5 nmol) was accomplished within 10 min at pH 4.4 and RT. In vitro characterisation included the evaluation of lipophilicity (LogD7.4), protein binding and stability in human serum. Internalisation assays were performed by using human FAP expressing cells. Biodistribution experiments in healthy mice were performed to evaluate pharmacokinetics up to 2h p.i.

Results

Hydrophilic properties, moderate protein binding (< 27.7 %) and elevated stability in human serum were observed over 4 hours for both radiotracers. Preliminary cell uptake studies showed comparable and receptor-mediated internalization (>22% after 1h incubation). Biodistribution data (2h p.i.) for [68Ga]Ga-ZW800FFAPI indicated slow clearance from blood pool (2.1% ID/g) and low kidney and liver uptake (<6.5% ID/g).

Conclusions

[68Ga]Ga-ZW800FFAPI showed promising in vitro properties and its comparison with the control indicated that these are minimally modified by the introduction of the fluorophore.

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Enhanced deep Raman microscopy by optofluidic adaptive optics.

The acquisition of weak Raman signals often requires time-intensive measurements, from minutes to several hours. Confocal Raman microscopy, designed for imaging volumetric samples, encounters spatial resolution and signal loss due to optical aberrations induced by the sample, thereby extending acquisition durations. Adaptive Optics (AO) offers a solution by compensating for these aberrations, restoring signal fidelity and resolution even at significant depths within the sample. In our study, we implement AO in confocal Raman imaging through an innovative optofluidic adaptive element, seamlessly integrated with a standard commercial confocal Raman system.

Employing an indirect wavefront sensing method, we conducted a proof of concept experiments involving the correction of pronounced refractive index mismatches and non-spherical aberrations induced by an artificial scatterer. Our findings demonstrate remarkable up to 5-fold enhancements in signal strength achieved by correcting the measured wavefront distortions.

These advancements hold significant promise for biological and medical applications. The improved spatial resolution and signal fidelity afforded by AO-enhanced confocal Raman microscopy could improve cellular imaging and tissue analysis. Enhanced imaging clarity may pave the way for more accurate diagnoses, deeper insights into cellular mechanisms, and potentially transformative strides in understanding and treating diseases at a cellular/tissue level.

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Poster abstracts

- Posters should stay up for the whole duration of the meeting and must be taken down immediately after the poster session on Friday.
- Poster presentations should last approximately 3 min.
- **Odd** poster numbers on **Thursday**
- **Even** poster numbers on **Friday**
- There will be poster prizes, so stay at your poster during your respective session!

Posters

Odd numbers on **Thursday, even** numbers on **Friday**

Presenting Author		#	Abstract title
Achrainer	Matthias	1	AN IN VIVO CHITIN BASED ADHESIVE SYSTEM
Rabensteiner	Fabian	2	Conserved Role of FoxH1 in Early Differentiation Processes
López-Amo alvo	Beatriz	3	Deciphering growth control in the development of human brain
Huber	Katharina	4	Investigation of the glioblastoma tumor microenvironment and expansion potential of tumor-infiltrating lymphocytes via single-cell transcriptomic analysis
Rigato	Irene	5	Transcriptomic analysis of molecular and cellular network rewiring during malignant transformation
Ausserhofer	Markus	6	NovumRNA: computational prediction of non-canonical tumor specific antigens from RNA sequencing data
Birstonas	Lukas	7	Elucidating the contribution of SrbA and AtrR in Aspergillus fumigatus triazole resistance
Nykiel	Kamila	8	Characterizing the m5C-dependent RNA protein interactome
Hanisch	Malou	9	Identifying preQ1 riboswitches in Listeria monocytogenes
Schwaighofer	Selina	10	Kinases in motion: impact of protein and small molecule interactions on kinase conformations
Shahbazi	Nargess	11	Deciphering the role of progenitor and stem cells in exocrine pancreas regeneration in zebrafish
Kugler	Valentina	12	Systematic identification of druggable PKA substrates involved in colon cancer progression
Van Goubergen	Jasper	13	The CLK2/SRSF9 axis is involved in an rs5918762 allele-specific manner in alternative splicing of androgen receptor variant 7
Zott	Melanie	14	Regulatory role of Mnx1 in beta-cell differentiation and maturation
Sathianathan	Marc	15	MNX1 acts as a fate regulator in human in-vitro beta-cell differentiation
Purwar	Astha	16	Organelle-specific assembly of the ESCRT machinery and their role in organellar repair

Posters

Odd numbers on **Thursday, even** numbers on **Friday**

Presenting Author		#	Abstract title
Kummer	Denise	17	Exploring the Interplay between Ether Lipid Metabolism, Tetrahydrobiopterin, and Lipid Peroxidation in the Gastrointestinal Tract
Egger	Anna-Sophia	18	Multi-omics investigation of central metabolism shifts in tuberous sclerosis complex
Weber	Leonie	19	The BASP1 signaling protein interferes with the oncogenic capacity of MYC
Monfort-Lanzas	Pablo	20	DoseRider: A multi-omic approach to studying dose-response relationships at the pathway level using mixed models
Marchet	Nikolas	21	Alpha-arrestin mediated control of cellular nutrient uptake and its role in metabolic signaling
Parrakova	Lucia	22	ExonSurfer
Bekdas	Baris	23	Differential Fates of Orm Proteins in Budding Yeast
Sammarco	Laura	24	VIP36 and VIPL trafficking and interactors
Plesche	Alexander	25	Characterization of the mechanical Influence of TPD52L2 on cellular Rigidity via the Actin-Cytoskeleton in Breast Cancer Cells
Mari	Martina	26	SZT2 Regulates Mitochondrial Function and Response to Oxidative Stress
Singer	Isabel	27	Protein interactions and metabolic signalling at the lysosome
Knoll	Gabriel	28	CD8+ HLA-DR+ CD45RC- T cells as a potential regulatory T cell population
Schweighofer	Paul	29	HPL AS SUBSTITUTE TO FCS TO CULTURE PRIMARY MONOCYTE-DERIVED CELLS?
Erckert	Sophie Ann	30	Characterization of DC migration from infected respiratory tissue
Schobert	Jan	31	Distribution of Aspergillus terreus in soil in Tyrol, Austria

Posters

Odd numbers on **Thursday, even** numbers on **Friday**

Presenting Author		#	Abstract title
Diem	Gabriel	32	A new perspective on cellular complement and how opsonized HIV 1 enhances dendritic cell maturation and survival via Mcl-1 stabilization regulated by anaphylatoxin receptors
Hatami	Jasmin	33	Investigating the therapeutic potential of an immune-modulatory cargo-expressing oncolytic virus in a mixed tumor model
Sarah	Danklmaier	34	Prediction and identification of MHC class I presented T cell epitopes of the oncolytic virus VSV-GP in BALB/c mice
Aufschnaiter	Andreas	35	Modifying oncolytic virus - induced cell death
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AN IN VIVO CHITIN BASED ADHESIVE SYSTEM

Various organisms evolved the ability to attach and detach form a wide range of surfaces in both fresh and sea water. This temporary attachment is based on a glue that is mainly composed of proteins and carbohydrates. A lectin screen revealed an abundance of N-acetylglucosamine in the secreted Hydra glue (called footprint). Further stainings confirmed the presence of a chitin-based meshwork. Chitin is a common biopolymer, which has been described to be involved in the attachment of barnacles. Thus, we propose that chitin forms a structural network and is interacting with a number of glue-specific proteins that adsorb to the substrate. To further investigate chitin function, we inhibit chitin synthase through knock-down experiments and chitinase treatments. Furthermore, we knock-down potential chitin binding proteins and enzymes that presumably modify the glue proteins (e.g. peroxidases). In conclusion, we start to unravel the Hydra glue composition, with emphasis on the role of chitin and associated proteins. Here we propose a naturally evolved in vivo model system to complement the medical research of chitin-based hydrogels

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Conserved Role of FoxH1 in Early Differentiation Processes

Gastrulation with the formation of the three germ layers followed by a step by step fate determination forms a keystone in the development of multicellular organisms. During this early process, cells undergo extensive chromatin remodelling which alters its structure and regulates gene transcription. FoxH1 is a known major key transcription factor which, during this early embryotic development, controls and fine tunes regulatory gene programs involved in mesoendoderm induction and left-right patterning. Recent studies further suggest FoxH1 as a potential pioneer-transcription-factor with more general function in chromatin remodelling during transition and early cell specification. Recent work from our group in zebrafish revealed interaction of FoxH1 with various DNA-sites lacking the FoxH1 consensus motif, suggesting that FoxH1 might indirectly influence the chromatin state as part of a bigger protein complex. However, how FoxH1 contributes to loci targeting, with which proteins it is interacting and the underlying kinetics during this early remodelling is still unknown. To address these questions, a set of human iPSC cell lines with domain-specific FOXH1 mutations was established. The DNA recognizing FH domain as well as the protein interacting SI domain are thought to be essential for chromatin remodelling events and available data suggests that the absence of either one of them will interfere with FoxH1's function of chromatin-looping. By the usage of these cell lines, the specific functions of each domain can be identified. Additionally, the lines are tagged with a 3xTY1 epitope, which will further be used for a molecular characterization of direct targets.

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Deciphering growth control in the developing human brain

During human brain development neural stem cells divide symmetrically to expand the neural progenitor pool before dividing asymmetrically to produce differentiated cell types, such as neurons. Variability among stem cells shifting between division modes generates heterogeneity in stem cell lineage sizes. Recent research has shown how stem cell lineages compete and compensate deficits among each other to ensure overall brain tissue growth and maintain tissue homeostasis. However, little is known about the genetic and molecular cues that lead to this growth self-control and cellular heterogeneity present in the brain tissue context. Additionally, a more comprehensive investigation on the variability of stem cell lineage contribution to brain development over time is needed. As a 3D model which recapitulates aspects of cellular architecture and functionality of early human brain tissue, human cerebral organoids offer a promising platform for this study. Furthermore, multifunctional genetic studies in a brain tissue model would help elucidate the mechanisms underlying the growth control of the human brain. Therefore, cell death assays in chimeric cerebral organoids are being performed to characterize the replenishment capacity of long-lasting stem cells during human brain development, focusing on tissue-mediated growth control across various developmental stages. Time-course differential gene expression analysis will help to identify potential candidate genes, which may be further validated in loss-of-function genetic screens conducted in brain organoids along with other literature-based genes associated with signaling pathways, transcription, cell cycle and fate determination to unravel the molecular mechanisms governing brain tissue-mediated growth control.

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Investigation of the glioblastoma tumor microenvironment and expansion potential of tumor-infiltrating lymphocytes via single-cell transcriptomic analysis

Glioblastoma (GBM) is a brain cancer known for its aggressive nature and dismal prognosis, which is partially attributed to a highly heterogeneous and immunosuppressive tumor microenvironment (TME) and scarcity of tumor-reactive T cells. Therefore, adoptive T-cell therapy (ACT) in GBM has long been considered unfeasible. However, recent findings showing the presence and activity of tumor-infiltrating lymphocytes (TILs) in GBM have challenged this notion. We have developed an optimized protocol for isolating tumor-reactive (tr) TILs from surgical material and expanding them in vivo, which is a cruical step in ACT. The reasons for failed expansion in 30% of samples remain elusive.

To investigate the determinants of TIL expansion, CD137+ tr-TILs were isolated from eight glioblastoma samples and enriched using immunomagnetic separation. Single-cell RNA-sequencing (scRNA-seq) was performed on tr-TILs and myeloid components, which remain strictly associated, allowing exploration of TIL-extrinsic factors affecting expansion.

To enable accurate cell-type annotation of our dataset, we collected publicly available GBM scRNA-seq data from seven studies and integrated them computationally to create an atlas of the GBM TME spanning ~500,000 cells. Leveraging this atlas for cell annotation, we contrasted expanded and non-expanded samples at the molecular and cellular level. We identified genes differentially expressed between expansion and non-expansion samples, with particularly marked differences in myeloid cells.

Our analysis sheds light onto the cellular and molecular determinants of TIL expansion success, which could be used to anticipate the feasibility of ACT in GBM patients and, ultimately, suggest potential targets for the ex vivo manipulation of TILs before ACT.

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Transcriptomic analysis of molecular and cellular network rewiring during malignant transformation

Evasion from programmed cell death, most notably mitochondrial apoptosis controlled by BCL2 family proteins, is a hallmark of malignant transformation. Despite our in-depth knowledge on apoptosis in established tumors, the molecular and cellular mechanisms that initiate and sustain the transformation of healthy B cells into malignant ones remain elusive.

In B cell lymphomagenesis, MYC overexpression is a causative oncogenic driver scenario despite inducing excessive apoptosis. Thus, BCL2 network rewiring is key for cell survival during transformation. To study the molecular reshaping during malignant B cell transformation, we analysed RNA-seq data generated from mouse models characterized by B cell-specific MYC overexpression along with wild type controls. To specifically investigate B cells and their immune cell environment, the data were generated from three organs: bone marrow, peripheral blood, and spleen.

In addition to regular gene expression analysis, we performed deconvolution-based cell-type quantification. Briefly, cell-type-specific fractions were deconvolved using quadratic programming, leveraging two publicly available single-cell RNA-seq datasets to derive cell-type-specific signatures. The analysis showed varying abundances in the EuMYC model compared to the wild type not only for MYC-expressing B cells, but also for other immune cells. The results of deconvolution were recapitulated by flow cytometry data generated from the same samples.

Through the integrated analysis of bulk RNA-seq and single-cell RNA-seq data, we aim to reveal mechanisms underlying B-cell malignant transformation, generating knowledge that will be further employed for the discovery of unexplored therapeutic vulnerabilities.

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NovumRNA: computational prediction of non-canonical tumor specific antigens from RNA sequencing data

Tumor-specific antigens (TSAs) are the major targets of T-cell responses. Besides canonical TSAs arising from single-nucleotide variants and short insertions and deletions, recent studies have shown that the aberrant transcriptomes of tumor cells can result in non-canonical TSAs (ncTSAs) that are recognized and targeted by T-cells. Thus, the computational prediction of ncTSAs can pinpoint new T-cell targets for anticancer immunotherapy, ultimately extending its benefit to a larger population of patients. However, the comprehensive pipelines for the prediction of ncTSAs from patients' tumor RNA sequencing (RNA-seq) are currently lacking.

We developed NovumRNA, a comprehensive ncTSA prediction pipeline. NovumRNA performs alignment of RNA-seq reads to the reference genome followed by transcript reassembly to identify tumor-specific transcripts from alternative splicing, endogenous retroviruses, and non-coding regions. Tumor specificity is ensured via filtering against an internal normal control database, which can be further augmented with healthy tissue data. NovumRNA performs sequence translation, chopping into peptides, followed by prediction of peptide binding affinity towards the patient's human leukocyte antigen (HLA) molecules.

By using publicly available RNA-seq datasets with matching mass spectrometry data (Laumont et al. 2018) and PCR-confirmed splicing events (Shen et al. 2014), we demonstrated NovumRNA prediction accuracy. Moreover, via NovumRNA analysis of RNA-seq data from human tumor cell lines treated with the splicing-perturbation drug indisulam, we showed an increase of ncTSAs upon treatment, suggesting an increased antigenicity for the treated tumor cells. These results underscore the value of NovumRNA for cancer immunotherapy and its utility for the identification of synergetic partners for combination therapies.

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Elucidating the contribution of SrbA and AtrR in Aspergillus fumigatus triazole resistance

Every day people inhale significant amounts of fungal spores in their regular dailylives and usually, those spores are neutralized by the person's immune system.However, people with a compromised immune system have a higher risk todevelop severe health problems. Out of more than 1.5 billion people affected byfungal diseases each year, in excess of 1.5 million end up in deaths. One of thedeadliest fungal species, responsible for a large proportion of these deaths, isAspergillus fumigatus. Treatment of infections caused by this pathogenic mold,generally termed aspergillosis, is limited to only four major drug classes withdifferent cellular targets and efficacy: azoles, polyenes, echinocandins andnucleobase analogs.First-line treatment of aspergillosis comprises azole antifungals that target sterol 14-α demethylase (Cyp51), a key enzyme in ergosterol biosynthesis. Similar tocholesterol in human cells, ergosterol stabilizes the fungal cell membrane,determines its fluidity and permeability. Inhibition of Cyp51 leads to theaccumulation of toxic intermediates and depletion of ergosterol, eventually growthinhibition. Ensuring adequate activation of several ergosterol biosynthetic genesincluding cyp51A, in A. fumigatus two major transcription factors involved in sterolregulation as well as azole resistance represent SrbA and AtrR. Employing tunableatrR and srbA strains as well as mutated versions of these transcription factors, inthis work we aimed to investigate common as well as independent functions ofthese proteins that play dominant roles in resistance of A. fumigatus to azoles.

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Characterizing the m5C-dependent RNA protein interactome

Background: RNA Binding Proteins (RBPs) drive the fate of RNA in a time and space dependent manner. Such a control begins in the nucleus where pre-mRNA undergoes a series of nuclear processing steps.Regulation might occur on the transcriptional and post-transcriptional level, when RNA is processed and exported to the cytoplasm. Thanks to the development of proteomic-based methods like RNA Interactome Capture (RIC) or physicochemical methods, the list of RBPs has been constantly growing. The modification of bases and/or ribose in RNA, specifically in mRNA, contributes to that proteome portrait. However, little is known about the reader proteins of specific RNA modifications. According to CLIPdb, several RBPs overlap with identified 5-methylcytosine (m5C). Our aim is to identify andcharacterize more of 5-methylcytosine readers.

Approach: We perform differential analysis of RNA-protein complexes isolated from wild-type mouse embryonic stem cells (ESCs) and ESCs mutant for the mRNA cytosine methyltransferases. To this end, we use in vivo UV crosslinking of RNA-protein complexes followed by TRIzol extraction. Complexes are purified and mRNA is enriched before mass spectrometry analysis of RNA-associated proteins.

Results: A preliminary proteome analysis confirmed the suitability of the method for the enrichment of mRNA-associated proteins. First comparative data suggest distinct differences in the RNA-interactome of wildtype and KO cells.

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Identifying preQ1 riboswitches in Listeria monocytogenes

Riboswitches are RNA-based gene control elements mostly found in Bacteria and Archaea, but also to a lesser extent in Eukaryota. They are typically located in the 5' untranslated regions (5'UTR) of bacterial mRNAs where they form secondary and tertiary structures that bind to a specific ligand with a high affinity. This highly conserved aptamer domain may undergo structural alterations upon ligand binding which triggers changes in the folding pattern of the expression platform. Therefore, riboswitches can regulate gene expression using several different mechanisms, mostly premature transcription termination and inhibition of translation initiation. More than 55 riboswitch classes have been identified in the last twenty years. Nevertheless, it is estimated that more than 1000 riboswitch classes remain hidden so far. New riboswitches have generally been predicted based on bioinformatics analysis of bacterial genomes. In preliminary experiments, we established an in vitro approach to identify preQ1 riboswitches in Listeria monocytogenes using a chemically synthesized preQ1 ligand by the lab of Prof. R. Micura. We generated a long list of candidate genes that are under the control of a potential preQ1 riboswitch and established methods to test those candidate riboswitches for translational or transcriptional riboswitch activity.

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Kinases in motion: impact of protein and small molecule interactions on kinase conformations

Protein kinases act as central molecular switches in the control of cellular functions. Alterations in the regulation and function of protein kinases may provoke diseases including cancer. In this study we investigate the conformational states of such disease-associated kinases using the high sensitivity of the Kinase Conformation (KinCon)-reporter system. We first tracked BRAF-kinase activity conformation changes upon melanoma drug binding. Second, we also use the KinCon reporter technology to examine the impact of regulatory protein interactions on LKB1-kinase tumor suppressor functions. Third, we explore the conformational dynamics of RIP-kinases in response to TNF-pathway activation and small molecule interactions. Finally, we show that CDK4/6 interactions with regulatory proteins alter conformations which remain unaffected in the presence of clinically applied inhibitors. Apart from its predictive value, the KinCon technology helps identify cellular factors that impact drug efficacies. The understanding of the dynamics of full-length protein kinases when interacting with small molecule inhibitors or regulatory proteins is crucial for designing more effective therapeutic strategies.

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Deciphering the role of progenitor and stem cells in exocrine pancreas regeneration in zebrafish

Exocrine pancreas displays an outstanding capacity for regeneration and cellfate plasticity. Using zebrafish models for exocrine cell ablation, we previously discovered a novel rare cell population displaying features of immature exocrine pancreas cells and identified these cells as a source of tissue regeneration after virtually complete removal of mature acinar cells.To better understand this progenitor pool, we established novel transgenic tools in zebrafish for conditional ablation and fluorescence-based lineage tracing. In addition, we developed and performed FACS-based protocols to enrich for acinar progenitor cells from conditionaly-ablated transgenic zebrafish larvae to enable the transcriptomic characterization of these cells under quiescent and regenerative conditions using balk SMART-seq. Furthermore, we have initiated a pharmacological investigation of the signaling pathways involved in the exocrine regeneration of zebrafish larvae. Towards this end, we selected small-molecule inhibitors of ADM- and/or PDAC-associated pathway components to investigate which of these might also play a key role in acinar cell regeneration. Quantification of sectioned and dissociated double-labeled pancreas from ptf1a:GFP/ela3l: E2Crimson zebrafish suggest that the proportion of ptf1a+/ela3l- progenitor cells is reduced from 5% in the larval pancreas to <0.2% in the adult exocrine pancreas.Preliminary results from bulk SMART-Seq show a rather distinct gene expression profile in ptf1a:GFP+ cells compared with ptf1a:GFP+/ela3l:mScarlet+ cells specially in expression of acinar mature markers e.g. elastase, amylase and early developmental markers or pro-cancer genes e.g., ptf1a,egr1, anxa4. Results from our primary drug screenings show a probable role of MAPK/P38 inhibition in decreasing the rate of mature acinar cell regeneration vs.control group.

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Systematic identification of druggable PKA substrates involved in colon cancer progression

Deregulation of G protein coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. Examples are activating mutations in the AC-stimulatory Gαs proteins (GNAS), which occur in 4,2% of all tumors. These lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA) pathway. In order to identify druggable PKA-effector proteins, we determined the phospho-proteomic composition of macromolecular PKA complexes from a collection of Gαs-mutated cancer cells and human glioblastoma biopsies. Using a substractive phospho-proteomic approach, we identified a multitude of proliferation-relevant PKA substrates and selected two druggable and cancer-implicated candidates for closer examination, namely the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and Tripartite motif 28 (TRIM28) respectively. PFKFB3 is a key modulator of glycolysis, implicated in maintaining cancer cell metabolism. We showed that nuclear PFKFB3 acts as a PKA substrate. Moreover, small molecule mediated inhibition of PFKFB3 reduced proliferation of Gαs-mutated colon cancer cells. Further, besides quantitative metabolite analyses of the cellular glycolytic flux, we revealed a nuclear function of PFKFB3. Using the RNAseq technology TUCseq we recorded immediate transcriptome changes upon PFKFB3 inhibition. Thus, we gained evidence for a possible link of PFKFB3 to p53 function in the studied colon cancer cell setting. TRIM28, the second novel PKA substrate, supports tumor progession through ubiquitination of the tumor suppressor p53. We investigated changes in protein stability of known anti-oncogenic TRIM28 ubiquitination substrates upon kinase activation. Currently, we explore a polypharmacology approach by inhibiting nuclear TRIM28 and PFKFB3 functions which may hamper proliferation of selected colon cancer cells.

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The CLK2/SRSF9 axis is involved in an rs5918762 allele-specific manner in alternative splicing of androgen receptor variant 7

Prostate cancer (PCa) is a leading cause of cancer-related deaths among men in the European Union. In advanced stages, androgen deprivation therapy (ADT) and androgen signaling inhibitors (ARSI), such as enzalutamide, are the mainstay of PCa therapy. However, most patients on ADT progress to a castration-resistant stage (CRPC), which is characterized by reactivation of the AR signaling pathway. One of the main drivers of CRPC is the upregulation of constitutively active androgen-independent AR-variants (AR-Vs), with AR-V7 being the most clinically relevant variant. Compared to the full-length AR (AR-FL), AR-V7 has a distinct and shorter 3' untranslated region (3'UTR) due to the inclusion of cryptic exon 3. Here, we aimed to identify players that are involved in the 3´UTR mediated regulation of AR-V7 mRNA. A common single nucleotide polymorphism (SNP), rs5918762, with a minor allele frequency of 30%, has been identified as the preferred allele for binding the RBP SRSF9. Depletion of SRSF9 leaded to decreased levels of AR-V7, indicating its critical role in AR alternative splicing. This interaction between SRSF9 and rs5918762 was confirmed through AR-V7 minigene assays and CLIP-qPCR analysis. The CLK/SRSF axis, involving CLK2 and SRSF9, is frequently dysregulated in metastatic PCa. Targeting this axis can be achieved by using CLK inhibitors like Lorecivivint, which inhibits CLK2 activity, thereby reducing SRSF9 phosphorylation and subsequently decreasing AR-V7 levels. In summary, this study highlights the impact of rs5918762 on the splicing of AR-V7 by modulating the binding capacity of the spliceosome component SRSF9 to the 3'UTR of AR-V7.

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Regulatory role of Mnx1 in beta-cell differentiation and maturation

The neonatal Diabetes factor Mnx1 is a key factor of fate-determination and –maintenance of insulin producing beta-cells in the pancreas. Loss of Mnx1 is known to cause a severe loss of beta cells accompanied by an increase in delta- and alpha-cells. Our studies in zebrafish have revealed that not only the majority of mono-hormonal beta cells is diminished but rather a new beta- / delta1-hybrid cell population becomes the dominant cell type in the pancreas. Further we have observed a strong mass increase in the whole pancreas area which mainly consists of duct-like structures shown by histological analysis. At earlier stages mnx1 mutant zebrafish larvae suffer from sever hyperglycemia and developmental issues which they recover from at 4-5 wpf. This time window correlates with a strong increase in hybrid cells and severe islet hyperplasia. We reason that the ectopic cell mass most likely is a secondary consequence of cellular mechanisms that aim to compensate the hyperglycemia caused by the loss of beta-cells. The origin of these hybrid cells is center of currently ongoing experiments. Immunohistological studies and scRNAseq data will hopefully help identify whether these ectopic cell population derives by migration from duct progenitor cells or by proliferation of differentiated endocrine cells.

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MNX1 acts as a fate regulator in human in-vitro beta-cell differentiation

Studies in different animal models and the association of MNX1 mutations with neonatal diabetes suggest that MNX1 has conserved functions in pancreas morphogenesis, beta-cell differentiation, and beta-cell fate maintenance. However, at present, neither the importance of Mnx1 in human beta cell formation nor the molecular functioning of Mxn1 is understood. Using a human in-vitro beta-cell differentiation approach, we now demonstrate that MNX1- deficient cells fail to differentiate into beta-like cells. We showed that the MNX1-/- clones were able to differentiate in pancreatic progenitors without significant differences in PDX1 and NKX6.1 expression compared to wild-type controls. However, consistent with a fate-shift of beta-cell progenitors into delta-like cells we find a significant increase in somatostatin-positive cells at the expense of insulin-positive beta-like cells in these mutants. Detailed analysis of this phenotype by qPCR and Bulk-RNA-seq at different stages identified HHEX as significantly upregulated in MNX1 mutants. HHEX is a transcription factor involved in delta cell development. Furthermore, Bulk RNA-seq revealed a multitude of differentially expressed genes. We currently aim to analyze the GRN (gene regulatory network) during differentiation to the beta-like stage. Analysis of these data will be presented as well as planned experiments such as and ChIP-seq analysis of MNX1 binding partners.

