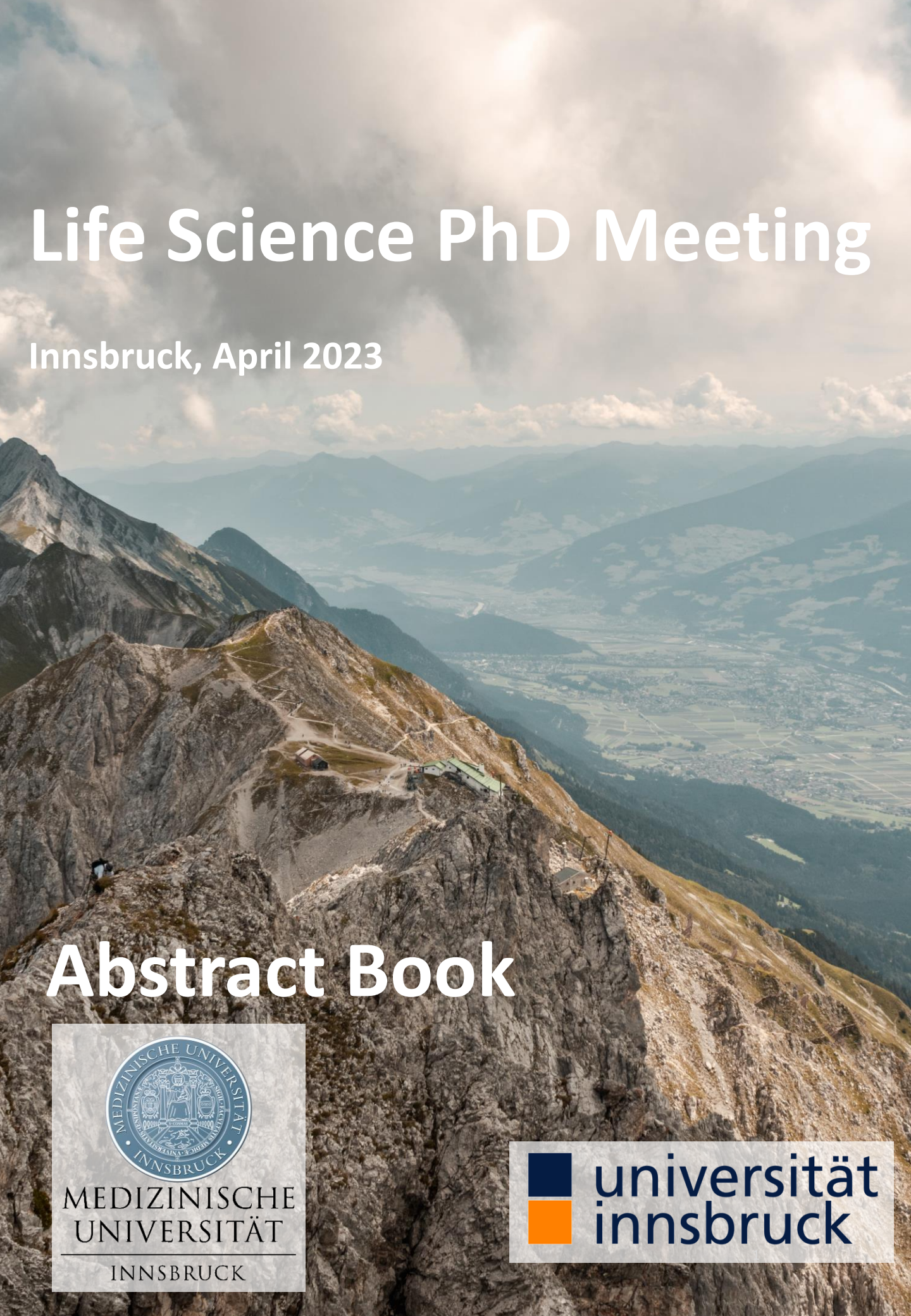
# Life Science PhD Meeting

**Innsbruck, April 2023**



# Abstract Book

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

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## This meeting is organized by:

Vincent Braun, Anna Schmit, Felix Eichin, Marina Schapfl, Ilaria Dorigatti, Xuechen Tang, Nargess Shahbazi, Anton Hörmann, Cristina Shöpf, Jiri Koutnik, Hussam Abd El Halim, Marion Lechable, Marcel Tisch, Lucas Hensen

Abstract book designed by Ilaria Dorigatti and Marina Schapfl

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# 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
- CMBI
- ARDRE
- HOROS
- SPIN
- CavX
- Clinical PhD program

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# Selected short talks

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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. These fusion events may contribute to pathogenesis and induce chromosomal instability (CIN), potentially promoting tumorigenesis. The cellular mechanisms preventing this are incompletely understood. Exploiting the ability of the vesicular stomatitis virus glycoprotein (VSV - G) to induce cell fusion of VSV - G - expressing cells in a pH - dependent manner, we provide evidence that the fusion of asynchronous cells triggers the activation of the PIDDosome multiprotein complex, leading to Caspase-2-mediated cleavage of MDM2, stabilization of p53, and upregulation of p21. Of note, cell fusion resulted in the clustering of the supernumerary centrioles and induced DNA damage in some nuclei, both stimuli which were suggested to trigger PIDDosome assembly in previous studies. However, as centriole depletion abrogated PIDDosome activation after cell fusion, the mechanism appears to depend mainly on the recognition of extra mother centrioles. Furthermore, cell fusion correlated to reduced S-phase activity and cell cycle progression. Taken together, our results indicate that the PIDDosome may act as an important sensor of pathological cell-cell fusion events and may act as a barrier against CIN by activation of the p53/p21 axis. The role of the PIDDosome in physiological cell fusion events, such as those seen in macrophages or osteoclasts, remains to be investigated.