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Organelle-specific assembly of the ESCRT machinery and their role in organellar repair

The endosomal-sorting complexes required for transport (ESCRT) were discovered in yeast for targeting the ubiquitinated transmembrane proteins into the vacuolar lumen for degradation via the multivesicular body pathway. Additional roles of ESCRT machinery in other cellular processes such as viral budding and autophagy have also been described. These seemingly unrelated processes require the assembly of ESCRT-III filaments and their interaction with Vps4 (AAA-ATPase) to drive organelle-specific membrane remodeling via mechanisms that are not fully understood. Despite the knowledge on specific recruitment processes for ESCRT-III to different membranes, it is unknown if the formation of ESCRT-III filament follows a set of general rules or if organization of ESCRT-III filaments is adapted to different biological tasks and hence organelle specific. We aim to investigate the assembly of ESCRT-III/Vps4 on different organellar membranes in S. cerevisiae. First, we identify and characterize the interface residues that mediate interaction of ESCRT-III protomers - Vps2-Vps24, and their interaction with Snf7 to form mixed filaments on the endosomes. Then, we will delineate how ESCRT-0, -I and -II contribute to the recruitment of ESCRT-III to the stressed plasma membrane upon TORC2 inhibition. Next, we will determine if the interaction of ESCRT-III subunits at the PM follows the same rules as on endosomes. Hence our work will reveal how ESCRT-III subunits assemble on endosomes for MVB biogenesis and at PM to preserve membrane integrity. Finally, we plan to extend our analysis to other stressed / damaged organelles to demonstrate either universal rules or organelle specific rules for ESCRT-III assembly.

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Exploring the Interplay between Ether Lipid Metabolism, Tetrahydrobiopterin, and Lipid Peroxidation in the Gastrointestinal Tract

Ether lipids are classified as plasmanyl or plasmenyl species, depending on the presence or absence of a vinyl ether double bond at their sn-1 position. These lipids, are prone to ferroptosis due to enriched polyunsaturated fatty acids (PUFAs) at their sn-2 position. Ferroptosis is an iron-dependent cell death limited by GPX4. Recent research highlights specific ether lipid metabolic enzymes in ferroptosis, such as plasmanylethanolamine desaturase (PEDS1), alkylglycerol monooxygenase (AGMO) and its cofactor tetrahydrobiopterin (BH4), which is synthesized from guanosine triphosphate (GTP) by the rate-limiting enzyme GTP-cyclohydrolase 1 (GCH1). In gastrointestinal disorders, heightened reactive oxygen species and GPX4 deficiency underscore ferroptosis' role, yet the interaction between PUFA-rich ether lipids and ferroptosis remains unclear.We aim to investigate AGMO, PEDS1 and GCH1 levels in various models ranging from cell lines to primary murine and primary human gastrointestinal cells. Utilizing sensitive HPLC-based assays, we quantified enzymatic activities in human gastrointestinal biopsies and cell lines. Additionally, we found gene expression for all three enzymes in human gastrointestinal biopsies. Among our tested cell lines, RAW264.7 cells exhibited the most robust activity for all three enzymes. Currently, we are generating enzyme knockouts using CRISPR/Cas9 technology, followed by genetic complementation through plasmid transfection. In parallel, we are optimizing live cell assays and flow cytometric approaches to examine lipid peroxidation in our cell lines.Our fundamental investigations will allow us to delve deeper into the roles of ether lipids and tetrahydrobiopterin in the gastrointestinal tract and their interplay in membrane homeostasis and lipid peroxidation.

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Multi-omics investigation of central metabolism shifts in tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is a congenital disease that affects approximately 1 in 6000 newborns. The disease is caused by mutations in the genes coding for the proteins tuberin (TSC1) or hamartin (TSC2). They form the TSC protein complex which serves as an inhibitor of the serine/threonine kinase mechanistic target of rapamycin complex 1 (mTORC1). TSC patients experience a variety of symptoms including tumor formation, lung disease and neurological manifestations (1). mTORC1 is a master regulator of metabolism that controls glucose and lipid metabolism. It stands to reason that a loss of TSC could dysregulate central metabolism, and in support epilepsy symptoms in TSC patients can be ameliorated by a ketogenic diet. In our study, we set out to investigate alterations in central metabolism in a TSC model system by simultaneous proteo-metabolomics. We conducted quantitative proteome analyses as well as targeted metabolomics analyses in TSC2 knockout and control cells, investigating a fundamental shift in energy metabolism induced by a loss of TSC2. We validated the findings in a TSC patient cohort.

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The BASP1 signaling protein interferes with the oncogenic capacity of MYC

The MYC oncoprotein represents a transcription factor that regulates crucial cellular processes like proliferation, differentiation, or apoptosis. While its activity is essential and highly regulated in healthy cells, MYC is found to be deregulated in about 70 % of all human tumors where it represents a potent cancer driver. One of the multiple transcriptional MYC targets is the brain acid-soluble protein 1 (BASP1), which is downregulated in a variety of MYC-dependent cancer cells. Recently, we found that ectopic BASP1 expression interferes with MYC-induced cell transformation.Using human colon cancer cells featured by high MYC expression and a silenced BASP1 promoter, we further investigated the putative tumor-suppressive property of BASP1. Metabolome and Proteome comparison of SW480 cells with those ectopically expressing BASP1 (SW480-B) was performed using liquid chromatography coupled to mass spectrometry (LC-MS). From 4,543 analyzed proteins, 278 were found to be specifically activated in BASP1-expressing cells including the tumor suppressor TP53. Among the 252 downregulated proteins are the MYC-associated factor X (MAX) and the metastasis-associated protein 1 (MTA1). In addition, re-activation of the dormant BASP1 gene using the CRISPRa technology was performed in SW480 applying a lentiviral system, and cells were tested for interference with human cancer cell growth. Further methods including RNA Sequencing and Immunoprecipitation coupled to LC-MS aim to further investigate cellular processes upon endogenous BASP1 expression. With regard to the tumor-suppressive role of BASP1 in human cancer, we also tested BASP1-mimetic peptides to develop strategies for the treatment of tumor cells featured by high MYC expression.

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DoseRider: A multi-omic approach to studying dose-response relationships at the pathway level using mixed models

Traditional toxicogenomic approaches focus on the mechanisms of action (MoA) and benchmark dose (BMD) at the gene level, using dose-response models for individual genes. This method, however, misses the complex gene interactions within biological pathways. To address this gap, we aimed to develop DoseRider, a more comprehensive method that employs mixed models with cubic splines for studying nonlinear dose-response relationships at the pathway level. This method overcomes the limitations of classical dose-response modeling (such as linear, polynomial, exponential, and Hill models) and is adaptable to multi-omics experimental designs. DoseRider is available as an R-package and a web application. DoseRider's capability to model all molecules within a pathway simultaneously not only provides a trend for the pathway but also identifies the trend change dose (TCD), the concentration at which significant changes in pathway activity occur. This methodology has proven successful in identifying significant dose-response pathways and related molecular patterns, thus offering deeper insights into pathway responses to toxic compounds or drugs, even at low doses. In conclusion, DoseRider marks a significant advancement in toxicogenomics by enabling a comprehensive analysis of dose-response relationships at the pathway level and facilitating the identification of the biological effects of a compound at specific concentrations.

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Alpha-arrestin mediated control of cellular nutrient uptake and its role in metabolic signaling

Uptake of nutrients is essential for cellular growth, proliferation and survival. Amino acid transporters are solute carrier proteins, which facilitate cellular nutrient uptake across the membranes of organelles and function as key elements in cellular homeostasis. The abundance of amino acid transporters at the plasma membrane determines the intracellular quality and quantity of amino acids and plays a critical role in in the regulation of cell growth and proliferation. We now demonstrate that cells entering quiescence selectively downregulated a subset of amino acid transporters by endocytosis and lysosomal degradation. The underlying molecular mechanisms are only partially understood, but appear to involve members of the alpha-arrestins protein family. We have now identified two alpha-arrestins ARRDC2 and TXNIP (Jennifer Kahlhofer; MS in preparation) that selectively target the glutamine transporter SLC1A5 (ASCT2) and the heterodimeric amino acid transporters HAT for essential amino acids SLC7A5(LAT1)-SLC3A2(4F2hc) for endocytosis and lysosomal degradation, when cells enter quiescence. In this project I address how the activity of ARRDC2 and TXNIP is regulated, how ARRDC2 and TXNIP target SLC1A5 and SLC7A5-SLC3A2 for endocytic degradation and how other alpha-arrestins contribute to endosomal degradation of amino acid transporters. Finally, we want to elucidate how cells use the alpha-arrestin mediated amino acid transporter degradation to adapt their metabolism during the transition from proliferation to quiescence, and study the consequences of failure.

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ExonSurfer

Background: Reverse transcription quantitative PCR (RT-qPCR) is a crucial method for assessing gene expression. However, designing high-quality primers is challenging with specific requirements for positioning, specificity, and avoiding genome amplification. We introduce ExonSurfer, a user-friendly web-tool addressing these challenges.

Results: ExonSurfer integrates primer design steps, including target selection, specificity assessment, and addressing polymorphisms. Primers on exon-exon junctions prevent genomic DNA contamination, and a genomic alignment filters primers. Tested on 26 targets, most primers accurately amplified intended targets.

Conclusion: ExonSurfer offers an end-to-end primer design for transcript-specific amplification. The interface is intuitive and supports web and command-line use. ExonSurfer is expected to simplify the setup of RT-qPCRs in different domains. For the detection of specific transcripts, designing primers that go beyond exon-exon junctions is critical. ExonSurfer, a web-based tool, combines primer design steps, resolves common polymorphisms, and verifies specificity. Users customize parameters for their procedures, ensuring highly accurate primer design for a variety of applications.

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Differential Fates of Orm Proteins in Budding Yeast

Sphingolipids (SL) play a major role in regulation of membrane structure and function. Moreover, their derivatives (e.g. ceramides) act as signaling molecules in cell growth, apoptosis and stress responses. Yet, SL biosynthesis intermediates are also toxic at elevated concentrations. Therefore, their synthesis in the endoplasmic reticulum (ER) must be tightly controlled. The conserved ORMDL family proteins (In budding yeast Orm1/2) are responsible for SL regulation by confining the rate-limiting enzyme serine-palmitoyl-CoA transferase (SPT) in an inhibited complex (called SPOTS complex). When SL levels are low or membrane stress is present, Orm1/2 dissociate from the SPOTS complex and the enzyme becomes activated. Despite sharing high sequence identity, Orm1/2 show some different features. E.g. after dissociation, Orm1 remains in the ER whereas Orm2 is exported for degradation. Whether Orm1/2 have additional functions that are independent of their role as SPT inhibitors is unknown. Using mass spectrometry-based interactome studies, we found interactions of Orm1 and Orm2 with several ER-localized enzymes involved in the synthesis of ergosterol (the fungal cholesterol analogue). These interactions are independent of the phosphorylation status of the Orm proteins, which regulates their interaction with the SPT to control sphingolipid homeostasis. Moreover, Orm2 mutants show different susceptibility to anti-fungal drugs that target sterol biosynthesis. Co-regulation of the biosynthetic pathways of SL and sterols was suggested previously, but the underlying molecular mechanisms are unknown. In this project, we investigate a potential new role of ORMDL family proteins in organizing sterol synthesis at the ER and coordinating it with the SL biosynthetic machinery.

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VIP36 and VIPL trafficking and interactors

The transport of correctly folded secretory glycoproteins out of the ER to the Golgi apparatus is enabled by the COPII vesicles. Soluble secretory proteins are sorted into COPII vesicles via cargo receptors. The project follows two main lines of investigation aimed at a better understanding of the intracellular function of two putative cargo receptors VIP36 and VIPL. These are transmembrane L-type lectins, which interact with glycoproteins via their luminal domain. We know little about VIP36 and VIPL with respect to the proteins they interact and how they exit the ER. The first line of research focuses on trafficking of VIP36 and VIPL within the secretory pathway. We use the RUSH system in combination with export/retrieval deficient mutants to elucidate trafficking regulation. I will show data describing which SEC24 paralog, part of the COPII responsible for binding to transmembrane proteins that exit the ER, controls the trafficking of VIP36 and VIPL. The second area of work focuses on the search for cargos for both VIP36 and VIPL. Since an IP based approach could suffer from limitations due to the weakness of lectin-glycoprotein interaction, new cargo proteins can be identified by screening the secretome of VIP36 and VIPL deficient cells. We will also use a protein-proximity labelling techniques. Complementary to this, we used the RUSH system to design versions of VIPL and VIP36 that exit the ER in a synchronized manner. Through this, we were able to identify proteins that selectively interact with VIP36 and VIPL after they exited the ER.

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Characterization of the mechanical Influence of TPD52L2 on cellular Rigidity via the Actin-Cytoskeleton in Breast Cancer Cells

Tumor protein D52 like 2 (TPD52L2) is part of the TPD52 protein family and is known to be overexpressed in a variety of malignant diseases. This including breast cancer, where its overexpression correlates with a reduced overall survival. Recently, TPD52L2 was reported to be associated with intracellular nanovesicles, thereby being involved in a variety of membrane trafficking pathways.During our study, the effects of TPD52L2 on the mechanical properties of the cell were investigated in BT-549 and MDA-MB-231 breast cancer cells. Depletion of TPD52L2 using siRNA resulted in an increase of cellular rigidity. This change to the stiffness profile is caused by aberrations of the actin cytoskeleton following depletion of TPD52L2. Specifically, alterations of the cortical actin layer, which is enlarged in these cells, are believed to contribute to the changes of mechanical properties. Due to these mechanical aberrations, TPD52L2 silenced cells have a mechano-adaptability defect. This is indicated by a reduced spreading area on hydrogels with different substrate rigidities. Furthermore, cells depleted of TPD52L2 display a significantly reduced migration speed in a 3D random migration assay. Remarkably, both the mechano-adaptability and migration defect were rescued by low-dose treatment with Y-27632, a ROCK-signaling inhibitor. Y-27632 reduces cellular stiffness, thereby reversing the effects of the cytoskeletal aberrations on cellular rigidity. This demonstrates that the mechano-adaptability and the migration defect are caused by the altered stiffness profile of the cell, implying TPD52L2 as a regulator of these factors via its control of the mechanical properties of the cell.

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SZT2 Regulates Mitochondrial Function and Response to Oxidative Stress

The SZT2 protein is a subunit of the KICSTOR complex and acts as a negative regulator of the amino acid sensing branch of mTORC1 signaling. In cells lacking SZT2, mTORC1 is constitutively localized to lysosomes and hyperactivated. SZT2 has been shown to influence epileptogenesis in mice and many reports describe biallelic SZT2 variants in DEE-18 patients. Uittenbogaard and colleagues have previously investigated the mitochondrial phenotype of fibroblasts from a patient with an heterozygous SZT2 variant. In these cells, the OXPHOs pathway was impaired and the mitochondria were elongated and with abnormal cristae morphology. Moreover, in our SZT2 interactome study, we identified many proteins involved in mitochondrial metabolism and neurological diseases. Our current goals are therefore to characterize SZT2's regulation of mitochondrial activity and to investigate its involvement in oxidative stress response.Reproducing the patient fibroblast results, ablation of SZT2 in HEK293 Flp-in-TRex cells led to reduced mitochondrial activity. We also re-examined the subcellular localization of SZT2 and found that it only partially localized to lysosomes. In brief, we found partial co-localization with several organelles including mitochondria, ER, endosomes and peroxisomes. Furthermore, our electron microscopy analysis suggests that SZT2 is a cytoplasmic protein.To mechanistically link SZT2 to mitochondria function / activity, we recently performed a differential interactome analysis of SZT2 patient derived mutations, focusing on interactors that are up- or down-regulated compared to SZT2-WT. Interestingly, we found not only misregulated mitochondrial proteins but also proteins involved in neurological disorders, stress response and intracellular transport.

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Protein interactions and metabolic signalling at the lysosome

In recent years, the perception of lysosomes solely as cellular waste disposal system has evolved with emerging data emphasizing its significance in coordinating cellular metabolism. Lysosomes house a sophisticated nutrient-sensing machinery that integrates information about both extracellular and intracellular nutrient availability. This machinery activates corresponding signaling pathways, leading to alterations in the cell's metabolic program.The LAMTOR-complex plays a central role in these processes by recruiting and activating AMPK, MAPK and mTOR on the lysosomal surface. To regulate these processes, LAMTOR associates with various partners, including the Rag-GTPases, SLC38A9, the v-ATPase, BORC, AXIN, LKB1, and others. We know that some of these associations are mutually exclusive, whereas others occur under the same physiological conditions. Nonetheless, how these interactions are regulated remains largely unclear. The aim of this project was to extend the functional characterization of LAMTOR's associations to its partners, with a special focus on regulatory mechanisms defining the interplay between the different signaling cascades. Using recombinant LAMTOR-complex, I confirmed by in-vitro phosphorylations followed by Mass-spectrometry that AMPK phosphorylates LAMTOR1 at S63. To investigate changes in binding partners of the LAMTOR-complex following AMPK activation, a mass spectrometry-based interactome study was performed under catabolic conditions. The interactome not only provides intriguing insights into processes affected by LAMTOR1 phosphorylation but also uncovers novel functions of LAMTOR beyond regulating previously recognized LAMTOR functions.Current work is focused on the validation of interactome data and determination of the effects of the aforementioned phosphorylation-site in-vivo, which will ultimately improve our understanding of lysosomal signalling.

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CD8+ HLA-DR+ CD45RC- T cells as a potential regulatory T cell population

Regulatory T cells (Treg) play an important role in immune homeostasis and disease. They maintain tolerance to self-antigens and regulate immune responses to pathogens. Dysfunctional or dysregulated Treg are linked to autoimmune conditions and cancer development. They were associated with impaired immune homeostasis and function in old age, leading to a higher risk for autoimmune disease, viral infection, cancer, and lower responses to vaccination. Suppression of T cell proliferation and production of anti-inflammatory cytokines are classical Treg functions. These functions were mainly studied in CD4+ Treg, but also CD8+ Treg were described to possess these key regulatory functions. While for CD4+ Treg, the phenotype and functions are extensively described, CD8+ Treg are contradictorily discussed due to a lack of precise markers. In our laboratory, an HLA-DR expressing CD8+ T cell population with regulatory capacities was described. Other groups proposed CD122+ or CD45RClow/- as CD8+ Treg markers, but none of the currently described CD8+ Treg phenotypes is widely accepted within the Treg field. We observed significant variation in the capacity of CD8+HLA-DR+ Treg from individual donors to inhibit T cell proliferation. These results and the absence of a widely accepted CD8+ Treg phenotype prompted us to characterize CD8+ Treg in more detail. We investigate functional molecules such as cytokines and inhibitory molecules together with several proposed CD8+ Treg markers to identify a bona fide CD8+ Treg population. Here, we suggest CD8+HLA-DR+CD45RC- T cells as a potential Treg population and show cytokine production and the expression of inhibitory molecules of CD8+HLA-DR+CD45RC- Treg.

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HPL AS SUBSTITUTE TO FCS TO CULTURE PRIMARY MONOCYTE-DERIVED CELLS?

Human platelet lysate as a medium constituent has been investigated and improved over decades and with the rising qualities in its production, the replacement of fetal calf serum for various cell lines gains feasibility and urgency. In this study, the quality and quantity of human monocyte-derived dendritic cells (DCs), cultured in (hPL)-based or (FCS)-containing media when infected with HIV-1 or stimulated with lipopolysaccharide (LPS), were monitored. The DC phenotype of immature, mature and HIV-infected dendritic cells was studied via flow cytometry and to monitor cell viability, DC differentiation, and maturation and infection levels. Confocal microscopy was applied as a qualitative and quantitative tool for virus particles and productive infection of DCs was quantified by ELISA (against the viral p24 protein). Confocal imaging and p24 ELISA as quantification tool for infection levels illustrated dendritic cells cultured in FCS superior compared to hPL-cultured DCs. Unspecific activation was higher in FCS-grown immature DCs. Calculations based on the viability of dendritic cells throughout the procedure relative to the seeded amount and the differentiation and maturation rates showed elevated yields of LPS-matured cells grown in hPL, while HIV-C infection led to significantly higher yields of matured cells in the FCS setting. So far, we found that hPL presents as a viable substituent to elevate the yield in activated dendritic cells. Since for HIV-infection as well as in immune-competent in vitro models, an immature DC phenotype is essential, FCS cannot be replaced from the differentiation protocol so far and thus, this process needs further optimization.

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Characterization of DC migration from infected respiratory tissue

Dendritic cells (DC) represent the bridge between the innate and the adaptive immune system. When they encounter a pathogen, they phagocytose it, mature and begin to express co-stimulatory receptors. The activated DCs migrate to the lymph nodes, where they present the antigen to naïve B and T cells. In this work, the migration of DC in SARS-CoV2 infected respiratory tissue is analyzed, including the influence of the lung epithelial cells on the DCs, as they can promote inflammatory responses.A human 3D air-liquid interphase respiratory model was used, with normal human bronchial epithelial cells (NHBE) from the upper respiratory tract cultured on Transwells. For the co-culture experiments with DC, monocyte-derived DC were applied to the basolateral side of the Transwell to allow the DC to adhere to and migrate through the Transwell and the tissue was infected with SARS-CoV2. DC activation and NHBE health were analyzed by various methods after 72h.Preliminary data from the still ongoing experiments indicate that the DC can be activated in this 3D co-culture model by contact with SARS-CoV2, showing a higher amount of activation markers compared to DC in uninfected tissue. Additionally, the migration of DC into the respiratory tissue does not lead to severe tissue damage of the tissue as seen by TEER measurement and the nucleus count, although both decrease with SARS-CoV2 infection.Human moDC can be activated in this 3D human respiratory co-cultivation model, probably by using their dendrites to recognize the apically applied virus.

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Distribution of Aspergillus terreus in soil in Tyrol, Austria

Aspergillus terreus is a filamentous fungus which, in addition to its occurrence as an environmental decomposer, is also as an opportunistic pathogen. While infections with A. terreus are rare, there are medical centers where they are more common, like in Innsbruck. In Tyrol, both infections and natural occurrence are more common in the eastern part, the lowland, than in the western part, the upland; a correlation is suspected.The aim of this study was to verify the higher occurrence of A. terreus in lowland soils and to find reasons for the higher occurrence of the fungus compared to upland soils. Soil samples were collected from multiple upland and lowland sites. These were tested for the presence of A. terreus by plate culture. Samples were also analysed for soil properties such as pH, soil moisture, organic matter and carbon and nitrogen (C/N) content. In 300 soil samples A. terreus was found more frequently in soils of the Tyrolean lowland (about 18%) than in soils of the Tyrolean upland (about 8%). Samples examined for soil properties of the lowland were significantly more humid (about 27 %) than those of the upland (about 23%) and had a lower pH (6.4) than the upland (6.6). The amount of soil organic matter (about 9%) did not differ between the upland and the lowland. Although differences were found between the soils of the Tyrolean up- and lowland, it remains to be investigated whether these parameters also influence the occurrence of A. terreus.

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A new perspective on cellular complement and how opsonized HIV 1 enhances dendritic cell maturation and survival via Mcl-1 stabilization regulated by anaphylatoxin receptors

The complement system is one of the oldest components of innate immunity. It has long been thought that the only functions of this system are the recruitment of other immune cells and the recognition/destruction of invading pathogens. However, studies in recent years have shed new light on the multiple roles of complement in immune regulation and crosstalk with other cellular effector systems. Recent evidence suggests that complement has additional functions within the immune system than just pathogen recognition and immune recruitment. Monocyte-derived dendritic cells (moDCs) were infected with HIV or complement-opsonised HIV (HIV-C) in the presence/absence of specific inhibitors for 24h. We measured expression levels of complement factors and cathepsins, intra- and extracellular anaphylatoxin generation, regulation of anti-apoptotic Bcl-2 family members as well as cell stress, maturation and survival. We could show that HIV-C promotes elevated anaphylatoxin production, maturation and increased survival by stabilizing the anti-apoptotic Bcl-2 family member Mcl-1, via phosphorylation at threonine 163. This effect is regulated by the anaphylatoxin C5a and the interaction with its receptor (C5aR), as upon inhibition, this stabilization was impeded. Upstream pathways controlling this specific phosphorylation event are dependent on MAP kinase and to a lesser extent protein kinase C, since inhibition of either way resulted in reduced Mcl-1 stabilization. In this work, we demonstrated a complement-dependent mechanism that caused improved DC survival and maturation, which could represent a novel approach for enhanced antigen presentation and protective immunity against viral infections.

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Investigating the therapeutic potential of an immune-modulatory cargo-expressing oncolytic virus in a mixed tumor model

Oncolytic virus (OV) therapy is an emerging anti-cancer approach to eliminate cancer cells due to their impaired antiviral defenses. Thus, the viral oncolytic activity releases tumor-associated antigens, which are captured by antigen presenting cells, triggering immune responses to eradicate remaining tumor cells. OVs can be armed with immunomodulatory cargos (IMCs) to support anti-tumor immunity. The vesicular stomatitis virus (VSV), pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) represents a potent OV. Despite defective intrinsic type I IFN pathways and good in vitro susceptibility of the most syngeneic mouse tumor models, the strong systemic type I IFN responses induced by VSV-GP therapies limit the susceptibility of tumors to VSV-GP infection in vivo. This not only reduces the virus's oncolytic capacity but also limits the IMC production encoded by the virus, making it challenging to assess the potential therapeutic effect. To overcome this limitation, we employed a hybrid tumor model by combining transformed murine lung epithelial cells (TC-1) expressing HPV-derived oncoproteins (E6 and E7) and the VSV-GP permissive IFNalpha-receptor knockout TC-1 (TC-1 ifn-/-) cells. Through this model, we examined the efficacy and CD8+ T cell responses subsequent to VSV-GP and VSV-GP-IMC administration. Although the oncolytic virotherapy of the wildtype TC-1 did not result in improved tumor control, virus treatment led to prolonged survival in the mixed tumor model. This together with the phenotypic differences of activated CD8 T cells between VSV-GP and VSV-GP-IMC treatments supports an improved effect of IMCs in the mixed tumor model.

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Prediction and identification of MHC class I presented T cell epitopes of the oncolytic virus VSV-GP in BALB/c mice

VSV-GP is a novel oncolytic virus (OV) platform that recently entered clinical phase I trials. The virus induced strong immune responses in preclinical studies. However, upon OV treatment not only antitumor but also antiviral T cells are activated. To be able to evaluate OV therapies, it is critical to distinguish antiviral and antitumor components of the immune responses. Therefore, we aimed to identify the antiviral CD8+ T cells upon VSV-GP treatment in the widely utilized BALB/c mouse model using a multilevel adapted bioinformatic viral epitope prediction approach. Viral epitopes presented on mouse MHC-I alleles H2-Kd, H2-Dd and H2-Ld were identified using the ELISpot assay, where the IFN-γ secretion upon T cell activation is a measure. To validate the identified VSV-GP epitopes, CD8+ T cells were further analyzed by using intracellular cytokine staining. In total eleven candidates significantly activated CD8+ T cells in the BALB/c mouse model. Custom peptide-MHC-I multimers using the newly identified epitopes allow the direct detection of virus-specific T cells and therefore provide an additional tool to measure anti-VSV-GP T cell responses. Additionally, the identified epitopes were used to compare the antiviral T cell dynamic between intravenous and intratumoral treatment routes in the tumor and the periphery of BALB/c mice. Taken together, the identified epitopes enable monitoring of the full repertoire of antiviral T cells upon VSV-GP oncolytic virotherapy in BALB/c mice. These findings contribute to preclinical development of novel VSV-GP variants by improving assessment of antiviral T cell immunity.

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Modifying oncolytic virus - induced cell death

Oncolytic virotherapy (OV) is a therapeutic concept in which a replication-competent virus lyses cancer cells via preferential infection and/or replication, while sparing normal tissue. VSV-GP is a chimeric oncolytic vesicular stomatitis virus (VSV) with the glycoprotein of lymphocytic choriomeningitis virus (LCMV-GP), which shows abrogated neurotoxicity compared to parental VSV. Infection of cancer cells with VSV usually induces apoptosis resulting in effective tumor control in vitro and in vivo in susceptible syngeneic mouse tumor models. In some models, however, rechallenge with the same tumor type leads to outgrowth, suggesting an insufficient or no activation of anti-tumor immunity. Here we show three concepts to modify VSV-GP – induced cell death facilitating molecular characteristics of necroptosis, an immune system activating, regulated cell death modality. Mechanistically, the three versions of VSV-GP-ICD (immunogenic cell death, ICD) differ in the hierarchic activation of the molecular necroptosis machinery: VSV-GP-ICD1 and -ICD2 make use of TNF receptor family member activation in combination with caspase inhibition. VSV-GP-ICD3 directly activates the downstream necroptosis executioner RIPK3. In contrast to VSV-GP, we found that VSV-GP-ICD induces cell membrane permeability and damage associated molecular pattern (DAMP) release (such as eATP, HMGB1, HSP70, HSP90 release and calreticulin (CRT) exposure). Furthermore, bone marrow – derived dendritic cells (BMDCs) cocultured with VSV-GP-ICD – infected cancer cells showed enhanced phagocytic activity compared to parental VSV-GP infection. Our results demonstrate that VSV-GP–induced cell death can be altered to resemble necroptosis in cancer cells in vitro. Subsequently, this might also translate into enhanced therapeutic outcomes in vivo.

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Tumor suppressor functions of TET2

Programmed DNA methylation patterning serves the generation and maintenance of cell identity, and represents a major barrier against cell transformation. TET2, an enzyme that contributes to the erasure of cytosine methylation via oxidation of the 5′ methyl group, is among the most frequent somatically mutated genes in human hematological tumors, commonly identified as loss-of-function versions. In mouse models, TET2 loss on its own is only weakly tumorigenic, but rather facilitates leukemic transformation driven by prototypic oncogenes such as AML-ETO or FLT3ITD. However, TET2 LOF is not limited to leukemias. In B cell malignancies, TET2 is found mutated in a fraction of diffuse large B cell lymphomas (DLBCL), which display a distinct gene expression profile. Using a MYC-driven mouse lymphoma model and in vitro culture systems, we show that TET2 loss fosters the transformation of B cells that express the MYC oncoprotein. Our data suggests that TET2 may exert tumor suppressor functions via controlling the expression of DNA damage repair genes, or via direct functions of oxidized cytosine species. In addition, TET2-deficient lymphomas select for high expression of the pro-survival BCL2 family protein BCLXL, potentially providing protection from MYC-driven apoptosis. Altogether, our data on the impact of TET2 loss-of-function on DNA damage repair and apoptosis mechanisms creates understanding of how TET2 loss conspires with MYC to overcome the barriers that prevent transformation. Ultimately, our work aims to define therapeutic entry points for hematologic and immune pathologies with impaired TET activity.

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Immune Modulatory Effects on Vessel Barrier Function in 3D-Bioprinted, Vascularized Neuroblastoma-on-chip Model

Neuroblastoma, the most common extracranial childhood solid tumor, accounts for the most cancer-related deaths in children. The progression and metastatic potential of neuroblastoma relies on shaping the tumor environment and stimulating vascularization. Understanding how immune cells affect vessel barriers and migration during metastasis is a vital clinical question, challenging to investigate using conventional 2D cell cultures or tissue samples.We developed a method to 3D-bioprint vessel-containing tissues with tumor spheroids into fluidic chips to study cancer metastasis in multi-organ-on-chip devices. Neuroblastoma tumor will be integrated into a diverse cell type vascularized connective tissue equivalent and connected to 3D-bioprinted liver and kidney tissue surrogates.In this neuroblastoma-on-chip model we will investigate the impact of macrophages, dendritic cells, and cytokines on vessel permeability and tumor cell migration. Furthermore, the impact of anti-angiogenic medications on metastasis will be explored. This approach employs iPSC differentiation, confocal live cell fluorescence imaging, flow cytometry, and RNA sequencing of metastasizing cells.This project will develop a novel 3D-bioprinted, perfused "tumor metastasis-on-chip" model to study vessel barrier function during metastasis-associated intra- and extravasation processes and how immune cells modulate different phases of metastasis, thus providing a platform for drug screening and personalized in vitro drug testing in precision medicine approaches

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Studying signal rewiring in DC subsets in 3D bioprinted skin-cancer-on-chip

Research in tumor immunology heavily relies on mouse cancer models, but translating these findings to the clinical setting poses challenges. There's an urgent need for skin cancer models that closely mimic human conditions. High-content microscopy of tumor patient samples provides only a snapshot, so we aim to develop 3D bioprinted skin cancer models for detailed investigations of cellular interactions. We'll create a disease-on-chip model for melanoma skin cancer and cutaneous metastases by introducing human melanoma tumor cell lines into the epidermal layers of 3D bioprinted skin. Incorporating Langerhans cells (LC) and dermal DC, we will examine interactions with tumor cells through high-content confocal live cell imaging. Skin cancer organoids/spheroids from patient material will also be explored for integration into the 3D bioprinted skin model. Intracellular signaling changes in DC subsets will be studied using RNA-sequencing, and phenotypical changes on the protein level will be assessed via multi-color flow cytometry. Functional consequences will be evaluated through cytokine measurements (Bioplex assays) and ex vivo DC-T cell cocultures. Additionally, autologous T cells will be introduced into the skin cancer on-a-chip model to observe, how tumor growth impacts DC-T cell interactions within the tumor tissue. These efforts aim to provide novel insights into interactions between skin DC subsets and tumor cells, informing the development of innovative immunotherapeutic strategies. The human skin cancer on-a-chip models may serve as valuable tools for future translational studies in immunotherapy.

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MED12 promotes prostate cancer (PCa) cell proliferation through c-MYC and androgen receptor (AR) signalling

Prostate cancer growth (PCa) is driven by the androgen receptor (AR) signaling, a target of therapeutic approaches such as treatment with enzalutamide. Some subunits of the Mediator complex promotes PCa by enhancing the AR signaling. Indeed, the Mediator complex is a multi-subunit protein that modulates gene expression by composing the pre-inititation complex (PIC). Its subunit MED12, which composes its kinase module, is overexpressed in advanced PCa. In this project, we aimed to investigate if MED12 promotes PCa and influences the response to enzalutamide. MED12 inhibition significantly reduced cell proliferation in three PCa cell lines (LNCaP, 22Rv1, and PC3) and in their respective 3D spheroids. MED12 knockdown significantly inhibited the c-MYC pathway, fundamental for promoting cell proliferation. Moreover, MED12 downregulation increased the cell/volume ratio in LNCaP and PC3 spheroids, suggesting that it modulated cell-to-cell contact. Loss of MED12 in 22Rv1 decreased the protein expression of AR-variant 7 (AR-V7) (60%), which is a ligand-independent AR splice variant that promotes enzalutamide resistance. Combining enzalutamide treatment with MED12 loss in 22Rv1 additively reduced prostate-specific antigen (PSA) secretion (i.e. AR signaling) in 22Rv1 cells. Inhibiting Cdk8/19, the kinase subunits of the mediator complex, also reduced PSA secretion in both LNCaP and 22Rv1 cells. Our data reveal that MED12 downregulation promotes multiple malignant processes in PCa, as cell proliferation, cell-to-cell contact and enzalutamide resistance. Further studies will elucidate if the inhibition of the Mediator complex kinase module (i.e., Cdk8/19 inhibition) produces similar effects.

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TFEB orchestrates stress recovery and paves the way for senescence induction in human dermal fibroblasts

In the realm of stress-induced premature senescence (SIPS), cells confront oxidative stress and widespread cellular damage, with mitochondrial integrity playing a crucial role. Central to mitigating this damage is the promotion of autophagy, particularly through an increase in lysosomal number. This study explores the dynamics of lysosomal quality control in this context, specifically investigating lysosomal signaling pathways during SIPS. Our findings delineate distinct signaling responses between the initial stress phase and subsequent senescent phase. In the stress phase, we observe an escalation in lysosomal damage, paralleled by an increase in reactive oxygen species (ROS) and mitochondrial dysfunction. This surge in ROS triggers AMP-activated protein kinase (AMPK) activation and Akt inactivation, leading to mammalian target of rapamycin (mTOR) suppression. The inactivation of mTOR during this phase facilitates the activation of Transcription Factor EB (TFEB), a key player in modulating ROS levels, augmenting autophagy, and enabling cellular survival. Interestingly, TFEB knockdown cells under stress showed increased apoptosis, highlighting TFEB's protective role in stress response. As cells transition into the senescence phase, the prior activation of TFEB, having facilitated the clearance of damage through autophagy, becomes less crucial; consequently, with the reduction in damage, TFEB activity is suppressed. The reduction in ROS levels normalizes AMPK and Akt signaling, reactivating mTOR. This reactivation of mTOR, pivotal in establishing the senescent state, mediates the inactivation of TFEB. Our results demonstrate a dynamic interplay between TFEB and mTOR, highlighting their critical roles in modulating cellular fate during the transition from stress response to senescence.

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Targeting mitochondrial defects in a 3D-bioprinted LCHADD/VLCADD model

Background: 3D bioprinting technologies are revolutionizing tissue engineering, especially in studying metabolic processes using patient-derived cells. Long-chain-3-hydroxy-acyl-CoA-dehydrogenase-deficiency (LCHADD) and Very-long-chain-acyl-CoA-dehydrogenase-deficiency (VLCADD) are rare disorders of the oxidation of long-chain fatty acids (LC-FA). Therapy mainly involves a diet restricted in LC-FA, supplement substitution, and fasting avoidance. Innovative strategies are needed to reduce mortality and improve quality of life.

Methods: The study examines mitochondrial rearrangement in β-oxidation-defective fibroblasts by exposing them to various rescuers in 2D. Mitochondrial morphology was analyzed using live cell fluorescence microscopy and quantified by counting the number of branches and dots. To mimic tissue physiology, a 3D-bioprinted, vascularized tissue model with healthy and patient-derived fibroblasts was developed.

Findings: Analysis of mitochondrial morphology in patient fibroblasts revealed significant alterations of mitochondrial morphology, reduced oxidative phosphorylation, but increased glycolysis and significantly increased intracellular ROS levels caused by NOX2. NOX2 inhibitors and other rescuers led to mitochondrial network refusion. 3D-bioprinted tissue surrogates showed impaired vessel formation in the presence of patient fibroblasts.

Interpretation: Our findings will improve the understanding and therapy of LCHADD/VLCADD, as there are no direct therapies for elevated ROS levels and mitochondrial dysfunction. 3D-bioprinted patient tissue models will be used for testing novel treatment modalities and tissue response during physiologic stress.

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The Role of Homologous Recombination Repair in Cisplatin-Resistant Bladder Cancer

Neoadjuvant cisplatin-based chemotherapy (NAC), followed by radical cystectomy is the golden standard for muscle invasive bladder cancer (MIBC) patients. However, there is a high resistance rate to the platinum-based chemotherapy treatment. Previous research from our group showed a correlation between a chromosomal amplification at location 7p12, and NAC non-responders. They showed that HUS1, RAD51 and other members of homologous recombination repair (HRR) are involved in the mechanisms behind the platinum-based chemotherapy resistance. The aim of this study is to unravel the role of HRR in cisplatin-based chemotherapy resistance in bladder cancer. Our preliminary data showed for the first time increased HRR in cisplatin-resistant bladder cancer cells, when compared to the parental cell lines. Additionally, we found increased basal RAD51 and CHK1 protein expression levels in the cisplatin-resistant cells. Besides this, western blot analyses indicated a direct correlation between two important members of HRR, HUS1 and RAD51. Furthermore, cell viability was inhibited when the parental cells were treated with cisplatin in combination with downregulation of HUS1 or RAD51. These results indicate the role of HRR in cellular viability in the presence of cisplatin. However, the complete mechanism of HRR in platinum-based chemotherapy resistant bladder cancer is still not fully known. Our HUS1 and RAD51 silencing results opened a new possible treatment strategy in overcoming platinum-based chemotherapy resistance. With this research, we are taking the first steps in unravelling the specific mechanisms involved, and we are moving towards new treatment strategies such as PARP/ATR inhibitors in bladder cancer.

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Cell-cell fusion can trigger the PIDDosome-mediated stabilization of p53

In pathological cell - cell fusion, as induced by certain viral or bacterial infections, asynchronous cells fuse to form multinucleated syncytia that exhibit supernumerary centrosomes. The PIDDosome, a multiprotein complex composed of PIDD1, RAIDD, and Caspase-2, responds to the presence of supernumerary mature centrioles and induces the Caspase-2-mediated cleavage of MDM2, allowing the establishment of a p53-dependent and p21-mediated cell cycle arrest. Besides, the PIDDosome was assigned a role as a cell death regulator in the DNA damage response by a yet unknown mechanism. To investigate whether the centrosome amplification induced by asynchronous cell-cell fusion induces the activation of the PIDDosome, we exploited the fusogenic properties of the VSV glycoprotein (VSVG). Here we show that cell fusion indeed triggers a PIDDosome response, which depends on the recruitment of PIDD1 to mature centrioles. Moreover, PIDDosome activation correlates to reduced cell cycle progression and enhanced protection of syncytia from cell death.

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Is cardiolipin remodelling a pivotal membrane lipid repair mechanism?

Cardiolipins are at the metabolic front line in the protection against reactive oxygen species that are generated in the mitochondrial membranes by the respiratory chain, thereby sacrifice themselves. To preserve mitochondrial functioning after peroxidation of their polyunsaturated side chains, these damaged lipids either have to be conveniently repaired, or degraded and replaced by de novo biosynthesis. We hypothesize that the cardiolipin repair mechanism is initiated by cleavage of damaged cardiolipin side chains by specific phospholipases and subsequent reacylation by the transacylase tafazzin, which is already described as a cardiolipin remodelling enzyme. However, tafazzin's contribution to repair processes has to be elucidated. Here, we used cell culture models for the monogenic diseases Barth Syndrome, MEGD(H)EL Syndrome, and Sengers syndrome, all impair enzymes contributing to the cardiolipin metabolic pathway, to obtain insights into the relative contributions of biosynthesis, remodelling and repair to cardiolipin homeostasis. A major step was the establishment of a 13C steady-state labelling assay, based on liquid chromatography mass spectrometry, to determine the incorporation rate of fatty acids into cardiolipins. In parallel, we characterized the mitochondrial morphology showing a strong phenotype in all diseases studied. Parallel oxidative stress measurements allow us to correlate cardiolipin repair rates with lipid peroxidation events and ROS levels in the same cells. In summary, we were able to observe strong mitochondrial phenotypes in all three monogenic diseases tested and set the basis for other steady-state labelling experiments with different fatty acids and glucose for precise quantification of the individual sub-processes and their stress-related dynamics.

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Membrane lipid alterations in β-oxidation disorders

Physiological cellular functioning requires the constant repair of damaged complex lipids in biological membranes. Alterations of membrane lipids have been reported in mitochondrial β-oxidation disorders, such as Very Long-chain acyl-CoA Dehydrogenase Deficiency (VLCADD) and Long-chain 3-hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD). However, it is still a highly challenging task to mechanistically link the membrane damage to certain pathologies of the respective metabolic diseases. Here, we characterized fatty acid metabolism in VLCADD and LCHADD patient-derived fibroblast and respective CRISPR/Cas9 knockout models in HEK-293T cells, using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We confirmed the expected phenotypes firstly by observing accumulation of long-chain carnitines in VLCADD/LCHADD and increased levels of hydroxylated long-chain carnitines in LCHADD cells, as well as by measuring fatty acid oxidation (FAO) via high-resolution respirometry. Previously reported elevated levels of ROS in the patient's fibroblasts were also detected in our HEK-293T models. Alterations of the membrane lipids were characterized by an untargeted lipidomics analysis approach, where nearly 600 lipids species were annotated across sixteen different lipid classes. Changes were detected in different lipid species, especially in ceramides, as well as in ether- and vinyl ether-linked phospholipids while for example the mitochondrially localized cardiolipins were unaffected. Based on our findings, we confirmed that pathologies affecting mitochondrial β-oxidation not only alter fatty acids metabolism, but also change the composition of membrane lipids, particularly ceramides and phospholipids. This represents the first essential step towards obtaining a better understanding for how membrane lipid damage contributes to the pathomechanism of β-oxidation disorders.

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Structural determinants of Dopamine Receptor Agonist selectivity

Dopamine receptors (DRs) are G protein-coupled receptors (GPCRs) expressed in the central nervous system. When activated, DRs can trigger different downstream signal transduction cascades based on which they are divided into two major classes: D1-like, which includes excitatory D1 and D5 receptors, and D2-like, including inhibitory D2, D3 and D4 receptors. Drugs acting on DRs are used to treat several neurological disorders, like Parkinson´s disease (PD), schizophrenia, depression or bipolar disorders. PD, for example, is caused by the early death of dopaminergic neurons in substantia nigra pars compacta. Therapeutic options for PD acting on DRs include the D2-like selective agonists ropinirole and pramipexole. Despite their approval over two decades ago, a comprehensive investigation of the structural determinants responsible for their D2-like DR selectivity has not yet been conducted. In this work we therefore aim to elucidate the molecular determinants conferring (un)selective binding of DR agonists, including dopamine, pramipexole and ropinirole. For this purpose, we investigated DRs signaling cascades through 16 Galpha-proteins (collectively named the transducerome) using bioluminescence resonance energy transfer (BRET) biosensors and a luminescence based cAMP assay. Computational analyses allowed us to perform information driven site-directed mutagenesis. Selected mutations in D2 and D1 receptors modified the efficacy and/or potency of pramipexole and dopamine dependent activation in comparison to wild-type receptors. In this study, we were able to identify hotspot mutations within the binding pocket of D1 and D2, which are involved in D2-like pramipexole selectivity, efficacy and potency.

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Exploring structure-activity relationships on new diphenethylamines interacting with the kappa-opioid receptor

The knowledge that the kappa-opioid receptor (KOR), opposite to the mu-opioid receptor, does not produce euphoria, respiratory depression or risk of overdose has stimulated the interest in discovering drugs acting on the KOR as potential therapeutics. Our group reported on diphenenthylamines as novel class of selective KOR ligands with distinct pharmacology. This study expands the structure-activity relationships (SARs) on our previous series of compounds by introducing structural variations in the lead molecules, 3-hydroxy, N-cyclobutylmethyl (HS665) and 3-hydroxy, N-cyclopropylmethyl (HS666) substituted diphenenthylamines. A library of new diphenethylamines was designed targeting different substitutions at positions 3, 3′, 4 and 4′, and modifications at the nitrogen. Binding studies showed the new diphenethylamine derivatives to bind to the human KOR with affinities in the nanomolar range. They also demonstrated KOR selectivity over the other opioid receptor types. The G-protein activation studies established their functional profile to the KOR, from full to partial agonists to antagonists. One diphenethylamine analogue was evaluated for antinociceptive efficacy and potential for inducing sedation/motor dysfunction after subcutaneous administration in mice. Dose-dependent antinociception and KOR-mediated mechanism was established in the writhing assay, together with the lack of sedation in the rotarod test. Remarkably, this compound had comparable or even higher antinociceptive potency than the lead molecules. The emerged SAR observations highlight the value of the diphenethylamine scaffold for the discovery of small molecules selectively interacting with the KOR, and as effective and safer therapeutics for the treatment of pain and other human diseases where the KOR plays a key function.

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Proteome analysis of mouse retinas carrying pathogenic Cav1.4 L-type calcium channel variants

Cav1.4 L-type calcium channels are predominantly expressed in photoreceptors, supporting tonic glutamate release. Mutations in the CACNA1F gene, encoding the Cav1.4 channel can cause congenital stationary night blindness type 2 (CSNB2), a rare X-linked retinal disease leading to a variety of symptoms, like nystagmus, photophobia, low visual acuity and variable levels of night blindness. Exemplary we focus here on two pathological Cav1.4 CSNB2 variants, R1827X (RX) and I745T (IT), which showed different biophysical channel characteristics. In this study we investigated whether the different phenotypes observed in mouse retinas could be associated with differentially expressed proteins and/or dysregulated pathways. We pooled six mouse retinas per genotype (wild type (WT) versus RX and IT). Retinal lysates (20 µg) were loaded on a SDS-PAGE and subsequently tryptic-in-gel digest was performed. Differential proteome analysis by LC-MS was performed by label free quantification using data-independent acquisition. We detected on average 4000 proteins in each condition. 345 proteins were differentially expressed in the RX retina compared to WT whereas in IT even 2167 proteins hit the criteria. Gene ontology analysis suggested similar downregulation of proteins important for vision in both mutant mouse model. Interestingly, we also observed dysregulated RNA binding proteins important for mRNA splicing. However, relevant clusters comprising retinal degeneration pathways or synapse interaction proteins were different in RX and IT. Our approach might not only bear an explanation for the differential retinal phenotypes that we observed but will also allow us to explore so far unknow pathways underlying the CSNB2 phenotype in the future.

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Hypersensitivity of a mouse model of autism spectrum disorder in response to the interoceptive effects of mild-hypercapnia

The subjective experience of internal sensation processing (interoception) which affects emotions, appears to be altered in autism spectrum disorder (ASD) and in anxiety disorders. Anxiety is a frequently co-occurring condition in patients with ASD, exacerbating ASD symptoms and causing functional impairments in these individuals. In contrast to anxiety in normotypic populations, our knowledge about the neuronal correlates of anxiety in ASD is very limited. Here we investigated behavioural responses of Setd5 haploinsufficient (Setd5+/-) mice, which demonstrate signs of ASD and altered anxiety-related behavior, to CO2 inhalation. This is a highly translational paradigm for inducing hypercapnia, i.e. elevation in the partial pressure of carbon dioxide, and is known to activate interoception and trigger anxiety. Exposure to 10% CO2 decreased locomotion and increased anxiety-related parameters indicated by center entries, center time, and rearing characteristics in both lines compared with control conditions. Relative to air, CO2 caused more pronounced behavioural changes in Setd5+/- than in wildtype controls. Sex-specific behavioral patterns were revealed. Taken together, the greater effect of CO2 on anxiety-related behaviours points towards hypersensitivity of SetD5+/- mice to the interoceptive effects of mild-hypercapnia. Thus, CO2 inhalation is suggested to be an interesting paradigm for studying neurobiological mechanisms underlying interoception-induced anxiety in ASD. To identify neuronal substrates of this difference, imaging studies using immediate early gene expression as a marker for neuronal activation are currently ongoing in CO2-exposed SetD5+/- and wildtype mice focusing on brain areas known to be involved in anxiety- and interoception processing, including the medial prefrontal cortex, insula, and hypothalamus.

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Computational identification of residues influencing kappa-opioid receptor coupling preferences

G-Protein coupled receptors (GPCRs) play a crucial role as targets in drug development and are involved in the mechanism of action of various drugs on the market. GPCRs in general consist of seven transmembrane helices connected by 3 intracellular and 3 extracellular loops and mediate the transfer of external signals into the cell. Extracellular ligand binding and subsequent conformational changes therefore result in intracellular coupling of either a G-protein heterotrimer, consisting of an α-, β- and γ-subunit, or arrestins. Depending on their downstream signalling, Gα-subunits can further be divided into 4 different classes, i.e. Gαi/o, Gαq, Gαs, Gα12/13. The kappa-opioid receptor (OPRK) represents a GPCR that mediates its downstream signalling primarily through inhibitory Gαi/o-subunits. To date, detailed structure-related explanations for specific interaction preferences have not been fully elucidated. We therefore aimed to identify residues influencing the coupling preferences of OPRK. For this purpose, various homology models of the k-OR together with different Gα-subunits were generated. Comparison of these models to experimental structures of GPCRs primarily signalling via other classes of Gα-subunits, helped formulating hypotheses for coupling preferences and identify potentially crucial residues. Subsequently, site-directed mutagenesis of selected residues was performed, and the signal transduction preference of the mutants in comparison to the wildtype receptor was investigated using a bioluminescence resonance energy transfer assay. Remarkably, various different alterations in the interaction preferences of the mutated receptors could be observed, which were investigated in detail using molecular dynamics simulations to further elucidate their molecular mode of action.

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Genetic deletion of stac2 adaptor protein alters electrical activity of mouse chromaffin cells

Electrical activity of mouse chromaffin cells (MCCs) is tightly controlled by voltage-gated L-type Ca2+ channels (LTCCs). Src homology 3 and cysteine-rich domain adaptor proteins (Stac) have recently been identified as new regulators of neuronal LTCC expression and biophysical properties. Upon overexpression in cultured hippocampal neurons Stac2 isoform abolished LTCC Ca2+ dependent inactivation via an allosteric inhibition of calmodulin binding. Additionally, deletion of the homologous gene in Drosophila reduced LTCC Ca2+ transients and neuropeptide release.

To elucidate the endogenous function of Stac2 we here investigate the first mouse model bearing a constitutive Stac2 deletion. To test if Stac2 deletion affects Ca2+ channels and neuron-like firing patterns we performed whole-cell patch-clamp experiments in isolated MCCs from wildtype (WT) and Stac2-/- mice.

Current-clamp experiments revealed that resting membrane potential and spontaneous firing frequency were not affected by Stac2 deletion. However, the AP depolarization threshold was significantly reduced in Stac2-/- MCCs compared to WT. Additionally, step current injection showed that Stac2-/- MCCs responded with a reduced rheobase and an increased AP firing frequency at the onset of the pulse, suggesting that they are easier to depolarize. While whole-cell Ca2+ current density was not different, the voltage dependency of activation was significantly shifted by ~6 mV toward more negative membrane potentials in Stac2-/- MCCs. Altogether, these data show for the first time that genetic deletion of Stac2 affects neuron-like firing behaviour and calcium currents in its native environment.

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Circumventing Imatinib Resistance in Chronic Myeloid Leukemia: Novel Telmisartan-based Chemosensitizers with Improved Efficacy and Stability

Chronic myeloid leukemia (CML) is a myeloproliferative disease caused by the mutation and proliferation of a monoclonal multipotent hematopoietic progenitor cell. Following gene products show a dysregulated tyrosine kinase activity resulting in enhanced proliferation and resistance to apoptosis of CML. Nowadays, targeted therapy of CML with tyrosine kinase inhibitors (TKIs) is available. However, the inability to attain a complete molecular response in most patients due to TKI-resistant cancer cells, has necessitated the exploration of new therapeutic methods to effectively combat these resistances. Previous studies performed by our group indicated that TKI-resistant cells can be efficiently targeted by a combination therapy of Imatinib and derivatives of the angiotensin 2 antagonist Telmisartan. In the present investigation, we continued designing chemosensitizers based on Telmisartan with the intention to improve their efficacy and particularly their stability while maintaining their selectivity. To evaluate the activity and selectivity of our developed compounds we performed a modified MTT assay with wild type K562 CML cells and their respective Imatinib resistant subclone K562-R. With the new compounds, we could increase the cytotoxic activity of Imatinib on resistant CML cells from 10% to >90%. In addition, the compounds were per se non-cytotoxic. The stability of the ester derivatives was investigated in the presence of porcine liver esterase using a validated HPLC method. Crucially, the designed ester derivatives demonstrated markedly enhanced stability against esterase cleavage. These advancements are a significant step towards future in vivo studies, as they promise to enhance the bioavailability and the therapeutic efficacy of these compounds.

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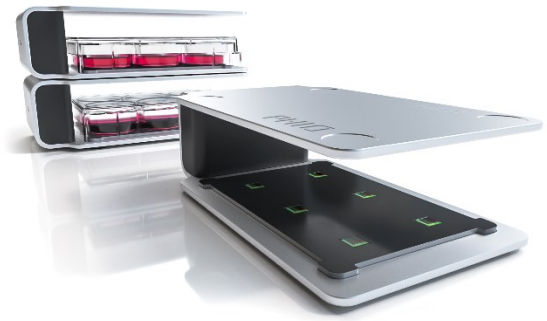

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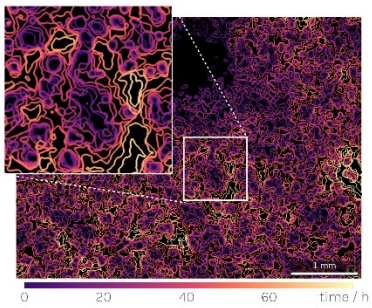
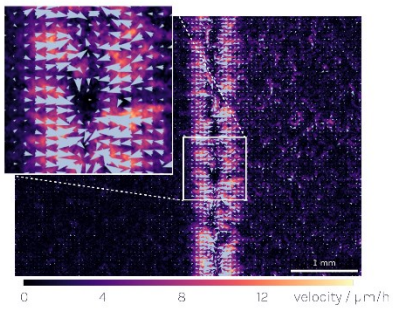
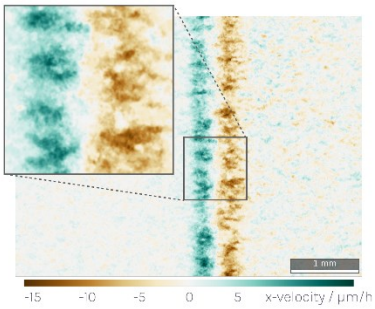


Cluster growth of stem cells



Spatially resolved cell motility of a scratch



Cell motility heatmap of a scratch

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Mitochondrial dysfunction induced by iron(III) salophene complexes

Iron(III)salophene complexes have already shown encouraging antitumor activity. However, detailed information on the effect on mitochondria are missing. Therefore, it was the aim of this study, to investigate the effect of methoxy-substituted iron(III) salophene complexes on the mitochondrial activity of the acute myeloid leukemia (AML) cell line HL-60 and the triple negative breast cancer cell line MDA-MB 231 by various cell-based assays. The iron(III) complexes significantly inhibited metabolic activity of both tested cell lines, analyzed by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Furthermore, the mitochondrial network was altered and mitochondrial function was impaired, detected by live confocal imaging, flow cytometry and high-resolution respirometry. The dysfunction of the mitochondria finally resulted in cell death which was analysed by flow cytometry using the Annexin V/propidium iodide staining. Additionally, the complexes released the detoxifying enzyme copper/zinc superoxide dismutase in the cell culture supernatant, whereas the thioredoxin system was not strongly affected by the compounds. The position of the methoxy group on the salicylidene moiety had no effect on the activity of the compounds. These data provide valuable insights into the mechanisms underlying the cellular effects of methoxy-substituted iron(III) salophene complexes.

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Complex gating changes of missense CACNA1D (Cav1.3) variants can cause diverse clinical phenotypes

Background: Ca2+-influx through voltage-gated Cav1.3 Ca2+ channels (encoded by the CACNA1D gene) is paramount for several physiological processes. In humans, homozygous CACNA1D loss of function (LOF) causes bradycardia and deafness (SANDD syndrome) whereas de novo (heterozygous) variants causing complex activity-enhancing gating changes are associated with a neurodevelopmental disorder (NDD). Herein, we aim to functionally characterize two missense mutations causing SANDD (inherited homozygous variant A376V, reported in 10 patients) or NDD (de novo mutation V1447L) to test if distinct gating changes could explain the different clinical phenotypes.

Methods: Whole-cell patch-clamp recordings were performed in HEK-293 cells transfected with wild-type, A376V or V1447L full-length Cav1.3 α1, β3 and α2δ1 subunits.

Results: Cells expressing the V1447L variant alone or together with wild-type revealed a significant hyperpolarising shift in the half-maximal activation/inactivation voltage by ~-9 mV together with other complex gating changes as previously observed with several NDD-associated CACNA1D variants. A376V, expected to cause LOF, unexpectedly showed robust currents and similar gating changes, with voltage-dependent gating shifted by ~-7.5 mV and faster inactivation kinetics.

Conclusion: Our data demonstrate the pathogenicity of the V1447L variant, and revealed a dominant effect on wild-type channels when expressed together. Surprisingly, A376V is the first complex gating-modifier leading to a clinical LOF phenotype (SANDD syndrome). Further studies using more physiological model systems and in silico modelling are required to elucidate how these complex gating changes can mimic the SANDD phenotype so far only described in individuals with non-functional Cav1.3 channels.

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Modulation of the membrane and functional expression of the voltage sensor of EC coupling CaV1.1 by ERC1

ERC1, a member of the family of CAST/ELKS scaffold protein, is crucial for the assembly of presynaptic active zones. ERC1 was reported to directly interact with the CaVβ subunit of voltage-gated Ca2+ channels (VGCC) and boost VGCC expression and activity. Here, we hypothesized that ERC1, endogenously expressed in skeletal muscle, might influence the membrane and functional expression of the voltage sensor of EC coupling CaV1.1, as well as the voltage-induced Ca2+ release from the RyR1. To analyse the effect of ERC1 on CaV1.1 functional expression, we performed patch-clamp experiments in HEK cells and found that ERC1 increases CaV1.1 current density by 63%. Conversely, the ERC1 fragment 1-404, which was suggested to bind CaVβ, only partially modulates CaV1.1 currents in HEK cells (29.4%). Next, to analyze the role of ERC1 in skeletal muscle, we generated two ERC1 knockout muscle cell lines with CRISPR/Cas9. First, we performed patch clamp coupled recordings on muscle cells reconstituted with CaV1.1 and ERC1. ERC1 reconstitution increased EC coupling but not CaV1.1 currents. We therefore examined the impact of ERC1 reconstitution on CaV1.1 and RyR1 expression levels in skeletal muscle using immunocytochemistry. Whereas CaV1.1 cluster intensity is mildy enhanced (15%), RyR1 expression remains unchanged. Conversely, overexpression of ERC1 in wild type muscle cells decreased CaV1.1 currents, without affecting EC coupling. Immunocytochemistry analysis revealed that the CaV1.1 cluster intensity was also enhanced with ERC1 overexpression. Altogether, our results suggest that ERC1 plays an important role at the triad in the assembly of the skeletal muscle excitation-contraction coupling complex.

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Functional anatomy of spiral ganglion neurons in auditory information processing

In the mammalian ear, sound signals in form of pressure waves, are picked up by neurosensory inner hair cells (IHCs) that translate them into neural code. They are then conveyed to spiral ganglion neurons (SGNs), which project onto the cochlea nucleus (CN) in the brain stem. The cochlea of the inner ear is organized in a tonotopic manner, with IHCs and SGNs encoding high frequency signals in the base of the spiral and the ones encoding low frequencies in the apex. The majority of SGNs are classified into three molecularly distinct subtypes, which likely correlate to three different firing behaviors identified using electrophysiology. These patterns of activity are believed to be the basis for sound amplitude encoding. Their crucial function in the auditory pathway in combination with their anatomical approachability makes SGNs one of the prime targets for treating hearing disabilities. Population wide profiling of SGNs regarding RNAs, proteins as well as electrical behavior over the last decades has revealed an astonishing diversity. Apart from their function in information transfer, it became clear that SGNs play crucial roles in filtering and preprocessing. In order to understand how they do so, my work aims to link together these findings in an anatomical context. By describing morphological landmarks like the axon-initial-segment, size and shape of the soma and patterns of myelination as well as establishing an anatomical comprehensive connectivity map reaching up to the CN, I hope to further the understanding of how SGNs are organized in their functional network.

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In vitro investigation of the role of specific miRNAs involved in neuronal injury and regeneration

microRNAs (miRNA) are short single-stranded non-coding RNAs (ncRNA) of approximately 22 nucleotide in length that function as master switches in almost all biological processes in health and disease by regulating gene expression, including neuroregeneration and neuropathic pain. Sequencing data from our lab identified dysregulated miRNAs in the dorsal root ganglia (DRG) of mice subjected to the spared nerve injury (SNI) of which some appear to be critical for the establishment of neuropathic pain whereas others seem to be involved in neuroregenerative processed. In order to identify the signaling pathways involved in the regulation of these miRNAs this project will pharmacologically induce or silence the expression of specific miRNAs in in vitro cultured neuron-like cells, such as the SH-SY5Y cell line. Furthermore, to elucidate the biological role of these miRNAs in humans, miRNA mimics and inhibitors as well as inducible plasmids and viral vectors will be applied on SH-SY5Y cells and, upon validating the aforementioned tools, on human induced pluripotent stem cell (iPSC) derived nociceptors (iNocs). miRNAs will be expressed or silenced and their target genes will be identified. The effects of the above will be analysed by utilizing a combination of methodologies, such as RNA sequencing, RT-qPCR, in situ hybridization, Western blot, immunocytochemistry and in vitro outgrowth assay. Overall, this project aims to provide evidence for the potential use of these miRNAs in therapeutic approaches for peripheral neuropathy and neurodegenerative disorders.

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Nuclear lamina–dependent mechanisms in neuroplasticity

The nuclear lamina is a filamentous meshwork of A- and B-type lamins attached to the nuclear envelope via interactions with inner nuclear membrane proteins. Emerging evidence from non-neuronal cell types implicates interactions between the nuclear lamina and chromatin, i.e., lamina-associated domains (LADs) in the regulation of both spatial genome organization and gene expression. A comprehensive understanding is currently lacking on whether these mechanisms also operate in neurons and, if so, which proteins serve as neuronal chromatin-nuclear lamina tethers and what their relevance is for activity-driven gene transcription. Previously, we have shown that the INM protein LEMD2 and the DNA binding protein SATB2 play a critical role in the regulation of nuclear shape, genome organization and activity-dependent gene expression programs in cortical neurons. Here, we hypothesize that LEMD2-SATB2 protein complex regulates the LADs dynamics upon pharmacological synaptic activation, thereby affecting both the radial genome architecture and the gene expression in pyramidal neurons.
To test our hypothesis, we use primary cortical cultures derived from WT, Satb2 cKO vs floxed mice, as well as Lemd2-deficient vs Lemd2 floxed cortical neurons. We are currently investigating activity-dependent changes on Lamin A/C- and Lamin B1-LADs and the effects of SATB2 and LEMD2 ablation on such dynamics. Our study will provide novel insights into the mechanisms of chromatin-nuclear periphery interactions in cortical neurons and their role in neuronal plasticity processes, underlying higher cognitive functions, e.g., learning and memory formation.

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Investigating and modulating κ-Opioid Receptor G-protein signaling.

The κ-Opioid receptor (OPRK) belongs to the family of G-protein coupled receptors (GPCRs), is mainly localized at axon terminals in different brain regions, and is involved in e.g., nociception, consciousness, or mood. Like all GPCRs, OPRK mediates downstream signaling via e.g. heterotrimeric G proteins, consisting of Gα-, Gβ-, and Gγ-subunits, or β-arrestins. OPRK is reported to primarily signal via the inhibitory Gαi/o family and also exhibits interactions with Gα12, however, how OPRK mediates this preference has not yet been elucidated. In this project we therefore aim to explore the molecular determinants underlying OPRK Gα subunit selectivity employing information driven mutagenesis. For this purpose, computational analyses were used to identify important amino acid residues (aa) responsible for OPRK Gα preference. Following OPRK mutagenesis, we employed the Bioluminiscence Resonance Energy Transfer based TRUPATH assay to analyse OPRK wildtype and mutants coupling selectivity to different Gαβγ subunit combinations. In addition to the already reported OPRK Gα12 interaction, we were able to show that OPRK wildtype can also signal via the Gα15- subunit. Furthermore, the characterization of mutations allowed us to identify several critical aa responsible for the observed OPRK signal transduction mediated by Gα12 and Gα15 . While it remains unclear if the acquired knowledge can be extrapolated to other GPCRs, our results significantly deepen our insights into OPRK-signaling.

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SATB2-dependent mechanisms in human cognitive ability and neuropsychiatric disorders

SATB2 is genetically associated with human intelligence and schizophrenia. Individuals with SATB2 haploinsufficiency suffer from SATB2-associated syndrome (SAS), defined by developmental delay, severe intellectual disability, and absent/limited speech. We have extensively characterized SATB2-dependent mechanisms in higher brain functions using mouse conditional knockout models, demonstrating an important role for SATB2 in late-LTP and long-term memory consolidation. At molecular level, our data have established SATB2 as a 3D epigenome organizer, which sets up the chromatin landscape of pyramidal neurons for cognitive processes. Here, we generate SATB2 + and - cortical glutamatergic neuronal models from human induced pluripotent stem cell (hiPSC)-derived neuronal progenitor cells overexpressing Neurogenin 2. In this study, we report differentialy expressed genes (DEGs) between the two populations of excitatory neurons dependent on SATB2. Based on GO enrichment analysis we find DEGs linked to synapses, behavior and membrane potential. Currently, we aim to further subject this model to assess the role of SATB2 regarding binding sites and chromatin accessibility regions. These analyses will uncover effects of SATB2-directed 3D genome folding as a mechanism of transcriptional control in cortical neurons and its evolution between the two species as a correlate of human higher cognitive ability. Furthermore, our data will provide novel insights into gene regulatory mechanisms associated with human intelligence and neuropsychiatric disease.

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Differences of Designer Receptor Exclusively Activated by Designer Drugs (DREADD) signaling preferences compared to wild type receptors

Differences of Designer Receptor Exclusively Activated by Designer Drugs (DREADD) signaling preferences compared to wild type receptors DREADDs have revolutionized neuroscience research and hold promise for therapeutic applications. While their ability to modulate neuronal activity is well established, the specific signaling pathways they can trigger remain only partially explored.

In this project, we therefore examine the signaling preferences of the human muscarinic acetylcholine receptor M3 and M4 (hM3/4) and the respective DREADDs (hM3D and hM4D). Additionally, we evaluate the impact of the individual single point mutations Y3.33C and A5.46G, which convert the wild type receptors to the DREADD. We investigated signaling of all receptors to 13 different heterotrimeric G-protein complexes and β-arrestin, using the bioluminescence resonance energy transfer based TRUPATH and luminescence based Presto-TANGO assays.

In this study, we identified substantial changes in signaling between hM3/4 and the associated hM3D/4D DREADDs. hM3D selectively couples Gαq/11 while hM3 couples to multiple subunit classes. hM4D gains Gα13/Q signaling and showed significantly reduced efficacy in Gαi activation in comparison to hM4. Additionally, we observed increased basal hM3D/4D β-arrestin signaling compared to hM3/4. Investigating the impact of the single point mutations in hM3/4 further allowed us to pinpoint the modifications responsible for the observed signaling alterations.

The activation of different G-protein subunit families leads to specific downstream signaling effects, and, subsequently, to a wide variety of physiological responses. A detailed characterization of DREADDs signaling is therefore pivotal for improved understanding of DREADD overexpression and activation dependent behavioral modifications, and essential for potential translational applications.

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Role of Zona Incerta Neurons in the Integration of Feeding and Fear

The zona incerta (ZI) is a subthalamic region in the brain, which comprises loosely packed albeit mixed populations of several neurons. However, an in-depth understanding of anatomical connections of neurons emerging 'from' and/or 'to' the ZI, and their functional implications, remain elusive. The ZI has recently gathered considerable research interest because of its role in primal survival-associated instincts, like appetitive drive, hunting, food intake and fear generalization. We thereby aimed to identify and characterize the neuronal populations and subpopulations, which may be involved in emotional (anxiety, fear etc.) and metabolic (fasting, feeding etc.) circuitries. For achieving this, we combined the widely known bacteria-derived tetracycline trans-activator controlled genetic tagging system (tet-tag) with Designer Receptors Exclusively Activated by Designer Drugs technology (DREADD). We injected C57BL/6N wild type mice (males and females) with tet-tag-DREADD vectors bilaterally into the ZI in order to 'tag' fasting-associated neurons (FANs) and assessed the effect of their activation and inhibition on anxiety and fear-related responses. Notably, chemo-genetic activation of FANs increased food and water intake in mice whereas, their inhibition led to exact opposite effects. Both, chemo-genetic activation and inhibition of FANs did not have an effect on anxiety-related behaviour. However, chemo-genetic activation of FANs promoted fear extinction. Since the ZI is well connected with many emotional and metabolic hubs in the brain, further investigations will reveal the identity and composition of the neuronal populations involved in integration of feeding and fear.

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A Characterization of Somatostatin expressing neurons in the Bed Nucleus of the Stria Terminalis

The bed nucleus of the stria terminalis (BNST) is a part of the so-called extended amygdala, and a sexually dimorphic forebrain area. Mostly studied for its involvement in emotional-affective behaviors (e.g. anxiety-like behaviors, fear-associated behaviors), its potential involvement in metabolic processing has drawn more attention recently. Consisting of several subnuclei with a wide variety of neuropeptide-expressing neurons, there is a need for a better understanding of the exact role of those subpopulations. Among these, inhibitory somatostatin (SOM) positive GABA neurons are still to this day elusive in their neurochemical identity, projection targets, and exact function. We therefore aimed to better characterize these neurons in terms of emotional and metabolic functioning. Using an available SOM-Cre mouse line to selectively transfect BNSTSOM neurons with chemogenetic "Designer Receptors Exclusively Activated by Designer Drug" (DREADD) or genetically encoded neuronal tracers, we could elucidate the effect of reactivation of these neurons. Interestingly, manipulation of BNSTSOM neurons did not alter metabolic processing, but instead led to aversion in both male and female mice. Additionally, activation of the BNSTSOM neurons in male mice resulted in enhanced states of phasic but not sustained fear. In contrast, activation of BNSTSOM neurons in female mice produced an anxiogenic effect without changing fear-like behaviors. Given the diversity of downstream hypothalamic and brain areas targeted by BNSTSOM neurons, further studies will need to investigate the role of those individual projections.

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Role of the charge transfer center as a bimodal regulator of voltage-dependence and kinetics of CaV voltage sensors action

The response of voltage-gated calcium channels to changes in the membrane potential is mediated by four distinct voltage-sensing domains (VSD I-IV) coupled to a common pore. Each of these VSDs consist of four transmembrane helices (S1-S4) with S4 containing four to five gating charges. Upon depolarization, these gating charges sense changes in membrane potential and initiate an upward movement of the S4 segment, resulting in the opening of the channel pore. This sliding S4 helix movement is enabled by the formation of consecutive ionic interactions between the gating charges and negative countercharges in the surrounding helices. The charge transfer center (CTC), a highly conserved structure of the voltage sensor, is crucial for catalyzing the movement of the positive gating charges across the membrane electric field. However, the exact mechanism how the VSD state transitions are regulated and the role therein of the interactions between gating- and countercharges is unclear. To investigate the functions of these transient ionic interactions in the CTC and their role in regulating calcium channel gating, we combined structure-guided site-directed mutagenesis with patch-clamp analysis in dysgenic (CaV1.1-null) myotubes reconstituted with mutated CaV1.1 constructs. Our findings indicate that interactions between the S4 gating charges and countercharges of the CTC serve two roles in the voltage-sensing process of CaV channels. The delicate balance between interactions stabilizing the resting or activated states fine-tunes the voltage-dependence of activation. Their action in catalyzing the sequential transitions of the gating charges across the hydrophobic constriction site determines the activation kinetics of calcium currents.

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VIP interneuron selective deletion of the autism spectrum disorder associated gene Setd5

Severe anxiety frequently co-occurs with the core symptoms of autism spectrum disorders (ASD); however, the determinants are unexplored (Kerns et al., 2021). Setd5, a gene that codes for a chromatin-regulating protein, has been identified as one of the genes linked to ASD. One of the most reliable anomalies reported in both ASD and anxiety disorder patients is an aberrant functional connectivity between the salience network and the default mode network (Uddin et al., 2015). Our data indicate that vasoactive intestinal polypeptide (VIP)-expressing interneurons (INs) play a fundamental role in salience detection and in gating sensory stimuli to adaptively shape behavior (Ramos-Prats et al., 2022). Our working hypothesis postulates that failure in the encoding of salience by impaired VIP-IN function in ASD leads to altered processing of sensory stimuli, contributing to the exacerbation of anxiety and ASD symptomatology.

To test our hypothesis, we have generated mice with a selective deletion of Setd5 in VIP-INs. We are currently testing these animals using a behavioral battery to investigate if VIPCre-Setd5-cKO mice display ASD-like dysfunctions and anxiety.

In particular, we are examining if VIPCre-Setd5-cKO mice have deficits in locomotion, spatial memory, or compulsive behavior and decreased social preference that are typical of ASD-like behavior. In addition, we are testing anxiety-like behavior using an approach/avoidance-based test, namely the elevated plus maze.

The preliminary data obtained with this set of experiments will be presented at the meeting.

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Exploring the Impact of SATB2 on Cocaine Relapse: Insights from a Mouse Model

Drug addiction poses a significant public health challenge, impacting individuals and societies at large. While substantial progress has been made in understanding the eurobiological underpinnings of addiction, certain aspects remain unresolved. Satb2 is a DNA-binding protein involved in gene expression regulation through chromatin remodelling. We propose that it can emerge as a potential player in addiction mechanisms. A Satb2 conditional mutant (cKo) (Satb2 flox/flox ::Camk2a-cre) that was generated, showed impairment in long-term memory and an absence of this gene in the cortex and CA1 region of the hippocampus from the third post-natal week on. The present study aims to characterize this mouse model on the behavioural and molecular levels. Using the Conditional Place Preference model of addiction, we compared einstatement to cocaine induced by cocaine priming, as well as stress (Forced swim test - FST), between Satb2 cKO and floxed control mice. These mice did not differ in the extinction or the reinstatement to cocaine, nevertheless, we observed a significant increase in immobility time in the FST between the two groups of mice. At the molecular level, we intend to identify the brain regions where Satb2 is expressed in mice. Moreover, activated Satb2 in different brain regions in mice that extinguished and did not extinguish cocaine preference, will be assessed. These efforts will provide significant insights into the molecular mechanisms behind cocaine addiction; furthermore, they have the opportunity to guide the development of efficacious drugs that target relapse in addition.

(In collaboration with the Institute for Neuroscience (MUI))

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Interleukin-4 Induces Glutamatergic Synapse Like Structures in iPSC derived Nociceptors

The prototypical pleiotropic anti-inflammatory cytokine IL-4 not only acts on immune cells but also has important roles in the nervous system mediating antinociception and neuroregeneration. Therefore, we explored the expression of IL-4, its receptors IL4RA and IL13RA1 and downstream signaling components together with morphological and functional assays as well as transcriptomic profiling in human nociceptors differentiating from induced pluripotent stem cells (iNocs).

IL-4 induced de novo formation of synaptic boutons immunoreactive for vesicular glutamate transporter vGLUT1 in iNocs which express both, components of the IL-4 receptor complex (IL-4RA and IL-13RA1) and signaling machinery (Jak1/2, STAT6, PKC isoforms, translation factor EiF4E) during differentiation. Pharmacological inhibition of these translational or cellular signaling components reduced the synaptogenic effect. IL-4 induced distinct transcriptomic changes associated with biological process ontologies for "neuron projection development", "axonogenesis" and "synapse", "cellular process involved in reproduction in multicellular organism", "regulation of membrane potential" and "calcium ion transmembrane transport". This partially reflected injury-induced transcriptional changes of mouse nerve injury models which contribute to regenerative processes in peripheral nerve but possibly also to reconnecting primary afferent neurons to their projections in the spinal dorsal horn and indicates a critical role for IL-4 in the control of developing and established neuronal networks.

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MRI-derived progression patterns predict future cognitive decline in Alzheimer's dementia – a retrospective longitudinal follow-up study

Introduction: Predicting cognitive decline in Alzheimer's dementia (AD) facilitates patient care. However, few studies have focused on predicting AD progression. This project addresses this knowledge gap using Magnetic Resonance Imaging (MRI) in search for markers of AD progression.

Method: AD patients underwent baseline MRI and neuropsychological testing (Mini Mental State Examination, MMSE) at baseline and follow-up. Disease progression index (PI) was measured as MMSE decline/ time to last follow-up. Structural T1-weighted images were segmented automatically with CAT12 into grey matter regions of interest (ROI) based on the Neuromorphometrics and Cobra Atlases. ROIs showing significant correlations ($p<.05$, FDR) with the PI after adjusting for covariates (e.g. age, sex) underwent Fisher z-transformation based on healthy controls (HC) data. Principal component analysis was applied on the selected ROIs. Linear regression ($p<.05$) was then conducted with extracted components as predictors and the PI as dependent variable while adjusting for covariates.

Results: 104 patients (mean age=78.61±5.82; 72% female, mean follow-up time=4.27±2.15 years) and 40 HC (mean age = 78.02±4.53; 70% female) were included. 33 ROIs were significantly negatively correlated with the PI. Four principal components were identified: hippocampal, temporal, frontal, and occipital. The regression model was significant ($R^2=.27$, $F(10,93)=4.75$, $p<.01$) with all components being significant predictors ($p<.05$) and indicating that more atrophy in these regions predicts higher PI.

Conclusion: This study reveals ROI based progression markers in grey matter areas, underscoring the importance of MRI-markers in AD. Future studies should also focus on white matter abnormalities as they also represent a pathological feature of AD.

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Interneurons in the nucleus accumbens alter their electrophysiological properties in a mouse model of neuropathic pain

Recently, research has focused extensively on the peripheral mechanisms that contribute to pain chronification, but our knowledge on the involvement of different brain regions remains limited. The Nucleus Accumbens (NAc) is a central hub for reward-related processes, with its interneurons having significant potential to control NAc output signals. However, their involvement in the chronification of pain has not been investigated until now.

In this study, we performed patch-clamp recordings of cholinergic interneurons (ChIs) and GABAergic interneurons positive for parvalbumin (PV-GaIs) and somatostatin (SOM-GaIs) in the NAc shell and core subregions of respective cre-reporter mice seven days after spared nerve injury (SNI).

We found that all interneuron types showed differences in electrophysiological properties between SNI and sham operated mice. SNI ChIs showed a more hyperpolarized resting membrane potential together with a decreased input resistance in the NAc shell, suggesting decreased excitability of these interneurons. On the contrary, NAc shell PV-GaIs of SNI mice exhibited increased input resistance and fewer action potentials in response to depolarizing current injections, suggesting altered neuron function. SOM-GaIs of SNI mice showed opposing alterations in excitability, with NAc shell neurons requiring more current to induce action potential firing, whereas NAc core neurons required less current.

Our findings indicate that NAc interneurons undergo electrophysiological changes in the early phase of pain chronification, exhibiting subregion-specific alterations. Since these interneurons have a significant impact on output signals from the NAc, this implies that they may play an important role in the development of mental comorbidities associated with neuropathic pain.

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Let the robots do your neuroscience

Our cognition is formed by a vast number of interconnected neural circuits that enable us to succeed in- and adapt to our daily challenges. However, the ability to investigate the neurocomputational circuitries foundational for information processing, learning, and actions is often limited by the difficulty of providing accurate, and scalable complex experiences with detailed behavior measurements.

Existing behavior environments are typically optimized for few, often simple tasks with constrained extendibility, and priced prohibitively for scale, while behavior tracking requires work intensive labeling to generate situation-specific training data. Here, we present our work on two novel open and flexible tools to enable easier pursuit of new experiment designs and their analysis.

Our newly developed behavior system enables any experiment designs, following any flow of events and conditions, that you can write in python. It can use any electronics you can connect to a Teensy® or Arduino. We provide tracking of task variables, live plotting, and camera capture for online or offline usage. 3D printable components allow for modular setups, which together with material costs in the 3 digits will help make complex neuroscience experiments more accessible. Our flexibility enables head-fixed- as well as freely moving experiments and pursuing insights through new experiment designs like a multi-step olfactory environment and a head-fixed explorable game environment.

We further automate positional tracking from videos by bringing breakthroughs in open-vocabulary-detection to neuroscience, enabling, for example, to find the positions and all respective pixels of a mouse and its paws by just prompting for “mouse, paw”.

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Modulation of L-type calcium currents associates with changes of dendritic growth in hippocampal neurons

L-type voltage-gated calcium channels (L-VGCCs) are upstream of competing pathways that promote or inhibit dendritic growth. How L-VGCCs achieve signaling specificity remains unknown. We hypothesize that modulation of ICaL determines distinct processes controlling dendritic growth. Murine hippocampal neurons were transfected with eGFP (DIV4), treated with L-VGCC agonists and antagonists for 48 hours (DIV5-7), and processed for Sholl analysis (DIV7). Our findings reveal that dihydropyridine (DHP) blockers suppress dendritic growth and that overexpression of DHP-insensitive CaV1.2 mutant prevented it, highlighting the crucial role of CaV1.2's ICaL for early dendritic development. Channel agonists BayK-8644 and FPL 64176 increased ICaL density, and FPL 64176 also slowed ICaL inactivation. Treatment with FPL 64176 enhanced dendritic complexity, while BayK-8644 had no effect. Immunostaining experiments revealed that only Bay-K 8644 increased activated CamKII levels (pCamKII) aligning with the idea that CamKII signaling restricts dendritic growth. To investigate the role of channel inactivation in the restriction of pCaMKII, we overexpressed STAC2-HA, which inhibits channel inactivation and increases ICaL density. Under these conditions, dendritic complexity was enhanced while maintaining basal levels of pCamKII. Biotinylation assays demonstrated that only FPL 64176 reduced membrane-expressed CaV1.2, suggesting that fewer available channels may constrain pCamKII and facilitate dendritic growth. We identified different Cav1.2 shRNAs and investigated their ability to reduce pCaMKII. Preliminary results confirmed the correlation between Cav1.2 membrane levels and pCaMKII. Our data suggest that CaV1.2's ICaL is necessary for controlling dendritic growth and that regulation of ICaL kinetics may modulate CaV1.2 membrane levels restricting CaMKII signaling and facilitating dendritic growth.

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Another Brick in the Wall: Increasing the Barrier Function of a 2D in Vitro Model of the Blood-Brain Barrier

Over the last decade, several rare autoimmune disorders affecting the central nervous system (CNS) have been identified, including neuromyelitis optica spectrum disorder (NMOSD), N-methyl-D-aspartate receptor (NMDA-R) encephalitis, and myelin oligodendrocyte glycoprotein antibody disease (MOGAD). These conditions are associated with autoantibodies targeting neuronal or glial antigens. The blood-brain barrier (BBB), typically a defense against CNS infiltration, can break down under immunological processes, allowing autoantibodies to induce inflammation and neurological damage. Current research focuses on understanding how these antibodies breach the BBB and their role in disease development. Human tissue culture BBB models show promise in overcoming limitations seen in animal models.

The study specifically targets NMOSD and autoantibodies against aquaporin-4 (AQP4) on astrocytes. The goal is to establish a BBB co-culture model with human brain microvascular endothelial cells for testing AQP4 autoantibody penetration. We are currently testing for suitable cell types for the brain tissue compartment and the role of complement activation after AQP4 antibody binding.

Once established, we aim to explore BBB-leakage and autoantibody penetration under different inflammatory conditions, providing insights into CNS tissue damage mechanisms. This exploration may contribute to novel therapies for autoimmune and neurological conditions, potentially aiding in transferring therapeutic antibodies through endothelial barriers for diseases like Alzheimer's, Parkinson's, and Stroke.

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Unraveling the role of a CD64+ DC population in melanoma

Dendritic cells (DC) play a pivotal role in orchestrating immune responses, acting as crucial mediators between innate and adaptive immunity. Our investigation of conventional DC (cDC) subtypes within the transplantable melanoma mouse models B16.OVA and D4M.3A, has unveiled a distinct DC population in tumors and lymph nodes marked by the expression of the FcγRI/CD64, traditionally associated with monocytes and macrophages. Remarkably, these CD64+ DC share phenotypic characteristics with cDC2, which we identified using our 26-marker flow cytometry panel.

We discovered that migratory CD64+ DC (MHC-IIhigh CCR7+) show an activated profile, expressing high levels of the co-stimulatory molecule CD40, hinting at their potential in eliciting robust T cell responses.

With the Zbtb46gfp reporter mouse model, we explored their DC-origin, finding that tumor-infiltrating and lymph node resident CD64+ DC were just partly pre-DC lineage derived while all migratory CD64+ DC expressed the Zbtb46 transcription factor, indicating their pre-DC origin.

Investigating CD64+ DC developmental fate, Flt3L treatment in D4M.3A tumor-bearing mice led to an in vivo expansion of CD64+ DC within tumors and tumor-draining lymph nodes, highlighting their pre-DC origin. Our ongoing investigation aims to decipher the developmental trajectory of CD64+ DC and determine if they represent a distinct DC subset with unique functional attributes. This exploration is crucial for understanding their role in triggering T cell responses, especially given the activated phenotype of migratory CD64+ DC, coupled with their co-expression of inhibitory receptors. This deeper comprehension of CD64+ DC biology holds promise for identifying potential targets in DC-based cancer therapy.

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Bridging research to clinical praxis

The existing disparity between fundamental research and clinical implementation remains substantial. Despite ongoing breakthroughs in computer vision, segmentation, registration, and related domains, the translation of these advancements to clinicians is often limited or nonexistent. While research outcomes are typically disseminated through papers, occasionally complemented by online repositories featuring source code, this conventional sharing method overlooks individuals without programming proficiency, hindering their ability to engage with the research and consequently preventing both clinicians and their patients from benefiting from the recent advances in the field.

In response to this challenge, we present a software that aims to cover this gap and creates a bridge between the basic research and the daily routine of physicians, thus, enhancing their performance and streamlining their current workflow.

Our software targets planning of a stereotactical liver tumor ablation procedure, a preferred choice due to its minimal invasiveness among both physicians and patients. The software is built on top of an open-source platform that is widely adopted for research prototypes. Utilizing C++ for fast performance and Python programming language which is very popular and prominent in deep learning and artificial intelligence fields, allows easy expansion and import of deep learning-based approaches and therefore, makes our software highly adaptable.

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Deep learning approach for improving prognostic value of dual CT imaging of brain ischemic stroke

Introduction:

Quick and accurate identification of brain stroke lesions in medical images is crucial for timely diagnosis and treatment planning. Computer tomography (CT) is typically the initial diagnostic test to differentiate between hemorrhagic and ischemic strokes. Following detection of ischemic stroke with CT, CT angiography (CTA) evaluates blood vessels for clots that may have formed in a vessel. Mechanical thrombolytic therapy is a common treatment for ischemic stroke, based on CTA findings. Dual energy CT (DECT) monitors treatment success by distinguishing contrast staining from intracranial hemorrhage. One of the most important factors for clinical outcome of ischemic stroke patients after EST is reliable prediction of hemorrhage and infarct size in future.

Method:

We used CNN, specifically a U-Net structure, as the segmentation framework for creating segmentation masks. For alleviating class imbalance, a 3D patch based deep learning approach for segmentation of hemorrhagic and infarction lesions from CT images has been used. Furthermore, we use all available data to train a deep neural network for identifying features from DECT brain images of stroke patients, aiming to predict the clinical outcome of endovascular thrombectomy.

Result:

Training on manually annotated segmentation masks from 60 patients and testing on 15 patients yielded promising initial results.

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Microbiome derived products inducing anti-cancer immunity in colorectal cancer

Although Immunotherapy holds promise, its effectiveness remains limited for certain cancer types. Colorectal Cancer is one such example, as it exhibits low response rates to immune checkpoint inhibitor treatment. Recently, it became evident that the drug response to immunotherapy is modulated by the host microbiome. Thus, It is of great importance to understand the mechanisms of the crosstalk of immune and epithelial cells with the microbiome in the gut.

In this PhD thesis the aim is to gain a deeper mechanistic understanding of a defined commensal consortium, which has recently been shown to be beneficial by improving the efficacy of immune checkpoint blockade when inoculated in germ-free mice subcutaneously engrafted with colon adenocarcinoma cells. In particular, we hypothesize that microbial-derived products could modulate the immune response thorough the synthesis of metabolites that interact with the immune cells' surface receptors. Our study employs murine colon organoids to understand the impact of metabolites found in the cell-free culture supernatant of those microbes on epithelial cell signaling.

Transcriptomic analysis and quantitative assessment of chemokine expression profiles reveals the upregulation of genes linked to cell migration, cytokine induction and neutrophil-attracting chemokines. Thereby indicating that soluble factors secreted by the effector strains directly stimulate epithelial cells to produce cytokines and chemokines. These findings suggest a potential mechanism orchestrated by those gut commensals, of IFN γ+ CD8+ T cell infiltration in the tumor microenvironment and subsequently improved immunotherapy efficacy.

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spacedeconv streamlines deconvolution analysis of tissue composition from spatial transcriptomics data

The investigation of cell-type abundances and spatial organization in tissues is key to understanding their (dys)function in health and disease. Sequencing-based spatial transcriptomics technologies enable whole-transcriptome measurements while preserving the tissue spatial organization. Data generated from spatially-resolved spots can be analyzed with deconvolution methods, revealing their cellular composition. A plethora of tools are available but produce differing results. Thus, a comparative analysis is needed but currently challenged by the different inputs, outputs, and implementations of existing tools.

We introduce spacedeconv, an R package for advanced deconvolution analysis of spatial transcriptomics data. spacedeconv unifies access to 14 first-generation methods based on pre-computed cell-type signatures and 17 second-generation tools that can be trained with annotated single-cell RNA-seq (scRNA-seq) data. Additionally, spacedeconv offers user-friendly modules for statistical analysis, including robust preprocessing, normalization, and subsampling to improve computational performance. spacedeconv streamlines the quantitative investigation and visualization of cell-type distributions, cell-cell colocalization patterns, and the characterization of multicellular niches.

We showcase spacedeconv capabilities through comprehensive spatial analyses performed in different tissues, illustrating how different deconvolution methods are optimally leveraged for specific applications. For instance, while first-generation methods leverage well-validated signatures for specific immune cells, second-generation tools extend deconvolution to additional cell-types and tissues where annotated single-cell references are available.

By providing a simplified interface to spatial cell-type deconvolution algorithms/methods, spacedeconv enables complex investigations of tissue architectures. Thanks to the possibility to run different classes of deconvolution methods, spacedeconv has applicability to numerous organisms, tissues, cell-types, and diseases, representing a valuable tool for different biomedical domains.

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Skeletal Muscular and Myocardial Adaptations to Iron Deficiency Anemia and their Reversibility after Intravenous Iron

Background: Fatigue is a cardinal symptom of iron deficiency anemia (IDA) that can rapidly improve after intravenous (IV) iron. Iron is essential for mitochondrial function and energy production. Hormonal control, training, substrate- and oxygen availability determine which nutrients are used for energy production in muscles. We aimed to investigate the effects of IDA on muscle cells and the reversibility of adaptive changes after IV iron treatment.

Methods: IDA was induced in three-week-old C57Bl/6 mice with dietary iron deficiency and phlebotomy. Animals were injected with three different IV iron formulations. Seven days after IV injection full blood count, gastrocnemius-, soleus-, plantaris-muscle, the diaphragm and the myocardium were analyzed. Gene expression was quantified by RT-qPCR. Metabolites and protein abundances were measured by untargeted LC-MS/MS. Fiber types were visualized by immunofluorescence staining.

Results: In IDA, energy production in skeletal muscle switched to anaerobic metabolism. Furthermore, oxidative fibers were significantly smaller, which correlated with hemoglobin concentration. Myocardial glucose metabolism was upregulated in IDA. However, pyruvate dehydrogenase was inactivated, resulting in increased lactate production. Respiratory chain and energy production were downregulated in both organs. IV iron treatment reversed most of the alterations caused by IDA, but the time course of the reversibility differed between the IV iron formulation tested.

Conclusion: The metabolic profile and preferred energy source differs between skeletal muscle and the myocardium in our IDA model. The structural and biochemical remodeling in skeletal muscle of IDA animals can be differentially reversed by IV irons. Signals controlling these adaptations will be further investigated.

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Unraveling specificities in iron metabolism during chronic kidney disease: a mouse model

Anemia is a prevalent consequence of chronic kidney disease (CKD), often characterized by impaired erythropoietin (EPO) production and progressive kidney damage. This study aimed to elucidate specificities in iron metabolism during CKD using a mouse model. An in-vivo CKD model was established in 3-week-old male C57BL/6N mice, inducing kidney damage through a diet containing adenine, phosphate, calcium, and varying iron levels. Healthy mice were fed a high-iron diet, CKD control group used adenine diet with a high- iron supplementation, while another group received a low-iron diet with or without adenine. The study analyzed blood parameters, gene expression, and protein levels to assess anemia markers. Results showed that the healthy low iron group developed microcytic, hypochromic anemia with reduced hemoglobin levels, leading to cellular respiratory dysfunction and organ-wide hypoxia. Kidney compensation in this group involved EPO production, influencing precursor cells in erythropoiesis, and elevating reticulocyte levels. In the CKD model, EPO production was hindered by adenine metabolite infiltration in renal tubules, preventing an increase in red blood cell levels. Iron target genes revealed suppressed hepcidin in the healthy low iron group, upregulating ferroportin for iron release. Conversely, CKD models exhibited hepcidin upregulation due to inflammation-driven IL-6, leading to intracellular iron sequestration. Hyperferritinemia, misleadingly associated with elevated iron stores, was observed in the CKD setting. Comparative analysis uncovered specificities in iron metabolism during CKD, providing valuable insights for tailored therapeutic interventions in CKD-associated anemia, distinguishing between iron deficiency anemia and anemia of chronic disease.

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New non-invasive, label-free monitoring approach for 2D and 3D cell culture

Two major issues of cell-based toxicological and drug response assays are the lack of the temporal component of endpoint assays, and the strong dependency of reproducibility and significance on the quality and condition of the cells used. Thus there is a tremendous need to provide insight into the usually inaccessible processes inside the incubator. We developed a novel lensfree imaging method exploiting the optical properties of the cell itself for imaging inside the incubator, which allows non-invasive, super compact, label-free, live-cell monitoring. By applying AI to determine key cell culture parameters such as confluence, proliferation, and cell motility [1], high-quality, automated, objective, and real-time data can be collected. Applying our lensfree microscopy (LM) method, we find that memory effects from heterogeneous cell culture conditions lead to an increase of variance during subsequent assays like e.g. omics-readouts [2] or other cell based assays, like wound healing assays, motility and proliferation assays significantly. Furthermore, our LM is also suitable for 3D applications and will enable quantification of organoid growth dynamics and interactions. Our approach dramatically increases control and processing speed. In the context of the reproducibility crisis, we hope to make a contribution in the direction of standardization of cell-based research in the future.

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Beyond bleeding: Exploring coagulation factors and cytokines in bone health in vitro

Introduction: Hemophilia A (HA) patients exhibit low bone mineral density (BMD), independent of conventional risk factors [1]. Recent research suggests that coagulation factor VIII (FVIII) may influence bone health directly or indirectly [2], prompting the need for deeper understanding. This study investigates the complex interplay among coagulation factors, cytokines and bone health in HA patients by using cell culture experiments.

Methods: Human and murine osteoblast cell lines, along with human osteoclasts, were cultured with human coagulation factors (FVIII, FIX, FX, vWF, vWF-FVIII complex, thrombin and FVIII-thrombin; 1 U/ml each) and cytokines (IL-6 and TNF-α; 50 ng/ml each). Assessments included cell viability, osteoblast mineralization and osteoclast tartrate-resistant-acid-phosphatase (TRAP) activity.

Results: Coagulation factors had distinct effects on human and murine osteoblasts: Compared to controls, thrombin reduced cell viability of murine osteoblasts by 18%, whereas vWF, vWF-FVIII, FIX, FVIII-thrombin, and FX increased viability in human cells by 33%, 24%, 26%, 23% and 31% respectively. Mineralization of murine osteoblasts was unaffected, but mineralization of human osteoblasts decreased with vWF, vWF-FVIII, thrombin and FX by 44%, 37%, 35% and 56%. In human osteoclasts, FX led to increased TRAP activity by 16%. TNF-α reduced viability in murine and human osteoblasts (29% and 53%) and mineralization of murine osteoblasts increased (119%). TNF-α and IL-6 reduced osteoclast TRAP activity by 50% and 60%.

Conclusion: This study reveals distinct responses of human and murine osteoblasts to human coagulation factors. Understanding the interplay between coagulation, inflammation and bone metabolism is pivotal for future therapeutic approaches.

Conflict of interest: None.

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Pathogenicity of de novo CACNA1D Ca2+ channels variants predicted from sequence co-variation

Voltage-gated L-type Cav1.3 Ca2+ channels support numerous physiological functions including neuronal excitability, sinoatrial node pacemaking, hearing and hormone secretion. De novo missense mutations in the gene of their pore-forming α1-subunit (CACNA1D) can lead to severe neurological disorder with and without endocrine symptoms. Here we employed evolutionary analysis on Cav1.3 α1-subunit sequences to predict the pathogenicity of human disease-associated CACNA1D missense variants. The co-variation model considering residue-residue couplings correctly predicted previously identified pathogenic variants, supported pathogenicity in variants previously classified as likely pathogenic and even led to the re-examination and re-classification of 18 out of 80 variants. Based on the prediction score, we electrophysiologically tested one variant (V584I) and found significant gating changes associated with pathogenicity. Thus, our co-variation model represents a valuable addition to complement the assessment of the pathogenicity of CACNA1D variants completely independent of clinical diagnoses, electrophysiology, structural or biophysical considerations and solely based on evolutionary analyses.

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Brain functional connectivity changes associated with learned focused attention meditation

INTRODUCTION: Focused attention (FA) meditation is a meditation practice that involves mainly sustained attention, vigilance, the ability to disengage from distractions and reorient attention. More and more evidence on the beneficial effects of even short-term meditation trainings on attention is emerging. This seems to be mediated by a reorganization of brain networks, including default mode network (DMN). In this paper brain network connectivity during meditation state after a seven-week FA meditation training has been explored in a group of novice meditators.

METHODS: 24 naive meditators underwent a seven-week meditation training based on FA meditation. All participants were scanned before and after meditation training. They were asked, respectively, to stay focused on their breath (BO) and to meditate (MED) inside the scanner. Three different analytical approaches were chosen to compare the two mental states (BO vs MED), namely independent component analysis, seed-based connectivity analysis and graph-based network analysis.

RESULTS: Well in line with the current literature, a trend toward an increase in the frontal and decrease in the posterior DMN connectivity and evidence for a reduction in mind-wandering were found. In addition, a substantial decrease in the cerebro-cerebellum connectivity (especially with DMN nodes) was evidenced, more likely suggesting a facilitation of attention. Contrary to expectations based on literature, no involvement of the striatum was observed.

CONCLUSIONS: Results demonstrated that functional connectivity analysis was able to differentiate the two conditions suggesting brain networks changes during FA meditation, even after a short-term meditation training. Controlled studies are needed for investigations on training effects.

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Automated Precise Planning of Transcatheter Aortic Valve Implantation using Aorta Unfolding method on contrast-free MRI

Purpose: The pre-procedural valve sizing in Transcatheter Aortic Valve Implantation (TAVI) hinges on precise measurement of the Aortic Annulus (AA).

Although contrast-enhanced Computed Tomography (CT) is conventionally

employed and well established, its applicability may be limited in patients with chronic kidney disease. This article presents an innovative strategy utilizing contrast-free Magnetic Resonance Imaging (MRI) for planning, addressing the challenges associated with diminished image contrast.

Methods: The proposed method leverages AA manual segmentation through an automatic unique aorta unfolding technique. The first step involves segmenting the aorta ascendens using a nnUNet on whole-heart MRI, followed by the application of an angular projection-based technique to simulate the unfolding of the aorta, optimizing the visualization of aortic leaflets. To calculate the STJ and AA virtual planes, we employ a 2D-UNet for the segmentation of aorta leaflet contours on the corresponding 2D-maps.

Results: The generation of unfolded maps took 1.116 seconds per MRI. A visual comparison between the proposed labeling methodology and the traditional approach illustrated a more precise definition in the former. Moreover, the examination of TAVI parameters using both methodologies indicated similar values for AA area, perimeter, and mean diameter with our unfolding method.

Conclusion: Our study presents an automatic technique to help identifying AA and STJ structures. It offers a faster alternative to traditional manual approaches, promising advancements in TAVI planning. Additionally, its applicability extends to CT and MRI images also in other anatomical structures. The absence of dedicated software for MRI-based planning emphasizes the imperative for advancements in this field.

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Quantification performance study of Magnetic Nanoparticles with MR Imaging modality for Magnetic Hyperthermia

Magnetic nanoparticles comprising of elemental magnetic iron oxide core(nm) surrounded by a biocompatible shell. Because of magnetic core, particles can be manipulated inside the body using external magnetic field. MNPs possess to convert electromagnetic energy into thermal energy and a high concentration of particles (in the order of several mg/mL) in the tumor to deposit sufficient heat quickly to annihilate tissues. Since the thermal dose is proportional to the injected concentrations, quantification of MNPs in the tissues allows to quantitatively evaluate the results for the treatments. MR imaging with a phantom featuring 8 different concentrations (0.00085-1.7mg/mL of 1mL agar+MNP(Perimag)) has been conducted, and the quantification performance is evaluated using two categorical quantification methods.

Presence of MNP distorts local magnetic field leading to a faster relaxation rate which changes in signal intensity. Relaxometry method uses magnitude information of MR-image to measure the concentration by mapping the relaxation rate. Model-based method quantifies by first modeling the magnetic field inhomogeneities and then fitting the model to the magnetic field variations obtained from the phase map generated by MNPs.

Both methods are used to be effective but provide information only up to a certain concentration. At high concentrations, signals become saturated, immoderate field inhomogeneities lead to image distortions affecting the accuracy of quantification.

MNPs are not detected directly, which makes it difficult to separate and quantify them accurately. The competence of MR imaging can be maximized by giving magnetic information from a reference modality, making it appropriate for the use of magnetic hyperthermia applications.

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Fitting of dentures fabricated on the basis of three-dimensional CBCT imaging

The PhD project aims to develop and validate an alternative to the intraoral scan (IOS) for dental restorations using three-dimensional digital volume tomography (DVT) imaging. DVT allows for non-invasive imaging of inaccessible tooth areas without the need for complex preparations. The study tests the null hypothesis that virtual tooth surfaces digitized through DVT (study group) or IOS (control group) show no geometric differences. The methodology involves segmenting DVT datasets, calibrating grayscale ranges, and addressing restoration artefacts. Calibration involves using a reference object with known dimensions and similar X-ray opacity to tooth enamel. The study compares the STL files of both imaging modalities through virtual co-registration and analyses mesh deviations using reverse engineering software. The three-phase project includes pilot investigations for grayscale calibration, transferring results to swine jaw models, and introducing restorative materials for clinical relevance.

The expected outcomes include insights into the accuracy of DVT compared to IOS, the influence of tissues and materials, and the challenges in meeting clinical requirements. The study anticipates that DVT data may not match IOS accuracy but aims to determine if the differences are clinically acceptable. Challenges involve achieving accurate segmentation without a calibrated grayscale scale for DVT and ensuring a reproducible measurement process. The introduction of restoration materials may lead to artefacts in DVT images.

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Identification of immunogenic neoantigens in microsatellite stable colorectal cancer patients

The majority of colorectal cancer (CRC) tumours are microsatellite stable (MSS). Because of their distinct tumour microenvironment (TME), MSS CRC patients are largely refractory to immunotherapy with checkpoint blockers. One promising strategy is targeting neoantigens, mutated non self-peptides of cancer cells, which can trigger T cell responses in patients. We developed a platform for testing immunogenicity of (non-canonical) neoantigens. Patient-derived tumour and healthy organoids from six patients were generated and autologous tumour-infiltrating lymphocytes (TILs) and peripheral blood lymphocytes (PBMCs) were isolated and expanded as well. Functional reactivity against autologous tumour antigens was investigated via a co-culture system. T cell subsets were characterized via an 18 multicolour Flow Cytometry panel and their tumour reactivity was measured additionally via an IFN-ɣ ELISA assay. T cell efficacy was monitored via IncuCyte measurements. RNA- and Whole Exome Sequencing (WES) data of tumour organoids and autologous PBMCs were analysed for in-silico neoantigen prediction. Co-cultures with different effector target ratios and durations revealed differences in IFN-γ secretion, which seem to be patient dependent (n=2). Live cell imaging data suggests that tumour reactive T cells, expanded from PBMCs, are able to kill autologous tumour organoid single cells. Taken together, our data shows for the first time that tumour organoids can be used as an 'unlimited' source to expand tumour reactive T cells from MSS CRC patients.

Thus, our workflow allows evaluating the immunogenicity of non-canonical neoantigens in order to develop neoantigen-specific T lymphocytes with the aim of evoking an immune response against the tumour in MSS patients.

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The association of cTnT and hs-CRP with infarct severity and outcomes in STEMI

Ischemic heart disease, such as myocardial infarction, is being regarded as the single most common cause of death worldwide. Although major advances in the therapy of myocardial infarction have been made, in-hospital mortality remains and 4 to 12 % in european countries. It is therefore necessary to develop strategies to identify patients that bear a great risk of adverse events. Cardiac magnetic resonance (CMR) has been proven to be a valuable tool for risk stratification in patients with ST-elevation myocardial infarction (STEMI) in various studies. Due to its excellent temporal and spatial resolution it is considered the non-invasive gold standard for morphological and functional tissue characterisation. The aim of this dissertation is to show an association between a combination of the cardiac biomarkers cTnT and hsCRP with clinical outcomes and CMR parameters like infarct size and left ventricular remodelling in patients with STEMI.

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Iron homeostasis and immune response in Gabonese children with acute febrile illness

Iron deficiency, anemia and infectious diseases have a complex relationship prevalent in Sub-Saharan African children. It was shown that the iron status shapes the immune response during infections while both factors can contribute to the development of anemia.

In this unexplored context in humans, we analyzed blood counts, CRP, iron parameters and cytokines in 416 Gabonese children (2-17 years) with fever or history of fever < 7 days. Symptom-based microbiological testing was performed classifying the etiology of infection according to Malaria status and bacterial and non-bacterial infections.

Anemia in general and iron-deficiency anemia in particular comprised 74.5% and 7.6% of cases, respectively, while 57.6% of children had iron-deficiency irrespective of anemia status and 24.0% anemia of inflammation. Increased body temperature and reduced duration of fever were significantly linked to higher odds of iron deficiency. Interestingly, malaria status did not yield significant results. Malaria positive (M[+]) children had increased ferritin and hepcidin, decreased transferrin levels as well as an increased ferritin: transferrin ratio compared to Malaria negative (M[-]) peers. TNF-α and IL-10 levels were elevated in M[+] children compared to M[-] children, with a higher TNF-α: IL-10 ratio in the latter. Additionally, transferrin saturation showed a negative association with IL-10, IL-6 and IL-2 in the Malaria group. In M[-] children with undetermined pathogens, high IFN-γ and IL-4 levels were positively associated with transferrin saturation.

These findings underline the significance of iron in infectious diseases and highlight diverse interactions between iron homeostasis and immune responses in relation to the causative infectious pathogen.

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Identification of females at risk after CABG by sex-specific cardiac troponin and creatine kinase-MB thresholds

Cardiovascular disease in women is often understudied, leading to underdiagnosis and inadequate treatment. This gap in knowledge extends to the context of sex-specific cardiac biomarker release during Coronary Artery Bypass Grafting (CABG) surgery, underscoring the need for sex-specific biomarker thresholds to better identify female patients at-risk.

Acknowledging the independent risk posed by female sex for adverse events and death upon CABG, our study aimed to examine the influence of sex on cardiac biomarker release post-CABG and to establish gender-specific thresholds for high-sensitivity troponin (hs-cTn) and creatine kinase-MB (CK-MB), in relation to 30-day Major Adverse Cardiac Events (MACE) and mortality.

We did a retrospective analysis of 3687 patients (17.4% female) undergoing CABG between 2008-2021 in two tertiary university centers, with serially postoperative hs-cTn and CK-MB analysis. Our primary outcome was a composite of myocardial infarction, all-cause mortality, and re-revascularization at 30-days, with secondary endpoints being 30-day and five-year mortality. Sex-specific thresholds for cTn/CK-MB were calculated using Youden's-J-Index.

We found lower cTn levels in women post-CABG. The optimal threshold for cTn was 94.36 times the upper reference limit (URL) for females and 206.07 times the URL for males. Females overseen by the general threshold demonstrated a heightened risk of MACE or death within 30 days post-CABG (cTn: MACE: OR3.78, CI:1.03-13.08, p=0.035; death: OR4.98; CI:1.20.-20.61; p=0.027) (CK-MB: MACE: OR10.04; CI:2.07-48.75; p<0.001; death: OR13.59; CI:2.66-69.47; p=0.002).

Our findings suggest significant sex-specific disparities in outcomes and biomarker release following CABG, highlighting the necessity of gender-specific thresholds for accurate diagnosis and optimal-treatment of female patients.

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Sex differences in cardiac autonomic function after acute ST-elevation myocardial infarction

Introduction: Periodic Repolarization Dynamics (PRD) and Deceleration Capacity are ECG-based risk markers that quantify sympathetic-activity associated low-frequency modulations of repolarization instability and vagally mediated regulations of heart rate, respectively. Previous research links increased sympathetic and decreased vagal activity after-myocardial infarction (MI) to a higher risk of malignant arrhythmias and overall mortality. Furthermore, studies showed that women show worse prognosis after MI than men do.

Methods: Consecutive patients with acute ST-elevation myocardial infarction (STEMI) presenting to the cardiology department of the Medical University Innsbruck were included. All patients underwent a 30-minute biosignal recording by a high-resolution (1000 Hz) electrocardiogram after successful coronary intervention (at 2-5 days, 4 months and 12 months after MI). PRD and DC were calculated using established methods.

Results: 491 patients were included (391 male vs. 100 female). PRD values at baseline, 4 months and 12 months differed significantly between sexes (mean [IQR]): 4.86 [2.18-6.96] vs. 3.33 [1.38-4.84] ($p < .001$), 4.27 [1.74-5.60] vs. 2.50 [0.86-3.20] ($p<.001$) and 5.28 [1.81-7.60] vs. 2.61 [0.87-2.73] ($p < .001$), respectively, at a corrected significance level of $\alpha <.016$.

In contrast, DC values were not different between sexes at any timepoint: 4.30 [2.81-7.00] vs. 5.43 [3.27-7.20] ($p = .043$), 6.92 [4.73-9.01] vs. 7.14 [4.92-9.06] ($p = .61$) and 6.55 [4.692-8.96] vs. 6.10 [4.68-9.30] ($p < .51$), respectively.

Conclusion: Elevated PRD values in women after STEMI indicate a more pronounced cardiac autonomic dysfunction and might account for the worse prognosis after STEMI in women.

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Intensified post-stroke care improves long-term swallowing function after ischemic stroke secondary analysis of the stroke card trial

BACKGROUND

Dysphagia is a common complication following ischemic stroke and considerably contributes to the rehabilitation process. Here we investigate the effect of intensified post-stroke care on the recovery of swallowing function within the first year after stroke.

METHODS

We conducted a secondary analysis of the STROKE CARD study, a randomized clinical trial. Out of 1419 patients with ischemic stroke at the study center Innsbruck (Austria), 236 patients (44.1% female) had dysphagia. Dysphagia was assessed through clinical swallowing examinations at four key time points: hospital admission, discharge, at 3-month intervention (only STROKE CARD care) and at 12-month follow-up. Health-related quality of life was measured via EQ-5D-3L and visual analogue scale after 1-year.

RESULTS

At hospital admission, 15.8% (STROKE CARD care; n=147) and 18.2% (standard care; n=89) of patients showed dysphagia and at hospital discharge 13.4% (n=125) and 13.1% (64). At 12-month follow-up, swallowing impairment was present in 3.4% (n=4) of patients with initial dysphagia in the STROKE CARD care group and 23.9% (n=17) in the standard care group ($p<0.001$) – the effect remained after adjustments for potential confounders including age, sex, functional disability or stroke severity at 12-months, initial dysphagia severity, residence in nursing home at 12-months and initial alternative feeding (OR 8.96 95%-CI [2.77,29.00], $p<0.001$). Furthermore, patients receiving STROKE CARD care reported a higher health-related quality of life after 1-year ($p=0.044$).

CONCLUSIONS

Intensified post-stroke care significantly improved long-term swallowing impairment and was associated with higher health-related quality of life after one year, highlighting the efficacy of comprehensive post-stroke care programs.

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Dynamics of Lipoprotein(a) after stroke, a prospective observational study.

Background: Lipoprotein(a) [Lp(a)] is an inherited, independent and causal risk factor for atherosclerotic cardiovascular diseases. Mechanistic, observational, and genetic studies support a causal role of Lp(a) in the development of coronary heart disease, peripheral arterial disease, aortic valve stenosis and ischemic stroke. Little is known about distribution of Lp(a) and changes in Lp(a) in stroke patients.

Objectives: The aim of the study is to assess describe Lp(a) in a representative cohort of stroke patients and evaluate Lp(a)-changes over time.

Methods: In December 2020 the STROKE-CARD registry was established to monitor patients with high-risk TIA (ABCD2-score ≥4) or ischemic stroke over the long-term. The registry collects clinical information including Lp(a) within 24 hours after hospital admission, 3 and 12 months. We will describe Lp(a) in a representative stroke cohort, as well as in subgroups based on age, sex, stroke etiology and comorbidities. We will explore determinants of changes of Lp(a) during follow-up (concomitant medication, comorbidities).

Results: So far, over 1400 patients were enrolled, and Lp(a) changes were tracked over 12 months.

Discussion: Investigations of effects on change in LP(a) after stroke are necessary, since Lp(a) is a risk factor for cardiovascular diseases and therefore plays a role in stroke follow-up care as well as stroke prevention. Several specific Lp(a) lowering therapies are currently tested in Phase I to III trials. More information about prevalence and dynamics of Lp(a) after ischemic stroke and its influencing factors is needed in order to choose patients that might profit most from future therapeutic options.

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Delayed orthostatic hypotension in Parkinson's disease compared to the aging general population

Background: delayed orthostatic hypotension (dOH) is a prodromal form of classic orthostatic hypotension, but its frequency in Parkinson's disease (PD) and aging individuals with history of syncope and orthostatic intolerance is still undetermined.

Objectives: to investigate the frequency and associated clinical-demographic characteristics of dOH in a cohort of individuals with PD and aging individuals referred for syncope and orthostatic intolerance.

Methods: This bicentric, retrospective study included 213 PD patients (35% women; 73 [69; 76] years of age; 6 [3; 10] years of disease duration) and 213 age-matched elderly individuals (45% women; 73 [69; 77] years of age) referred to the Department of Neurology of the Medical University of Innsbruck and to the Department of Geriatrics of the Careggi University Hospital (Florence) between January 2008 and September 2016. All recruited individuals underwent a head-up tilt examination of at least 10 minutes under continuous non-invasive hemodynamic monitoring using CNSystem® devices.

Results: dOH occurred in 18% of the PD and 9% of the aging individuals. Multivariate analysis showed that dOH was significantly associated with a diagnosis of PD [OR=2.32 (95% c.i.: 1.08-4.99), p=0.031] and polypharmacy (OR=1.18, 1.03-1.35 95% c.i., p=0.016), while negatively associated with pressor agent intake (OR=0.12, 0.02-0.65 95% c.i., p=0.014). PD individuals showed a significant fall in systolic BP during prolonged head-up tilt with respect to aging individuals ($p<0.001$).

Conclusion: dOH is a more frequent finding in PD than in other aging individuals referred for orthostatic intolerance and syncope. The fall in systolic BP drives dOH development.

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Effects of Chemical Warfare Agents on Hb-O2 Affinity - an ex-vivo Experiment

This study focuses on investigating the effects of chemical warfare agents (CWAs) on hemoglobin-oxygen (Hb-O2) affinity in red blood cells. Although there is substantial information on the acute and chronic impacts of CWAs on multiple organ systems, the specific influence on the capacity of hemoglobin in erythrocytes to load and unload oxygen remain insufficiently studied. The oxygen dissociation curve (ODC) is used to measure Hb-O2 affinity. A rightward shift in the ODC suggests a decrease in Hb-O2 affinity, facilitating oxygen release to tissues, whereas a leftward shift indicates an increase, enhancing oxygen uptake in the lungs. This affinity is affected by both internal cell metabolism and external environmental factors. The aim of this study is to delve into the potential effects of CWAs, enhancing our understanding of their pathophysiological effects. A key aspect of this investigation is how CWAs may alter erythrocyte function and systemic oxygen transport in an in-vitro setting

The study design consists of tow trials: an initial pilot study and a subsequent dose/time dependency trial. In the pilot phase, venous blood samples will be carefully collected, stored, and processed, ensuring pH consistency, and then exposed to supramaximal CWA dosages for Hb-O2 affinity analysis. Should the pilot study show significant effects, the second trial aims to further explore the impact of different CWA concentrations and exposure durations on Hb-O2 affinity and methemoglobin levels.

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Primary NSCLC patient-derived microtumours (PMTs) for clinically relevant prediction of (cancer) therapy efficacy considering gender-specific aspects

Background:

The outcome of cancer patients after therapy depends on many different factors, on the one hand on histological and molecular characteristics, and on the other also on gender-specific aspects. Therefor, the response rate is very individual, which emphasises the need for a customised therapeutic approach. To this end, we have developed a functional 3D in vitro model to enable the assessment of the response of different patient groups.

Methods:

Freshly resected NSCLC tumour material from 20 patients with different gender-specific backgrounds was mechanically and enzymatically digested to obtain a single cell suspension. PMTs were then generated by seeding the suspension into plates with extremely low adherence. Afterwards the PMTs have been treated with different therapy combinations. The dynamic response to the drugs was monitored over 14 days using bright-field imaging

Results:

Our main objective was to create a model that reflects patient- and society-specific aspects as accurately as possible, in order to better predict their influence on the response of different patient groups to treatment. To this end, we comprehensively analyzed the included cohort with regard to gender-specific distributions. In a second step, in-depth analyses of the clinical outcomes in the model were performed with regard to these gender-specific aspects. Both reflected the clinical routine, categorized by objective response rates, clinical and social outcome distribution.

Conclusion:

The present PMT model is one of the first to show typical clinical outcome distribution according to social and gender-specific aspects and thus enables an approach for individualized therapy in the future.

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2. PreComb Therapeutics Ag

Sex differences in individuals with persisting COVID-19 associated loss of smell: data from the SMELL-trial

Background: Up to 70% of individuals experience persistent olfactory dysfunction (OD) after SARS-CoV-2 infection, the virus responsible for the COVID-19 pandemic. Former studies identified risk factors such as female sex and increased age. OD is generally associated with impaired quality of life, anxiety, and depression.

Methods: The SMELL-trial is a monocentric prospective randomized controlled trial including individuals with COVID-19 associated OD (> 3 months post-infection). Multiple questionnaires regarding self-reported health, physical and mental function (SF-36), olfactory dysfunction (measured by Visual Analog Scale), and clinical global impression scale of severity (CGI-S) were collected. We assessed olfactory function perception using the Sniffin´ Stick test battery, with OD defined as a score of < 13 on the identification subscale.

Results: We consecutively included 57 individuals (37 women, 65%) with an average OD-duration of 18.4 months (SD 4.4). There were no sex-differences identified on the SF-36 questionnaire, or on olfactory VAS or CGI-S ($p > 0.05$). No differences in demographic data or comorbidities were seen ($p > 0.05$). Male participants had a lower score on the olfactory test battery compared to female participants (15.2 vs 18.9, $p < 0.01$).

Conclusion: Although the prevalence of COVID-19 associated OD is more prevalent in women, we found men to perform worse on olfactory testing. No sex-differences regarding physical or mental function, health, and self-reported OD or symptom severity were found.

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YOUhealTH - a cluster randomized, controlled interventional study with blinded outcome assessment

Background: Cardiovascular diseases are the leading cause of death worldwide, with unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol being the most important behavioral risk factors. Cardiovascular health promotion is particularly useful in adolescence, as certain behaviors regarding these risk factors are established in this stage of life.

Objectives: Aim of the study is to evaluate the efficacy of a one-year health promotion intervention focusing on diet and physical activity in adolescents and adults.

Methods: The YOUhealTH study is being conducted at six Tyrolean schools between January 2023 and January 2025. Schools are randomly assigned to an intervention or control school. The adolescents are actively involved in clinical research by participating in all steps of the research project as a student study team. They develop the health promotion with focus on healthy diet and physical activity. Approximately 150-200 students, aged 14-17 years, and at least one legal guardian are included in the study. Baseline and outcome examination include an extensive assessment of vascular risk factors as well as a vascular phenotyping. The primary outcome parameter is change in diet and physical activity score of the 2022 cardiovascular health metrics update of the American Heart Association.

Results: 118 participants were included in the study and completed baseline examination. Among adolescents 50.8 % were male and among adults 76.3 % were female.

Discussion: Several studies highlight the importance of health promotion in managing cardiovascular health. However, specific strategies and their efficacy can vary and need to be further investigated.

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Immune response after SARS-CoV2 vaccination in patients with inflammatory bowel disease

Background: Vaccination against SARS-CoV2 has been used worldwide to prevent severe courses of Covid-19. In the past, immunomodulatory therapy has already been shown to weaken the immune response to the SARS-CoV2 vaccination. The aim of our study was to study humoral and cellular immune responses after SARS-CoV2 vaccination in patients with inflammatory bowel disease over a period of one year.

Materials and Methods: 71 patients with inflammatory bowel disease were included in our prospective monocentric, open phase IV study carried out at the Innsbruck University Hospital from April 2021 onwards. The immune response to SARS-CoV2 was evaluated at the day of the first of three vaccination, and 3, 6, and 12 months thereafter.

Results: Of the inital 71 patients with IBD, 68 were included in the final analysis (57 patients with Crohn's disease and 11 with ulcerative colitis). We noticed a significant increase in antibodies (S1-RBD) three month after vaccination (median for Crohn's disease and ulcerative colitis : 416.8 and 757.6 BAU/ml) that declined over time (6 month follow-up (median for Crohn's disease and ulcerative colitis : 120.45 and 123.35 BAU/ml). After one year patients antibody titers (median) increased up to 1276.2 and 6167.6 BAU/ml for Crohn's disease and ulcerative colitis probably caused by patients SARS-CoV2 infections indicated by antibodies against nucleocapsid protein.

Conclusion: Patients with chronic bowel disease respond to the SARS-CoV-2 vaccination with an increase in vaccination titres (S1-RBD). A boost of the immune reaction could be observed due to infections indicated by positive antibodies against nucleocapsid protein.

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The role of Wnt and KRAS signaling pathways in lung cancer

Lung-cancer remains the leading cause of cancer death worldwide. Lung-cancer develops due to well-characteristic molecular changes in selected oncogenes and tumor-suppressor genes. One of these mechanisms is underlying the Ras and Wnt/β-Catenin pathway. Mutations in the Ras pathway lead to uncontrolled proliferation and survival. The Wnt/β-Catenin pathway was found to be activated in tumor cells where it leads to activation of target-genes like c-Myc, leading to proliferation. A possible crosslink between these two pathways should be considered.

Studying the possible crosslink between KRAS and Wnt/β-Catenin signalling pathway a lung cell-line was used with stable transfection with KRAS G12V, MEK-DD and a vector control. Transfected cell-lines were treated with specific MEK-, ERK- and Wnt-inhibitors. To investigate changes in protein levels and/or phosphorylation during inhibition of KRAS or Wnt/β-catenin pathway, Western Blot analysis were implemented.

Inhibition of all cell-lines with specific MEK-inhibitor (U0126) leads to decreased cMyc levels.

It can be assumed that inhibition of MEK also has an effect on the Wnt signaling pathway.

Again, all cell-lines were inhibited with a specific ERK-inhibitor (SCH772984). Western-Blot results could show decreased LRP-6 levels. LRP-6 might acts as a linking factor of both signaling pathways.

Inhibition with LGK-974 shows no effect on LRP-6 levels both in activated MEK-cells and KRAS mutated lung-cells. These results assume a possible link between both pathways and Wnt is activated via RAS-pathway.

A manipulation of the KRAS-pathway shows an effect on Wnt-Pathway in lung-cancer cell-lines. Despite Wnt-inhibtion, Wnt-pathway remains activated. LRP-6 can be assumed as a possible link.

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High-resolution single-cell atlas reveals diversity of the tumor microenvironment in colorectal cancer

Colorectal cancer (CRC) is characterized by molecular heterogeneity with diverse immune cell infiltration patterns, which has been linked to therapy sensitivity and resistance. However, full understanding of how immune cell phenotypes vary across different patient subgroups is lacking. Here, we dissect the CRC tumor microenvironment by integrating 2.2 million single cells from 1143 samples and 437 patients and 27 studies representing 4 billion expression values. Additionally, given the scarcity of granulocyte single-cell data, we complemented the atlas by analyzing samples from 12 patients with CRC using a platform that captures cells with very low transcript count. We provide a high-resolution view of CRC with 44 major cell types/states and show different cell-type composition patterns in CRC subtypes. The atlas enabled refined tumor classification and patient stratification into four immune phenotypes: immune desert, myeloid, B cell, and T cell subtypes. These findings may have important implications for improving cancer immunotherapy in CRC.

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Retrospective analysis of pelvic MRI in the diagnosis of endometriosis and adenomyosis in 100 women using the #ENZIAN classification score

Endometriosis (extrauterine endometrium-like proliferation) and adenomyosis (intramyometrial endometrium-like proliferation) are common, heterogenous, overlapping, hormone-dependent gynecological diseases that primarily affect women of reproductive age. Frequently associated with dysmenorrhea, chronic pelvic pain and infertility, they can cause a broad spectrum of symptoms and there is usually a considerable delay between the onset of symptoms and diagnosis.

Surgical visualization with histopathological correlation remains the diagnostic gold standard and transvaginal ultrasound (TVUS) is considered the imaging modality of choice. Routine performance of an MRI in the preoperative assessment of endometriosis/adenomyosis is not yet established, despite of its high diagnostic accuracy, especially when using structured reporting, and its great potential contribution to preoperative planning and staging.

The aim of this retrospective study-project is to further asses the advantages of including MRI in the diagnosis and preoperative planning of endometriosis and adenomyosis. For this purpose, we propose a multifocal approach, starting with a retrospective analysis of existing unstructured MRI reports and a blind structured review of existing imaging by several radiologists, with correlation of the respective (un-)structured #ENZIAN-MRI-Scores to the histopathological #ENZIAN-Score and determination of the interobserver agreement when using structured reporting. Further, we intent to demonstrate the standardised inclusion of Diffusion weighted imaging (DWI) in pelvic MRI-sequence-protocols aimed to detect and characterise endometriosis/adenomyosis as being obsolete.

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Female patients spend longer time on the waiting list, but live longer after liver transplantation than male patients

Background: Sex-related disparities in liver transplant recipients exist in relation to the underlying disease, post-transplant outcome and overall mortality.

Aim of the study: The aim of the present study was to investigate sex-associated differences in patients who underwent liver transplantation.

Methods: For this aim, 847 patients who received a liver transplant at the University hospital in Innsbruck and their respective donors were retrospectively studied. Baseline characteristics, waiting list time, post-transplant survival as well as the impact of gender mismatch were investigated.

Results: Male patients got transplanted with a higher median age (58 vs. 55 years, p= <0.001), whereas female patients got listed with a higher median MELD-Score (16 vs. 14, p = 0.027). HCC (Hepatocellular Carcinoma) was significantly more frequent among male transplant recipients (40.6% vs 17.2%, p = <0.001). This in combination with MELD-Score was associated with a shorter waiting time in male transplant recipients (median 75.5 days vs. 107.0 days, p = 0.0015). Cox regression analysis showed post-transplant survival rate was higher in female patients (Median survival of 13.5 vs. 18.5 years, p=0.0043), but after multivariable adjustment for age this association was no longer significant.

We could not find a significant impact of gender mismatch in our cohort.

Conclusion: The observed gender disparities among liver transplant recipients are mainly attributable to differences in age-, MELD score at listing and HCC prevalence.

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Gender differences in sleep apnea, cardiac autonomic dysfunction and physical activity in pacemaker patients

Background: In the ACaSA trial, the presence and burden of sleep apnea, cardiac autonomic dysfunction and physical activity are prospectively evaluated in pacemaker patients. We thought to evaluate gender differences.

Methods: Patients with dual-chamber pacemakers (Microport Borea DR) were enrolled. Continuous monitoring of the so called respiratory disturbance index (RDI) via the thoracic impedance sensor of the pacemaker facilitates precise sleep apnea assessment. PRD, an ECG-based risk marker, determines cardiac autonomic dysfunction. Physical activity was recorded via both, the pacemaker acceleration sensor as well as and thoracic impedance sensor.

Results: Between November 2021 and January 2024, 93 patients were enrolled (median age 74, 40% female). Male patients exhibited a higher incidence of moderate to severe SA (34.5% vs. 21.1% in females) with significantly higher mean RDI values (18.8 vs. 13.7 events/hour). Cardiac autonomic dysfunction was more prevalent in females (82.6% vs. 54.5% in males), although PRD values showed no statistical gender difference. Physical activity displayed no gender-based distinctions.

Conclusion: Sleep apnea manifested in a noteworthy percentage of individuals with pacemakers, exhibiting discernible sex-based variations consistent with existing literature. The ongoing ACaSA trial will show, whether females with pacemakers and autonomic dysfunction face an elevated risk of adverse cardiovascular events such as heart attacks, strokes, or mortality in comparison to men. Notably, marginal disparities between the sexes were observed concerning physical activity, implying a lack of gender-based distinctions in the impact of this risk factor on clinical outcomes.

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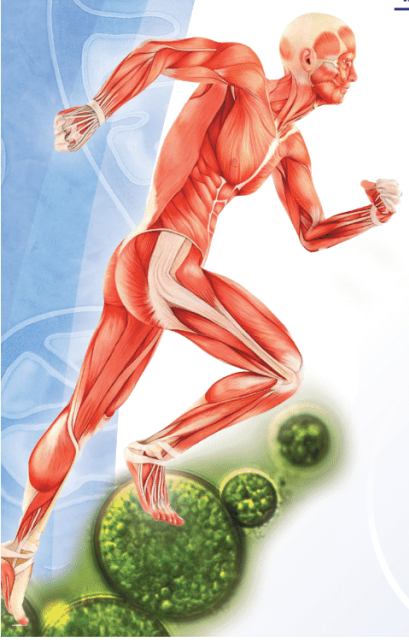

»Combine measurements in the O2k

- O_2 consumption
- Q-redox state
- NAD(P)H redox state
- Oxygen dependence
- Hypoxia and O_2 kinetics
- H_2O_2 production
- mt-Membrane potential
- ATP production
- pH, Ca^{2+}, $NO^{\cdot}$
- Photosynthesis
- Dark respiration
- Light-enhanced respiration

» Bioenergetic studies in various sample types

- Isolated mitochondria
- Tissue homogenate
- Permeabilized muscle fibers
- Permeabilized cells
- Living cells
- Chloroplasts

University project collaborations

- ALAS
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Investigating the Migraine Cycle over 21 Consecutive Days Using Proton Magnetic Resonance Spectroscopy and Resting-State fMRI: A Pilot Study

Recent neuroimaging studies have revealed important aspects of the underlying pathophysiological mechanisms of migraine suggesting abnormal brain energy metabolism and altered functional connectivity. Proton magnetic resonance spectroscopy (1H-MRS) studies investigated migraine patients in the interictal or ictal state. This first-of-its-kind study aimed to investigate the whole migraine cycle using 1H-MRS and resting-state functional magnetic resonance imaging (fMRI). A migraine patient underwent 1H-MRS and resting-state fMRI for 21 consecutive days, regardless of whether he was in an interictal or ictal state. Metabolite ratios were assessed and compared to the intrinsic connectivity of subcortical brain areas. Probable migraine phase-dependent changes in N-acetyl aspartate (NAA)/total creatine (tCr) and choline (Cho)/tCr levels are found in the left occipital lobe and left basal ganglia. NAA reflects neuronal integrity and Cho cellular membrane turnover. Such abnormalities may increase the susceptibility to excitatory migraine triggers. Functional connectivity between the right hippocampus and right or left pallidum was strongly correlated to the NAA/Cho ratio in the right thalamus, suggesting neurochemical modulation of these brain areas through thalamic connections. To draw statistically significant conclusions a larger cohort is needed.

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Post Stroke Osteopathy

Background: Falls and fractures are a frequent problem in post-stroke care leading to a considerable morbidity. It is assumed that the high fracture risk of these patients is caused through a systemic bone altering process initiated by the event itself.

Methods: A high-resolution peripheral quantitative Computer Tomography imaging (HR-pQCT) was performed of the distal radius and distal tibia right and left site on four different time points. Blood samples were drawn at five time points. Patients have been recruited from the Stroke Card Registry Study from March 2021 to May 2023.

Results: 122 patients have been included in the Post Stroke Osteopathy study, 35 women (28,7%) and 87 men (71,3%), with a mean age of 72 years. In total 404 HR-pQCT scans with four extremities each have been performed. In a follow up period of 12 months 31 (25,4%) patients reported at least one fall, 11 (31,4%) women and 20 (23%) men. 7 (5,7%) fractures have been documented, 2 (5,7%) women and 5 (5,7%) men. There was no difference in functional recovery at discharge, after three months, and after twelve months, neither in neurological recovery (NIHSS) at these time points (results no shown here). The increased risk of falls in women is unclear at this point and merits further research. The HR-pQCT showed a decrease of the Bone Mineral Density in all four extremities in women and men. Cortical Porosity in the lower extremities slightly decreased in women but increased in men. Analysis of the HR-pQCT data is ongoing.

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Ex Vivo Lung Perfusion: A New Clinical and Translational Model for Prolonged Ex Vivo Lung Perfusion and Oncological Testing

Ex vivo lung perfusion (EVLP) is an already accepted technique in specialized centres to evaluate borderline donor lungs for organ transplantation. Nevertheless, the duration of EVLP is still too short to allow for sophisticated oncological research models calling for according research in EVLP prolongation.

With prolonged perfusion time also the implementation of oncological research platforms is possible. If the duration of EVLP can be prolonged to several days, such as is done with livers, new therapies can be tested on human tissue ex vivo to accelerate their development and clinical use. As a secondary effect this assists in abandoning animal testing and reduce unsuccessful or possible harmful clinical trials.

With certain protocols it is already possible to perform EVLP for up to 24 hours with stable organ functions. Options for prolonging this time frame involve the optimization of the perfusate by adding nutritional factors and dextran, positioning maneuvers, negative pressure ventilation, perfusion pressure/volume or modifying the perfusion temperature.

Based on the extensive experiences with successful prolonged ex vivo liver perfusion at our institution and available literature this study aims to significantly prolong EVLP in a porcine EVLP protocol by using these resources. To achieve this, we aim to perform EVLP with ten porcine lungs with a modified EVLP protocol. The outcome will be measured with functional parameters to allow for the fast implementation of our results in clinical, oncological and transplant applications.

This study delivers the basis for prolonged EVLP to promote and enable an oncological and transplant research model.

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Gender differences in pain in people with multiple system atrophy

Background: Pain is a frequently reported non-motor feature of multiple system atrophy (MSA). Nonetheless, gender differences in pain have not been explicitly addressed in MSA to date and a pooled meta-analysis was not able to detect any difference in pain prevalence across female and male persons with MSA.

Objective: Here we explore differences in pain prevalence across female and male individuals with MSA.

Methods: Based on evidence retrieved from a systematic review of literature, on inputs from patients and their caregivers, from pain and movement disorders experts, we developed a questionnaire addressing individuals with MSA. The questionnaire explored clinical-demographic features, presence and characteristics of pain, pain-related burden, pain treatment strategies and

was available online between February and May 2023.

Results: Two hundred and seventy-one individuals with MSA accessed the questionnaire. After checking for data completeness and plausibility, questionnaires completed by 190 individuals were retained for final analysis. Among them, 46% [n=87, age: 63 (57; 69), disease duration: 5 (4; 7)] were male and 56% [n=103, age 62 (55; 69), disease duration: 5 (4; 7)] female subjects. Age (p=0.434) and disease duration (p=0.725) did not differ across the two groups. Pain occurred more frequently in female compared to male persons [OR: 6.38 (95% C.I. 1.27-32.08), p=0.025].

Conclusion: Pain is more frequently reported by female as compared to male individuals with MSA. Both biological, psychological, and social factors may contribute to the observed difference. A better characterization of gender differences may help developing tailored screening and management strategies for pain in MSA.

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Neuroimmuneprofiling in a schizophrenia cohort

Inflammation being involved in the pathogenesis of schizophrenia is largely discussed in the literature with evidence coming from clinical, genetic and epidemiological studies. However, reported findings of cytokine profiles and neurotransmitter precursor amino acid data are largely heterogenous and knowledge on associations between inflammatory status and response to antipsychotic medication is scarce.

As a high percentage of patients shows treatment resistant symptoms, identifying prognostic biomarkers in order to identify those patients that would benefit from add-on anti-inflammatory treatment is an important further step in individualising antipsychotic treatment.

The presented study is an add-on project to the European Long-acting Antipsychotics in Schizophrenia Trial – EULAST which compared depot and oral antipsychotics treatment in a longitudinal, randomized, pragmatic trial. Serum samples of peripheral blood from a total of 352 schizophrenia patients from 40 centers in Europe and Isreal were analyzed at up to four different timepoints after initiation of the study medication. Both cytokine and metabolic parameters were assessed and neuroimmuneprofiles will be related to response and remission criteria measured by Positive and Negative Syndrome Scale (PANSS). An interim analysis of results shows the neuroimmune profile heterogeneity of the study population and allows first insights in associations of parameters and symptom severity.

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Sex-based analysis of systolic blood pressure in migraine patients with prophylactic monoclonal antibodies treatment

Migraine is a neurological disorder that affects nearly 20% of the general population, making it the second leading cause of Disability Adjusted Life Years in young adults aged 25-49 years. It is predominantly diagnosed in women. In the last decade, targeted therapy options have been approved in the European Union. Four monoclonal antibodies (mAbs) targeting the Calcitonin gene-related peptide (CGRP) pathway have been approved for prophylactic treatment of episodic and chronic migraines. CGRP is one of the most potent vasodilators in humans and animals, blocking its pathway may result in elevated systolic blood pressure, as suggested in a previously published retrospective study by Saely et al.

The purpose of this retrospective data analysis is to assess the presence of elevated systolic blood pressure (BP) after prophylactic treatment with mAbs in our study cohort (n = 508), with a focus on sex-based differences.

We retrospectively enrolled all patients who visited the outpatient clinic of the Neurology Department at the Medical University Innsbruck between 01.01.2015 and 30.04.2023. Systolic blood pressure is routinely measured during outpatient visits. Blood pressure levels were collected before starting the mAb therapy, at baseline, and after starting the mAb.

The retrospective cohort consisted of 83.8% females and 16.2% males. There were no significant differences in systolic blood pressure levels before and after starting the mAb treatment. Furthermore, no sex-based differences in systolic blood pressure were observed.

In our cohort, we found no correlation between mAb prophylactic treatment in migraine therapy and elevated systolic blood pressure, regardless of sex.

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Cardiovascular health profiles and the impact of being born preterm in adolescence – Gender Aspects and Results from the EVA-Tyrol Study

Preterm birth has been linked with an increased risk of cardiovascular (CV) disease in later life. Here, we aimed to (I) investigate differences in CV health profiles between former term- and preterm-born infants (II) with a special focus on sex related aspects in a cohort of healthy Tyrolean adolescents.

The Early Vascular Aging (EVA)-Tyrol study is a population-based non-randomized controlled trial, which prospectively enrolled 14- to 19-year-old adolescents in North and South Tyrol between 2015 and 2018. Metrics of CV health (body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP), smoking, physical activity, dietary patterns, total cholesterol and fasting blood glucose) were assessed and compared between former term- and preterm-born girls and boys.

In total, 1,491 study participants (59.5% female, mean age 16.5 years) were included in the present analysis. SBP and DBP were significantly higher in former preterm-born adolescents (mean gestational age 34.6 ± 2.4 weeks) compared to term-born controls ($p<0.01$). Spearman correlation showed an association between SBP and sex in former preterm (<0.01, -0.524) and term (<0.01, -0.400 born adolescents as well as an association between DBP and sex in former term born adolescents (0.013, 0.067). After adjustment for sex multivariate regression analysis revealed significant higher SBP and DBP in former preterm born adolescents after adjustment for sex.

Preterm birth is associated with elevated SBP and DBP in adolescence. Furthermore, our data show that blood pressure in preterm and term born individuals is influenced by sex. This must be kept in mind in targeted health promotion.

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The effects of acute ischemic stroke on cerebral energy metabolism in male and female patients

Introduction: Acute ischemic stroke (AIS) is a vascular brain disease characterised by a sudden ischemic infarction within the central nervous system (CNS), caused by a rapid reduction of the cerebral blood flow (CBF). Currently it is assumed, that depending on the CBF-rate, this reduction leads to serious metabolic and biochemical changes in affected neurons and ultimately to neuronal dysfunction and cell death. However, the precise effects of AIS on cerebral energy metabolism are currently unknown.

Methods: 30 patients (20 male, 10 female) presenting with an ischemic stroke of the supra-tentorial brain within 6h after symptom-onset were examined after medical treatment by using P31-MRS imaging. For further analysis voxels were distributed according to their localization relative to the infarct core into four regions: stroke, adjacent to stroke, healthy ipsilateral and healthy contralateral.

The phosphorus-compound metabolite ratios (PCr/ATP, Pi/ATP and PCr/Pi) of each region were then calculated, compared and correlated with diffusion-weighted-imaging signal intensity (TRACE, ADC).

Results: Adjacent voxels showed a significantly lower PCr/ATP-ratio compared to healthy voxels on the ipsilateral side. Voxels of the infarct core on the other hand showed a significantly higher Pi/ATP-ratio and a significantly lower PCr/Pi-ratio, than the voxels of any other brain region.

The regression analysis further revealed a significant correlation between the Pi/ATP-ratio and the TRACE signal intensity, as well as the PCr/Pi-ratio and TRACE signal intensity.

There were no significant differences between the overall metabolic ratios of both sexes.

Conclusion: AIS has serious effects on cerebral energy metabolism, especially phosphorus-compound metabolites.

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Impact of Inflammation during Normothermic Machine Perfusion of the Liver Graft on the Recipient

Background and Goal of the Study:

Normothermic machine perfusion (NMP) represents an innovative technique in liver graft preservation post-explantation. This method not only allows for real-time monitoring of graft functionality and metabolic activities but also provides insights into the influence of circulating substances in the perfusate on both the organ's condition and the recipient's perioperative health trajectory. This study explores the effects of Neutrophil Extracellular Traps (NETs), where activated neutrophils release a web-like chromatin structure into the extracellular space, and several inflammatory markers during NMP on recipients.

The aim of this research is to establish a correlation between specific inflammatory profiles during NMP and the subsequent postoperative course in recipients. Identifying detrimental inflammatory mediator patterns could pave the way for early detection of patients at heightened risk for postoperative complications.

Methods:

Plasma and perfusate samples from 30 patients who underwent liver transplantation following NMP will be analyzed. The quantification of NETs will be conducted using a sandwich ELISA format with anti-myeloperoxidase as the capture antibody and a labeled DNA-specific detection antibody. Additionally, inflammatory cytokines, interleukin (IL)-1, IL-6, IL-8, IL-10, and tumor necrosis factor-alpha, will be assessed through ELISA. Analysis will be conducted at the the start, after six hours, at the end of NMP and in the first three postoperative days. Inflammatory mediator levels will be correlated with the occurrence of early allograft dysfunction and other postoperative complications such as myocardial infarction and kidney injury.

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Gender differences in ECG-derived QRS microfragmentation for risk stratification in patients presenting to the emergency department

Background: QRS microfragmentation (QRSμf) is a novel ECG-derived parameter that quantifies irregularities within ventricular depolarization. Previously, increased values of QRSμf (>3.5%) were found as a strong predictor of mortality in patients presenting to the emergency department. The aim of this work was to evaluate gender specific differences in values of QRSμf and its predictive power in these patients.

Methods: We retrospectively collected ECG recordings from unselected patients presenting to the emergency department and calculated QRSμf as previously published. The gender of the patients was defined as specified within the hospital information system at date of admission. Survival status was retrieved for all patients after one year.

Results: In total, 10915 patients were included in our analyses. Median age was 69 (IQR 54-80) years, 5643 (52%) were male and 5272 (48%) were female. Values of QRSμf in women were significantly lower than in men (1.5 [IQR 1.1-2.1]% vs. 1.8 [IQR 1.3-2.5]%, $p<0.001$, Figure 1). In Cox regression analysis QRSμf was a strong predictor of mortality in both, female and male patients, with hazard ratios of 1.54 [1.40-1.69] and 1.45 [1.33-1.59] ($p = 0.379$ for interaction). In exploratory analysis, the optimal cut-off value for maximizing the chi-square statistic was 2.43% for women and 3.18% for men.

Conclusion: In patients presenting to the emergency department, numerical values of QRSμf as well as the optimal cut-off point were significantly lower in women compared to men. However, the predictive value of QRSμf was present in both groups and did not significantly differ between them.

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Imaging Findings and Outcomes in CT- or CMR-guided TAVR According to Sex: a Secondary Analysis of the TAVR-CMR trial

Background & Objectives: Previous studies have reported sex differences in pre-procedural imaging characteristics of patients undergoing transcatheter aortic valve replacement (TAVR) evaluation. We aimed to evaluate sex-based differences and outcomes of computed tomography (CT)-guided or cardiac magnetic resonance (CMR)-guided TAVR for severe aortic valve stenosis.

Methods: This was a secondary analysis of the TAVR-CMR trial, a randomized clinical trial comparing TAVR planning by CT or CMR. Outcomes (based on the Valve Academic Research Consortium (VARC)–2 definition) with each imaging strategy were compared according to sex.

Results: 267 of 380 patients randomized eventually underwent TAVR (133 women (49.8%) and 134 men (50.2%), p=0.457). Imaging findings differed between the sexes for both imaging modalities. The comparison between CT and CMR to assess the access route and landing zone showed no difference in both women and men (all p>0.05). Implantation success was not significantly different between imaging strategies for both women (84.7% (CT group) vs. 93.2% (CMR group), p=0.16) and men (95.7% (CT group) vs. 93.8% (CMR group), p=0.71). All-cause mortality at 6 months was not significantly different between imaging strategies for both women (10.2% (CT group) vs. 8.1% (CMR group), p=0.77) and men (4.3% (CT group) vs. 9.4% (CMR group), p=0.31).

Conclusions: This secondary analysis has confirmed sex-related differences in pre-procedural imaging characteristics, with no influence of the imaging modality used. Similar outcomes were observed in both female and male patients when the TAVR was guided by either a CMR or a CT scan.

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MR Spectroscopy in well-characterized individuals with and without post COVID condition prior to and following a Yoga breathing intervention- an Explorative randomised controlled trial

From a clinical perspective, we find that many patients with Post COVID condition suffer from severe and debilitating shortness of breath, while routine pulmonary investigations fail to find the cause of the problems experienced. If dyspnea is associated with palpitations, dizziness or anxiety, patients are commonly diagnosed with "dysfunctional breathing". From a psychosomatic perspective, the symptom of dysfunctional breathing can be classified as a "functional symptom" under the umbrella term of somatic symptom disorder. Therefore, Yoga interventions with special emphasis on breath-guided relaxation are a promising approach. We aim to investigate the psycho-somatic and somato-psychic pathophysiology on a morphological, psychological, functional and biological basis underlying the symptom of dysfunctional breathing. Furthermore, we plan to investigate the mechanism of Yoga intervention on the mental and somatic symptom burden of participants with Post COVID condition. Then, we aim to compare the impact of Yoga on other groups - healthy individuals, patients with chronic obstructive lung disease (COPD), as well as those with somatic symptom disorder. As a control intervention to Yoga guided breathing exercises a social contact group will be used.

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Subjective outcomes in schizophrenia patients using oral vs. long acting injectable antipsychotic drugs

Background

Schizophrenia classifies as a long-term neurodevelopmental and neurotoxic disorder, characterized by recurrent phases of relapse, which propagate mental as well as physical deterioration. In order to acquire and maintain a status of illness remission, antipsychotic medication plays a key role in the therapy management of schizophrenia

Methods

The "Efficacy of oral versus long-acting antipsychotic treatment" (EULAST), a pragmatic, large-scale, open-label, randomised trial was conducted at 50 general hospitals and psychiatric specialty clinics in 15 European countries and Israel in patients with early-phase schizophrenia, comparing time to all-cause discontinuation in patients randomly allocated to LAI versus oral medication during 19 months of treatment. Utilizing data collected during the EULAST trial, my doctoral thesis aims to compare of subjective well-being (SWN-K) and quality of life (EQ-5D-5L) in patients treated with oral versus long acting injectable antipsychotics. Additionally, we plan to investigate to what extent psychopathological symptoms (PANSS), side effects of medication (SMARTS, AIMS, St. Hans), and adherence (MARS) are associated with subjective well-being and quality of life in patients treated with depots vs. orals.

Objectives

Up to now, no study has investigated subjective outcomes in patients treated with oral vs long-acting antipsychotics. Therefore, this analysis will hopefully contribute to a better understanding of patients perspectives and experiences in order to further improve therapeutic alliance, medication adherence, long-term prognosis and ultimately to have a positive impact on schizophrenia patient´s life overall.

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Changes in Psychopathology and peripheral inflammation in Chronically Ill Schizophrenia Patients – A 6-months survey

Background: There is an increasing body of literature indicating a correlation between neuroinflammation and schizophrenia. However, there is a lack of long-term studies. Additionally, previous discoveries regarding a possible longitudinal connection between psychopathology at the initiation of antipsychotic monotherapy and changes in both psychopathology and peripheral inflammation have been inconsistent.

Methods: 116 (52.6% male) cases diagnosed with schizophrenia commencing monotherapy with a new-generation antipsychotic were included in this study. Alongside the baseline evaluation of sociodemographic and clinical data, the Positive and Negative Syndrome Scale (PANSS) was utilised at the beginning and after 12 and 26 weeks of therapy. Blood samples (full blood count, C-reactive protein [CRP]) were collected simultaneously at these intervals. Next to CRP levels, the neutrophil- to-lymphocyte ratio (NLR), the monocyte-to-lymphocyte ratio (MLR), and the systemic immune-inflammation index (SSI) were considered as integrative immune inflammation markers. Baseline analysis data were available for 116 cases, while data for 45 and 26 cases were available at 3 and 6 months of treatment, respectively.

Results: PANSS (sub)scores decreased significantly from baseline to follow-up assessments, and there were no differences between males and females. Moreover, no significant differences were found between sexes in CRP levels, NLR, MLR, and SSI. Spearman rank correlation analyses revealed no statistically significant associations between PANSS (sub)scores, markers of neuroinflammation, and sex during any point of the investigation.

Conclusions: These preliminary findings did not reveal a link between peripheral inflammation and the severity of symptoms in schizophrenia. Further extensive studies are required to clarify this matter.

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Influence of intracranial arterial calcification on stroke, subsequent stroke and related complications.

Background: Stroke is one of the leading causes of death in the European Union. Intracranial arterial calcifications (IAC) are a common incidental finding on computed tomography (CT) scans used in the diagnosis of stroke. Due to the severe consequences of stroke, IAC has received increased scientific attention as a risk factor and potential predictor of future stroke and related complications.

Objective: To evaluate the influence of intracranial arterial calcifications on stroke, subsequent stroke and related complications.

Methods: Retrospective extraction and analysis of patient data from the local database implemented for the VASCage subproject "Imaging Biomarkers For Vascular Diseases and Vascular Aging". Common characteristics of the patient collective are at least one cerebral event and being of legal age. In addition to cerebral events, cardiovascular and peripheral events as well as risk factors are recorded. Sociodemographic information comprises sex and year of birth. IAC is analyzed by non-contrast CT and computed tomography angiography (CTA) using quantitative and qualitative methods.

Quantitative calcium measurements comprise volume and density. The Agatston-score is calculated additionally on non-contrast CT.

The qualitative Kockelkoren Method can be used to determine the affected vessel wall layer of the intracranial internal carotid artery on non-contrast CT.

Discussion: Overall, IAC might present a promising marker for risk stratification or even a predictor of future stroke, about which little is known. IAC may also play a role in recanalization failure. Ideally, IAC could be used as a biomarker for risk stratification of stroke and complications in the future.

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Improving safety of phacoemulsification cataract surgery in high-risk patients using real-time anterior chamber pressure sensing and regulation

Cataract is the most common cause of blindness in the world, accountable for 50 % of all cases. The gold standard therapy, called microincision phacoemulsification surgery (MICS), is associated with an intraoperative complication rate of 1 - 3 % for experienced surgeons. The most common complication is the rupture of the posterior capsule (PCR).

Instability of the anterior chamber during surgery with protrusion of the posterior capsular rupture are the major risk factors for PCR. Existing pathologies, such as zonular dehiscence, pseudoexfoliation, trauma, phacodonesis, advanced cataract and poor preoperative visual acuity also favour the occurrence.

While latter factors are given, anterior chamber stability is a variable that can be partially influenced, with a direct negative association to intraoperative complications and postoperative inflammatory reactions in the eye. The Alcon Centurion Vision System with Actice Sentry handpiece specifically is designed to improve this stability. The density of inflammatory cells in the anterior chamber as well as central corneal thickness are established parameters to determine the postoperative immune response, and are related to surgical time, energy used, and surgical trauma.

This system was compared with the Eva phacoemulsification device from DORC. The primary outcome parameters measured were postoperative inflammatory response as anterior chamber flare (ACF) und central corneal thickness (CCT) on days 1 and 7. ACF was measured by laser-flare-photometry which is an objective method to quantify cells and proteins in the anterior chamber, CCT by optical coherence tomography (OCT).

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Cytokine values in nasal lavage samples of patients with cystic fibrosis indicate a primary mucosal immune response in patients with mild lung disease

Objectives: Measuring cytokines in induced sputum (IS) and nasal lavage (NL) samples to investigate inflammatory state in people with cystic fibrosis (pwCF) has been in use for many years. The aim of this study was to directly compare IS and NL samples in patients with mild and severe lung disease.

Methods: pwCF were primarily categorized into mild and severe based on structural abnormalities by their lung computed tomography and secondarily on lung function parameters. Serum inflammatory markers and neutrophil elastase (NE), IL-1β, 2, 6, 8, 10 and 17a were locally measured in each IS and NL samples.

Results: 32 sample-pairs of 29 patients were included in the study (11 were classified as mild and 18 as severe). All patients classified as severe were chronically bronchopulmonary colonized and systemic inflammatory markers as well as sputum cytokines were significantly higher compared to mild. However, all markers measured in NL were higher in mild patients ($p = <0.05$ for NE, IL-6, and IL-8).

Conclusion: Major differences in cytokine levels were shown in IS and NL although samples were obtained at the same time in the same patient. Advanced structural lung disease closely related to systemic and sputum inflammation whereas preserved lung function was associated with higher cytokine levels in the upper airways. We hypothesize, that the main part of the immune response takes place in the nasal mucosa in patients with minor pulmonary changes. Our results suggest that inflammation must be interpreted individually depending on the compartment where it was measured.

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Sex differences in serum neurofilament light chain levels in amyotrophic lateral sclerosis.

Introduction: Neurofilament light chain (NfL) is a cytoskeletal protein expressed in neurons, which is released into the extracellular space upon neuro-axonal injury. NfL has been proposed as a diagnostic and prognostic biomarker in several neurodegenerative conditions, including amyotrophic lateral sclerosis (ALS). Sex-related differences in neurodegenerative diseases are multifactorial and may be determined by factors such as (epi)genetics, gonadal hormones, immune response, lifestyle and gut microbiota. Therefore, sex potentially has an effect on serum NfL concentrations. We aim to investigate the relationship between serum NfL and sex in ALS.
Methods: Using validated enzyme-linked immunosorbents assays (ELISAs), NfL was measured in serum in patients with ALS as part of routine clinical work-up early in the diagnostic process, and was evaluated retrospectively. Clinical and demographic data were obtained at the time of NfL sampling. Serum NfL levels in men and women were compared.

Results: n=50 women (mean age 64.2±10.5 years) and n=50 men (mean age 61.5±11.5 years) with ALS were included. ALSFRS-R, as marker of disease severity, was 39.7±0.9 in men and 39.6±0.8 in women. Age and disease severity were not significantly different between sexes ($p = 0.241$ respectively $p = 0.610$). Mean NfL in women was 129.0±91.9 pg/mL while it was 108.3±62.0 pg/mL in men. No sex difference in serum NfL levels was observed.

Conclusion: In cohorts of similar age and disease severity, no evidence was found for sex differences in the serum NfL level in ALS patients.

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Determinants of Diet Quality in Adolescents: Results from the Prospective Population-Based EVA-Tyrol and EVA4YOU Cohorts

(1) Background: Unhealthy dietary behaviors are estimated to be one of the leading causes of death globally and are often shaped at a young age. Here, we investigated adolescent diet quality and its predictors, including nutrition knowledge, in two large Central European cohorts. (2) Methods: In 3056 participants of the EVA-Tyrol and EVA4YOU prospective population-based cohort studies aged 14 to 19 years, diet quality was assessed using the AHEI-2010 and DASH scores, and nutrition knowledge was assessed using the questionnaire from Turconi et al. Associations were examined utilizing multivariable linear regression. (3) Results: The mean overall AHEI-2010 score

was 42%, and the DASH score was 45%. Female participants (60.6%) had a significantly higher dietquality according to the AHEI-2010 and DASH score. AHEI-2010 and DASH scores were significantly associated ($p < 0.001$) with sex, school type, smoking, and total daily energy intake. The DASH-score was additionally significantly associated ($p < 0.001$) with age, socioeconomic status, and physical activity. Participants with better nutrition knowledge were more likely to be older, to attend a general high school, to live in a high-income household, to be non-smokers, and to have a higher diet quality according to the AHEI-2010 and DASH score. (4) Conclusions: Predictors of better diet quality included female sex, physical activity, educational level, and nutrition knowledge. These results may aid focused interventions to improve diet quality in adolescents.

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Hippocampal Avoidance Whole Brain Radiotherapy and Stereotactic Radiosurgery in Patients with Multiple Brain Metastases

Introduction: Whole brain radiation therapy (WBRT) has long been the primary treatment for patients with multiple brain metastases, offering improved overall survival and rapid symptom relief. However, it can lead to permanent neurocognitive decline by affecting the radiation-sensitive hippocampal region. This study aims to assess if Hippocampal Avoidance Whole Brain Radiotherapy with Simultaneous Integrated Boost (HA-WBRT+SIB) can enhance intracranial control compared to Single Isocentre Dynamic Conformal Arc Stereotactic Radiosurgery (SIDCA SRS), while preserving neurocognitive function.

Study Design: This is a randomized, controlled, single-center trial involving 100 patients with multiple brain metastases. Patients will be randomly assigned to receive either HA-WBRT+SIB (Arm A) or SIDCA SRS (Arm B). HA-WBRT+SIB will be administered through VMAT with a prescribed dose of 30Gy in 12 fractions and a simultaneously integrated boost of 51Gy to each brain metastasis. SRS will deliver 20Gy to the tumor. Primary endpoint: intracranial progression-free survival. Secondary endpoints: quality of life, neurocognitive function, overall survival, local control, safety, and toxicities.

Current status and early results: Among 49 enrolled patients (47 completed therapy, maximum follow-up: nine months), nine remain alive (Arm A: 4, Arm B: 5). Mean overall survival: Arm A 359 days, Arm B 278 days. Five new metastases in Arm A, three in Arm B post-treatment. Mean intracranial control: Arm A 454 days, Arm B 161 days. All intracranial failures were due to new metastases, with three in Arm B occurring in the hippocampal avoidance area.

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Gender differences in the virtual non-contrast density evaluation of diffuse liver disease

Introduction. Dual Energy CT (DECT) has the advantage of material decomposition algorithm providing a broad spectrum of diagnostics. DECT enables determining the iodine content of the liver on enhanced CT images and creating virtual non-enhanced image (VNC). If validated, this could offer the opportunity to reduce patient`s radiation dose by omitting true non-enhanced images (TNC) and improving efficiency of scans.

Methods. Data of 184 patients (141 male, 43 female) is analysed. DECT is performed at the Siemens Somatom Drive with pre-set of 100/140 keV and post processed with Siemens Syngo.Via software. To address differences in attenuation of liver and spleen mean density of each organ is measured on TNC, arterial VNC (aVNC) and delayed VNC (dVNC) and compared. These differences are stratified for gender and likewise compared.

Results. The mean difference of attenuation of the liver is -7,78 ± 7,75 HU (TNC-aVNC) and -7,57 ± 7,25 HU (TNC-dVNC) and of the spleen -12,76 ± 12,47 HU and -10,34 ± 12,45 HU. Men showed an average offset of -8,9 ± 8,29 HU and -8,50 ± 7,84 HU, whilst women showed a smaller difference of -4,77 ± 4,97 HU and -5,05 ± 4,22 HU. A significant difference between genders is detected.

Discussion. Overall showed VNC a small difference of liver attenuation of less than 10 HU compared to TNC, a cut-off value often used in literature to determine a clinical relevant deviation of VNC. The significant offset between genders could be explained by gender-specific differences in prevalence of liver disease.

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Sex Insights into Clonal Hematopoiesis of Indeterminate Potential within a Vast Stroke Cohort

Background: Clonal Hematopoiesis of Indeterminate Potential (CHIP) denotes the acquisition of certain mutations in hematopoietic cells, impacting health outcomes. CHIP, characterized by leukemia-associated mutations (e.g., DNMT3A, TET2, ASXL1), unveils heightened risks of hematologic cancers and cardiovascular diseases, including stroke. This study delved into the relationship between CHIP, stroke, and sex disparities within a cohort of stroke patients.

Methods: Blood samples from participants 1 to 7 days after stroke underwent deep targeted next-generation sequencing, enabling the precise identification of CHIP-associated mutations. Additionally, plasma was isolated to profile cytokines, shedding light on inflammatory signatures.

Results: Preliminary analysis of our 399 sequenced patients (269 males, 151 females) revealed age-associated escalation in CHIP frequency. Despite similar ages (men: 70.7 years, women: 71.4 years), gender-based disparities emerged: Women exhibited a higher prevalence of CHIP mutations (28% vs. 22% in men). DNMT3A variants were notably more prevalent in women (22.1% vs. 17% in men), while ASXL1 variants predominated in men (3.9% vs. 1.75% in women); TET2 variants demonstrated comparable occurrence between genders. Also, the level of IL22 were higher for men than for women (p=0,034), while the other analyzed cytokines showed no significant gender-specific differences.

Conclusion: These findings highlight how genetics and sex intersect in stroke patients with Clonal Hematopoiesis, emphasizing the need for personalized approaches to understand and address associated risks.

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Proximal junction and transitional mechanics and the effect of a novel Tether Pedicle Screw in long- segment spinal instrumentation.

Objective

Correction of adult spinal deformities carries a high risk of junctional failure. A soft-landing construct at the end of a rigid construct might reduce the risk of proximal junctional kyphosis (PJK) and failure (PJF). Therefore, a novel tether pedicle screw (TPS) was designed to mitigate the risk of PJK/PJF. That screw is characterized by a tether between the threaded shaft and the screw head enabling motion among parts.

Methods

For initial flexibility tests three instrumentation pattern were tested: Representing conventional instrumentation, a standard thoracolumbar pedicle screw-rod instrumentation T10-L2 was used (STD-group). The TPS were tested at T9 (TPS+1 group) one level above the upper instrumented vertebra and two level above in T9 and T8 (TPS+2 group).

Flexibility tests (±5 Nm) were performed and repeated after cyclic loading (250 cycles, 1-10Nm). Finally, STD-group and TPS groups were subjected to screw pullout at index level analysing stress-shielding effects by the TPS.

Results

The TPS+2 group demonstrated largest ROM decrease at T9-10 in the flexibility tests, with a smaller effect in the second adjacent segment at T8-9. There was no significant change in the ROM observed in the upper most segment T7-8 among all instrumentation pattern studies. Pullout-test revealed higher mean forces at the T10 end-level in TPS+2 compared to STD group.

Conclusion

TPS effectively distributed the loads across three adjacent levels and softened the load transition compared to a rigid construct. TPS also showed the potential to stress-shield the upper instrumented vertebra T10 and lower the risk for end-level screw loosening.

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ECT-Induced Increases In Limbic Volumes In Patients With Treatment-Resistant Depression — Pilot Data From The Innsbruck ECT-MRI Study

Electroconvulsive therapy (ECT) is a safe and effective treatment for affective and other mental health disorders. Its mechanisms of action and effects on the brain are topics of active research.

The literature shows increases in cortical grey mader volumes (GMV) and cortical thicknesses (CT) aser ECT.

Recently, the hippocampus and limbic regions known for their role in emotional and memory processing have been brought into focus as particular areas of interest. We hypothesized ECT- induced increases in the GMV and CT in limbic and memory regions.

In a longitudinal-observational study setting, 3T MRI is acquired in patients with treatment- resistant depression (major depressive [MDD]/bipolar disorders [BD]) before and after a course of ECT. T1-weighted MPRAGE images are analyzed with Computational Anatomy Toolbox 12. Longitudinal ROI and surface-based statistical analyses ($\alpha<0.05$, Holm-Bonferroni corrected) are applied using Neuromorphometrics-, CoBra-, and Destrieux-Atlases (2019) and are corrected for total intracranial volumes.

We will present pilot morphometric MRI results (GMV and CT) from the ongoing Innsbruck ECT-fMRI study.

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Sex specific body composition and difference in FMI and BMI categorization for overweight and obesity in Tyrolean adolescents: Data from the prospective EVA4YOU cohort study

Background: Obesity is an increasing problem in adolescents. Body mass index (BMI) inadequately distinguishes between fat and lean mass, impacting accurate obesity classification.

Objective: To develop age- and sex-specific reference percentiles for fat mass index (FMI) and fat-free mass index (FFMI) in adolescents aged 14 to 19 years and to determine differences in overweight/obesity classification by FMI and BMI.

Methods: 1422 adolescents aged 14-19 from the Early Vascular Ageing in the YOUth study underwent anthropometric measurements and BIA assessment. Reference curves for FMI and FFMI were then generated and classification differences between BMI and FMI percentiles were analyzed.

Results: Body composition classification by FMI and BMI percentiles show a concordance for the <75th and >97th percentile. Based on FMI 15.5% (221/1422) of the whole population and 29.4% (92/313) of those between the 75th and 97th percentile are classified one category higher or lower than those assigned by BMI.

Conclusion: BMI is limited in accurately assessing adiposity, showcasing substantial reclassification when utilizing FMI. FMI reference values provide a more nuanced understanding of obesity in adolescents, emphasizing the potential clinical relevance of BIA in assessing body composition, but further research is warranted to define standardized FMI cut-off values on a biological basis.

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Subgaleal Drainage with One Burr-hole for Chronic Subdural Hematomas: Our 2-Years' Experience

Objective: Subgaleal drainages have a lower risk profile compared to subdural drainages. It has not yet been reported, that it is as efficient with only one burr-hole. Therefore, the aim of this intervention is to evaluate the efficacy of subgaleal drainage placement with one instead of two burr-holes.

Methods: The main measured outcome at the end of the clinical trial was to determine whether surgery for recurrences is needed. Secondary endpoints were outcome based on modified Rankin Scale scores. Furthermore, seizures, infections, and parenchymal brain injuries were evaluated. The findings were compared to outcome data from the literature of the same institution.

Results: This case-series included 123 patients (38 female/85 male). The reoperation rate was 13 percent. 2 percent of the patient had a parenchymal bleeding, 2 percent a seizure, and none of them an infection. None of the drainages were misplaced. Compared to a retrospective analysis of a same institution cohort, the reoperation rate using subdural drainages was 15 percent. 2 percent suffered from an drainage related infection, 8 percent suffered from a seizure, 5 percent from a parenchymal bleeding. None of the drainages were misplaced. The incidence of a chronic subdural hematoma was smaller in female patients in both groups. (30 percent females in the subgaleal group and 30 percent females in the subdural group.)

Conclusion: We demonstrate in this case series that one burr-hole might be as efficient and safe as two burr-holes with the use of subgaleal drainages.

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Fibrinogen and FXIII in vitro substituion in Dilutional coagulopathy- Gender analysis

Introduction. Immediate substitution of coagulation factors is essential in dilution coagulopathy in order to stop vital bleeding. This in vitro study aims to find a substitution threshold for FXIII in relation to different fibrinogen levels.

Methods. 20 citrate blood samples (10 females, 10 males aged 18-85) were diluted in a 30:70 ratio with 50% colloid and 50% crystalloid to simulate dilution coagulopathy. Once diluted, samples were divided in 25 aliquotes and each aliquote was spiked with different concentrations of fibrinogen and FXIII. Coagulation tests PT, aPTT, Fibrinogen levels (as measured by Clauss and immunologic) and FXIII levels as well as ClotPro® (Fib-Test) and a clot retraction assay were performed in each spiked sample. In 5 of the 20 blood samples Ex-Test and TPA-Test were additionally analyzed in each spiking step. The main endpoint was defined as the first Fibrinogen to FXIII ratio to first reach a Fib MCF of 9mm.

Results. To restitute the minimum desired FIBtest MCF to 9 mm, a median FBG level of 135 mg/dL (Clauss method, IQR 124 – 225 mg/dL) and a FXIII level of 49 % (IQR 41 – 79 %) was necessary. Regarding clot composition indices (clot weight/serum weight) in the clot retraction assay, only addition of FXIII showed a consistent improvement. No significant differences were seen across gender.

Conclusion. According to this in vitro dilution model, we could recommend the substitution of 10 IE/kg FXIII for each 50 mg/kg Fibrinogen. That would correspond to a ratio of Fibrinogen to FXIII of 5:1.

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The role of Malassezia globosa in the development of Pancreatic Cancer

Introduction: the fungus Malassezia globosa recently gained interest in the pathogenesis of pancreatic ductal adenocarcinoma (PDAC). However, the evidence in a clinical setting is still under discussion. This study aimed to evaluate different human pancreatic tissues for the presence of M. globosa.

Methods: We retrospectively collected formalin-fixed paraffin-embedded (FFPE) pancreatic specimens from patients undergoing pancreas surgery. The detection of M. globosa was performed via rt-qPCR after DNA-extraction. The expression of fungal DNA was related to clinical and oncologic patients´ features.

Results: a cohort of 137 patients was established. Among them: 56 PDAC, 32 precursor lesions, 29 chronic pancreatitis, and 20 benign entities. For each patient, specimens of lesional as well as perilesional tissue were available (258 FFPEs in total). PDAC showed significantly higher Malassezia expression than benign entities ($p=0.010$) and chronic pancreatitis ($p<0.001$). Of note, in both low-stage PDAC (UICC I-IIa) and high-grade precursor lesions, fungal colonization was higher than in PDAC UICC ≥ IIb and low-grade precursors ($p=0.016$ and $p=0.014$, respectively). Regarding PDAC, Malassezia was related to negative nodal status, lack of vascular and lymphatic vessel invasion, lower grading, and lower UICC-staging ($p=0.021$, $p=0.005$, $p=0.012$, $p=0.009$, and $p=0.021$ respectively). On the other hand, sex, tobacco and alcohol consumption, and invasive preoperative interventions, like endosonographic biopsy and endoscopic retrograde cholangiopancreatography were not related to fungal DNA expression.

Conclusion: a significantly higher presence of M. globosa was characterized in UICC I-IIa PDAC and high-grade precursor lesions. These findings suggest a possible role of Malassezia in the malignant transformation of pancreatic cysts.

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Diagnostic and prognostic value of Interleukin 6 in the pediatric emergency department - a retrospective analysis.

Background:

Interleukin 6 (IL-6) has been established in neonatology as an important marker for early diagnosis of sepsis. Based on this success the usability of IL-6 has also been studied in various other fields of pediatrics like in the context of febrile neutropenia, community-acquired pneumonia or acute appendicitis. All this data led to a frequent routine use of IL-6 in the pediatric emergency medicine. However, the prognostic value of measuring elevated levels of IL-6 in children, who otherwise show no sign of an acute severe illness, is still up to debate.

Methods:

The main objective of this study is to evaluate the utility and potential impact of IL-6 measurements in a pediatric emergency department. Various laboratory parameters, including inflammatory markers such as Il-6, procalcitonin, and C-reactive protein, infectiological diagnostic as well as surrogate parameters for patient outcome, such as hospital admission data or vital signs, will be collected and correlated.

The study will be conducted in a monocentric, retrospective design. The pediatric department at the University Hospital Innsbruck will serve as the study center. Data extraction will be performed for the years 2019 to 2023. During this period, an expected number of 5000 patients will be included. Due to the retrospective design, no active intervention is planned for the study participants.

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Determinants of non-alcoholic fatty liver disease in young people: maternal, neonatal, and adolescent factors

Aims: To assess the impact of maternal, neonatal, and adolescent factors on the development of non-alcoholic fatty liver disease (NAFLD) in a cohort of 14- to 19-year-old Tyrolean adolescents.

Methods: This study is part of the Early Vascular Ageing in the YOUth study, a single-center cross-sectional study conducted in western Austria. Maternal and neonatal factors were extracted from the mother-child booklet, adolescent factors were evaluated by a face-to-face interview, physical examination, and fasting blood analyses. Liver fat content was assessed by controlled attenuation parameter (CAP) using signals acquired by FibroScan® (Echosense, Paris, France). The association of maternal, neonatal, and adolescent factors with CAP values was analyzed using linear regression models.

Results: In total, 595 adolescents (27.2 % male) aged 17.0 ± 1.3 years were included. 4.9 % (n = 29) showed manifest NAFLD with CAP values above the 90th percentile. Male sex ($p < 0.001$), adolescent triglyceride levels ($p = 0.021$), Homeostatic Model Assessment for Insulin Resistance index and BMI z-score ($p < 0.001$, each) showed a significant association with liver fat content in the multivariable analysis. Maternal pre-pregnancy BMI was associated with CAP values after adjustment for sex, age, and birth weight for gestational age ($p < 0.001$), but this association was predominantly mediated by adolescent BMI (indirect effect b = 1.18, 95% CI [0.69, 1.77]).

Conclusion: Components of the metabolic syndrome were the most important predictors of adolescent liver fat content. Therefore, prevention of non-alcoholic fatty liver disease should focus on lifestyle modification in childhood and adolescence.

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No Sex difference in Infarct Size post STEMI

Background: Myocardial infarct size is a predictor of patient outcome post ST-elevation myocardial infarction (STEMI). This can be quantified via cardiac magnetic resonance imaging (CMR) by calculating the percentage of the injured myocardial mass of the left ventricle (IS in % LVMM). Identifying patients more at risk of larger myocardial infarctions post-STEMI, could result in better risk stratification. Sex as assigned at birth (sex) has been associated to differences in the clinical presentation of patients suffering from STEMI. An association between sex and IS in % LVMM could lead to tailored personal medicine.

Purpose: Aim was to determine, whether there is an association between sex and IS in % LVMM in STEMI patients treated with primary percutaneous coronary intervention (pPCI).

Methods: 97 STEMI patients treated with pPCI, included in the prospective MARINA-STEMI cohort study (NCT04113356), were investigated. IS in % LVMM was quantified via contrast enhanced CMR a median of 4 (interquartile range [IQR] 3–5) days post-STEMI. Patient sex (male/female) was determined via questioner.

Results: 19% (n=18) of the study population were of the female sex. Female patients had a significantly lower body mass index (p=0.017), more frequently had hyperlipidemia (p=0.026), and had a lower systolic (p=0.042) and diastolic blood pressure (p=0.011 than male patients. These parameters were not significant in the logistic regression analysis. IS in % LVMM was not statistically significant across the two groups (p=0.224).

Conclusion: In STEMI patients treated with pPCI, sex as assigned at birth was not associated with IS in % LVMM.

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Serum α-synuclein-aggregation assay (SAA) in neurodegenerative parkinsonian disorders - a pilot study.

Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The differential diagnosis to multiple system atrophy (MSA), another α-synucleinopathy can be challenging as their clinical features overlap, especially in the early stages. The neuropathological hallmark of PD and MSA is the detection of abnormal α-synuclein as intracytoplasmic inclusions (Lewy bodies or Papp-Lantos bodies).

Recent studies could show that the misfolded α-synuclein in PD differs structurally from the one in MSA. This disease specific abnormal α-synuclein could be detected in various tissues. Recently, an immunoprecipitation-based real-time quaking-induced conversion (IP/RT-QuIC) assay was developed and was able to detect pathologic α-synuclein. It showed good diagnostic efficiency in differentiating between PD, atypical parkinsonism, and controls.

Despite the future promise of this method, it remains to be seen whether this assay can be validated independently.

Methods

As part of a biomarker study (MUI ethics vote AN1979, 336/4.19 401/510), blood samples were taken and 500µl serum samples were pseudonymised and preserved. 45 patients (25 MSA and 20 PD) diagnosed according to the current diagnostic criteria were included. Within the framework of a bilateral agreement, the samples are shipped to the Juntendo University, Tokyo, Japan. α-synuclein will be extracted by IP and analysed for abnormal α-synuclein using RT-QuIC.

Aim

This pilot study aims to determine the diagnostic accuracy of IP/RT-QuIC based α-synuclein-aggregation assay to distinguish PD from MSA patients.

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Evaluation of risk markers of Parkinson's disease and prodromal scores in two prospective population based studies

Background: Parkinson's disease (PD) continues to be diagnosed based on its characteristic motor symptoms. However, accumulating evidence from clinical, neuropathological, and imaging studies suggests that PD-related pathology begins before the onset of motor signs, manifesting with a broad variety of non-motor symptoms (NMS). Several risk and protective markers for PD have been identified, yet the predictive accuracy of individual markers is insufficient to reliably assess the overall risk for PD. In response to this challenge, the International Parkinson's Disease and Movement Disorder Society (MDS) introduced research diagnostic criteria for the prodromal phase of PD in 2015. These criteria are based on epidemiological data encompassing a wide range of risk and prodromal markers. However, further prospective validations of these criteria are still needed. There are other proposals for ideal identification of at-risk subjects, but also their potential to date remains unknown.

Methods: Two different prospective population-based studies (Healthy Brain Aging [HeBA] and Bruneck Study) will be used to evaluate different single risk and prodromal markers as well as comprehensive risk scores to detect ideal combinations for the identification of prodromal or incident PD.

Discussion: The objective of the present thesis is to evaluate different strategies in detecting the prodromal stage of PD. Understanding this latent period of PD is crucial, especially concerning the advancement of therapies aimed at modifying or protecting against the progression of the disease.

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Right ventricular pacing decreases periodic repolarization dynamics

Background: Periodic Repolarization Dynamics (PRD) is a novel ECG-based risk marker which refers to efferent sympathetic-activity associated low-frequency (≤0.1Hz) modulations of cardiac repolarization instability. Past research revealed that increased PRD is an independent predictor of malignant arrhythmias and sudden death in both, ischemic and non-ischemic cardiomyopathy.

Methods: The study enrolled patients with a dual chamber pacemaker (Microport Borea DR), preserved atrioventricular conduction and a low median right ventricular pacing rate of 1 (IQR 1 – 6) %. In all patients, a high-resolution (1000 Hz) ECG recording in Frank leads configuration was obtained over a 30-minute period, consisting of initial 15 minutes without right ventricular stimulation (AAI) and thereafter 15 minutes with continuous right ventricular stimulation (DDD) slightly above the spontaneous heart rate, excluding a possible cardiac memory effect.

Results: Between November 2021 and December 2023, a total of 62 patients were enrolled. Median age was 75 (IQR 68 - 79) years, 25 (40 %) patients were female. 22 (36 %) had coronary artery disease. Native PRD without right ventricular stimulation (AAI) was 8.4 (IQR 3.7 - 13) deg2. Upon new-onset continuous right ventricular stimulation (DDD programmed with a short AV delay), PRD decreased to 5.8 (IQR 1.7 – 9.1) deg2 ($p = 0.00006$, paired Wilcoxon test). Overall, PRD decreased in 46 out of 62 patients.

Conclusion: In pacemaker patients with preserved atrioventricular conduction, new onset right ventricular stimulation significantly decreases PRD. Our results suggest that new thresholds should be considered to assess the arrhythmic risk in right ventricular paced patients.

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Response to treatment of epiretinal neovascularization secondary to ischemic retinal vein occlusion in proliferative MacTel 2

Purpose

We report a case of unilateral epiretinal neovascularization (ERN) secondary to a non-recent ischemic retinal vein occlusion (RVO) in a patient with bilateral proliferative macular teleangiectasia type 2 (MacTel 2; Toto stage 4) as documented by multimodal imaging.

Observations

A 75-year-old woman with a six-year-history of MacTel 2 developed proliferative subretinal neovascularization (SRN) in both eyes over time with exudation only in her right eye and was therefore treated with anti-vascular endothelial growth factor (anti-VEGF; aflibercept 2mg) intravitreally, before she was lost to follow-up in 2022. At her last consultation one year later, an ERN was noted in addition to neovascularization of the optic disc, delayed filling of the retinal veins, peripheral capillary ischemia and intravitreal hemorrhage only in her left eye. Meanwhile, the SRN complex remained unchanged in both eyes. Diagnosis and treatment with anti-VEGF were observed via optical coherence tomography angiography.

Conclusions

We believe that the ERN formation was a reaction to ischemia of the inner retinal capillary plexus due to a preceding RVO. The ERN responded well to a single intravitreal injection of anti-VEGF whilst the SRN complex remained unchanged.

Importance

The herein presented case provides a new perspective on the development and treatment of ERN in MacTel 2.

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Soluble PD-L1 shows no association to relapse and overall survival in early stage non-small cell lung cancer (NSCLC)

Background: High soluble (s)PD-L1 is associated with reduced survival and treatment failure in advanced stages. Here, we evaluated sPD-L1 in early stage NSCLC for its impact on relapse free survival (RFS) and overall survival (OS).

Methods: sPD-L1 plasma levels from 74 and additional 73 (control cohort) NSCLC patients (stage IA-IIIB) was collected prior to curative surgery. Data from The Cancer Genome Atlas (TCGA) were investigated for PD-L1 splice variants and enzymes involved in proteolytic cleavage via ADAM10. In-vitro T cell stimulation was performed in the presence of sPD-L1 to evaluate its immunomodulatory activity.

Results: In vitro, sPD-L1 inhibited IFN-γ production, proliferation of T cells and induces a terminal effector CD4 T cell subtype expressing CD27. This effect could not be translated although in the initial cohort sPD-L1 levels were significantly higher in NSCLC patients who experienced disease relapse (median: 966.44 vs. 448.24 pg/mL, p = 0.038). Multivariate analysis revealed high sPD-L1 (> 1000 pg/mL) as an independent predictor of RFS and OS. However, these findings could not be validated in an independent control cohort, which showed lower levels and no association to disease relapse.

Discussion: We could show a negative correlation of sPD-L1 and clinical outcome, which was supported by the inhibition of T cells in-vitro and data from the TCGA databank. However, we were not able to reproduce these results in an independent control cohort. Therefore, the impact of sPD-L1 is controversial and further standardization of sampling and quantification might be needed.

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Gender Aspects of the Study: Effects of polishing procedures on colour stability of dental tissues and restorative materials

Objectives

This study aims to investigate the colour changes of standardised tooth samples repeatedly exposed to tobacco smoke in vitro and to examine the efficiency of air-polishing to remove tobacco stains.

Materials and methods

Twenty-four extracted human teeth were exposed to standardized cigarette smoke in an automated smoking chamber, involving two repetitions of a 14-days-smoking-cycle (five cigarettes per day) and cleaning every two weeks, either with air-polishing using two experimental powders or rubber cup and pumice stone as control. Spectrophotometrical colour changes and profilometric surface roughness measurements were carried out pre- and post-treatment. The experiments were done in triplicates.

RESULTS

The tested cleaning procedures effectively removed tobacco staining from dentine and enamel samples, however, with each smoking cycle, the cleaning efficiency decreased. Tooth colour after two cycles was not statistically significantly different between male and female groups ($p > .05$) but was statistically significantly darker compared to baseline values ($p < .001$). No significant differences in enamel and dentin roughness compared to baseline were observed after repeated air-polishing with experimental powder 1 or 2 ($p > .05$). There was no statistical significance between the male and the female specimen, whether on enamel nor on dentine ($p > .05$).

Conclusion

There is no difference between human male and female dental hard tissue on the cleaning efficiency with MAPD. Wheter experimental powder one nor two could restore the colour completely on enamel or dentine. Pumice stone achieved the best result but with the highest wear.

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A Prospective Study to investigate Renal Pathology in Early-Stage Chronic Kidney Disease in Type 2 Diabetes – Study Design of the Innsbruck Diabetic Kidney Disease Cohort

Introduction

About 20-40 % of patients with type 2 diabetes (T2D) develop chronic kidney disease (CKD), which aggravates cardiovascular morbidity and mortality. The underlying histopathology is known to be heterogeneous, but usually remains unknown due to a lack of routine kidney biopsy in diabetic CKD patients. As clinical parameters are unreliable predictors of renal pathology, timely histologic assessment in T2D patients with CKD could improve therapeutic management and therefore prognosis.

Aim

We aim to determine the prevalence and the disease course of typical diabetic, non-specific, non–diabetic and coexisting kidney pathologies among T2D patients with mild-to-moderate kidney impairment (KDIGO CKD stage G3a/A1-3 or G2/A2-3; i.e. eGFR 59-45 ml/min irrespective of albuminuria or eGFR 89-60 ml/min and albuminuria >30 mg/g creatinine).

Methods

At least 65 T2D patients with mild-to-moderate kidney impairment shall undergo a diagnostic kidney biopsy. 6-monthly clinical follow-up for 5 years will provide clinical and laboratory data to assess cardio-renal outcomes. Blood, urine and kidney tissue samples are collected to establish a biobank dedicated to the identification of diagnostic and prognostic biomarkers.

Discussion

Currently, risk assessment is solely based on clinical features in this population. Our study aims at determining the epidemiology and prognosis of the underlying renal pathology as well as the risks and benefits associated with early renal biopsy. The study may provide evidence for a potential change of the diagnostic standard towards routine kidney biopsy and individual risk prediction regarding cardio-renal risk.

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Sex as a Significant Predictor of Long-Term Pulmonary Outcomes Assessed by Chest CT Following COVID-19: Insights from a prospective Cohort Study

Background:

Emerging evidence suggests that sex-related differences may influence the clinical course and outcomes of COVID-19. This study investigates the role of sex, radiologic findings and various clinical parameters in predicting persistent pulmonary abnormalities one year after COVID-19 infection, as assessed by chest computed tomography (CT).

Patient and Methods:

A comprehensive analysis was conducted on the CovILD study cohort, consisting of 143 patients, who were followed for up to 12 months after COVID-19 infection. Various parameters including sex, age, clinical presentation (outpatient treatment, hospitalization on a regular ward or intensive care unit), calcium score, body mass index (BMI), pack-years of smoking history and radiological findings from chest CT scans were assessed.

Binary logistic regression analysis was employed to assess the significance of these variables in predicting persistent pulmonary abnormalities one year after COVID-19.

Results:

A total of 91 patients, comprising 35 women and 56 men, participated in the 12-month follow-up assessment. Binary logistic regression analysis demonstrated treatment on ICU (Wald = 9.6, p = 0.002; Exp [B] = 24.9) and sex (Wald = 10.4, p < 0.001; Exp [B] = 0.126) as significant predictors on persistent pulmonary abnormalities one year after COVID-19. Baseline differences in radiological findings, hospitalization rates, calcium scores, and BMI between sexes were noted but did not diminish the robust association between female sex and reduced risk of post-COVID-19 pulmonary abnormalities.

Conclusion:

Female sex emerged as a protective effect against persistent pulmonary abnormalities one year after COVID-19 infection.

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An evaluation of the Elekta cone-beam CT software XVI's MV radiation isocentre calculations

Ensuring the quality assurance of medical linear accelerators is crucial in radiotherapy, especially in terms of the geometry of a linear accelerator (Linac) and verifying the radiation isocentre with imaging systems. This study aims to assess the accuracy of Elekta's software tool for MV radiation isocentre calculation using independent software. For this purpose, MATLAB software was implemented and the positioning errors of five linacs in our department were investigated. To assess the positioning errors, the ELEKTA ball-bearing phantom was positioned on the Linac table using lasers to ensure the ball center was at the laser's crossing point. Then, portal images were acquired at the main gantry angles of 0°, 90°, 180°, and 270°. Two shots were taken with collimator angles of 90° and 270° at each angular position, respectively, resulting in 8 PIs. Using the PIs, the positioning deviation of the sphere from the radiation isocentre was calculated using the ELEKTA software and corrected using the vernier screws on the phantom to ensure the center of the sphere was positioned at the radiation isocentre. Data from five Elekta linacs was used in this work. Using the XVI and in-house developed (IHD) MATLAB code. The presented results were obtained by analyzing 89 series (712 images), with 8 images in each series. Nowadays, Image-guided radiation therapy is the standard practice for the routine QA of the linac isocentre calibration. The Elekta XVI software inaccurately calculates the MV radiation isocenter position in the longitudinal direction by 0.23 ± 0.14 mm towards the Gun_Target.

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The iRAVi Study - What can we learn from Aviation?

Background: Despite the established use of checklists in various medical fields, such as anesthesiology, the incorporation of checklists during procedures in interventional radiology remains a novel concept inspired by practices in aviation. This study aims to investigate the impact of introducing intraprocedural checklists and a Standard Operating Procedure (SOP) in the training of medical students without prior angiographic experience on an angiography simulator.

Methods: Participants are divided into three groups: Group A, receiving traditional training with a demonstration where the trainer progressively steps back, providing less and less assistance on each run of the thrombectomy task; Group B, provided with a simple SOP as a training app for preparation and execution; and Group C, trained using a detailed SOP with an intraprocedural checklist in a cockpit-style approach, that was designed for this intervention. In Group C, training involves a trainer actively forming a team with the participant, working together according to a specific procedure guided by the intraprocedural checklist, similarly to how aviation training for pilots is performed.

Focus: The primary objective is to evaluate whether the implementation of intraprocedural checklists and SOPs contributes to a reduction in procedural errors and a reduction of intervention time, as well as contrast agent used during simulated thrombectomy procedures on an angiography simulator.

Outlook: The findings may contribute to optimizing training protocols in interventional radiology, allowing for a change in training culture for future doctors.

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Update of clinical and transnational data of the INNWOP-1 study including gender aspects

The INNWOP1 study is an investigator initiated Phase II trial exploring the neo-adjuvant use of the immune checkpoint inhibitor Pembrolizumab in combination with the anti-angiogenic tyrosine kinase inhibitor Lenvatinib in patients with resectable non-small cell lung cancer (NSCLC). Primary endpoint of the study is major pathological response (MPR) in the resected lung tumors. In parallel, we perform an extensive biomarker project including in-depth single cell RNA sequencing (scRNAseq) of the tumor microenvironment (TME) in biopsy samples and resected lung tumors as well as multi-parameter flowcytometry (mp-FACS) of tumor tissues and peripheral blood (PB), cytokine analysis in PB and stool microbiota typing. Here we present the current update of the clinical study and preliminary data of the TME mapping using mp-FACS and scRNAseq as well as longitudinal immune cell phenotyping in PB under cancer immunotherapy using mp-FACS.

First author: Laurenz Nagl; Last author: Andreas Pircher (supervisor); Further authors to be named

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Innsbruck, April 2024

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