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# Lysosomal repair mechanisms and cytosolic acidification control during stress-induced cellular senescence

Aging is characterized by a decrease in tissue function and increased number of senescent cells. These cells contribute to the decline in tissue function, since cell replacement needs are not met, and the senescence-associated secretory phenotype (SASP) induces a pro-inflammatory environment and facilitates the non-physiological remodelling of the extracellular matrix. Senescent cells are characterized by permanent cell cycle arrest, mitochondrial dysfunction or loss of proteostasis. Research on mitophagy and mitochondrial quality control have been undertaken, but lysosome damage and repair has not been adequately studied. Therefore, we investigated lysosomal quality control in stress-induced premature senescence (SIPS) in dermal fibroblasts. Fibroblasts were irradiated twice daily with UVB (0.06 J/cm2) for four days, and senescence was studied using Western blotting and β-gal staining. Lysosomal membrane disruption was studied by immunofluorescence. A fraction of the SIPS cells showed Galectin3 puncta, indicating lysosome repair. Cytosolic acidification could arise from lysosomal leakage, as they are acidic organelles and proton leakage contribute to acidification. Cytosolic pH was measured using the BCECF probe, showing a slight acidification in senescent cells. We checked if the expression of glutaminase, a protein reported to counteract acidification by producing ammonia, was altered. We found that the KGA isoform of glutaminase was upregulated during SIPS. Previously, it has been found that KGA is upregulated in nutlin3a drug-induced senescence. BPTES, a glutaminase inhibitor, killed these drug-induced senescent cells. However, in our SIPS model, BPTES treatment does not induce senolysis, suggesting that these cells possess redundant mechanisms to control cytosolic pH during senescence.

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# Water proton resonances reset the circadian clock in mammalian cells

The magnetic responsiveness of biological systems has regained scientific attention and several studies have demonstrated the potential of low intensity electromagnetic fields (EMFs) for wound healing, cell proliferation, differentiation and metabolic reprogramming. EMFs are also suspected to affect cellular clocks via the core clock protein Cryptochrome (CRY). The mechanism to allow for magnetic responsiveness has been described as the Radical Pair mechanism (RPM). Thereby the circadian clock protein CRY, and most probably also other Flavoproteins, binds to Flavin Adenine Dinucleotide (FAD). After excitation with blue light or radio frequencies (RF), FAD-CRY forms radical pairs which consequently alter the amounts and ratios of reactive oxygen species (ROS) via an entangled electron transfer upon a tryptophan chain. However, the RPM is predicted for RFs in the MHz range. Here we show, that water proton resonances induced by a therapeutic "low dose" Nuclear Magnetic Resonance device (tNMR; 0.4mT, 17kHz) exposure for 6 hours alters the cellular ROS signature of NIH3T3 cells in an RPM like manner. In addition, tNMR affects the cell autonomous clocks, as demonstrated by Per2::LUC mouse reporter gene assays. tNMR therein appears to have an even stronger capacity to synchronize cellular clocks than the commonly used cortisone derivative Dexamethasone, increasing the amplitude and lengthening the period of the Per2 oscillation. All EMF induced alterations follow a nonlinear, binary dose response relationship. Hence, the strength of the EMF as well as the duration of the exposure are both crucial, and lead to opposite outcomes.

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# Protein interactions and metabolic signalling at the lysosome

Lysosomes harbor a complex nutrient sensing machinery that integrates information about extra- and intracellular nutrient availability and activates corresponding signaling pathways, causing changes in the cell's metabolic program, rendering proper lysosomal function indispensable for cellular homeostasis.

The LAMTOR (late endosomal/lysosomal adaptor and MAPK and mTOR activator) complex plays a central role in these processes by scaffolding different signalling cascades to the lysosomal surface.

In order to regulate these processes, LAMTOR associates with a number of partners including the Rag-GTPases, SLC38A9, the lysosomal v-ATPase, MEK, BORC, AXIN, LKB1, and many more. 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 and the regulatory mechanisms defining the interplay between the different signaling cascades. Special focus is laid on phosphorylations present in the N-terminus of LAMTOR1, both in terms of the triggered changes in the interactome of the complex, as well as signaling downstream of LAMTOR.

I found that phosphorylation of LAMTOR1 has a broad range of effects on cells, including the regulation of binding to the RagGTPases, and regulation of the AMPK and MAPK signaling cascades.

Using recombinant LAMTOR-complex, I could confirm in in-vitro phosphorylations followed by Mass-Spectrometry, that AMPK, MAPK and CDK2 phosphorylate LAMTOR1 at S63, S45, and S27, respectively. Present work focuses on determining the effects of the aforementioned phosphorylation-sites in-vivo.

This work is funded by the FWF funded PhD program Cellular Basis of Diseases: Molecular Control of Metabolism and Inflammation (DOC 82 doc.fund)

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# tRNA superwobbling - impact of tRNA modifications and sequence on decoding of synonymous glycine codons

Transfer RNAs (tRNAs) allow the ribosome to decode the genetic information and provide the cell with sufficient amounts of proteins. The number of tRNA types essential for the decoding process varies in bacteria from 28 to 46. This variation results from different numbers of tRNA isoacceptors for decoding of synonymous codons. In E. coli three tRNA isoacceptors with the anticodon GCC, CCC, and UCC are required to decode the four glycine codons GGA, GGC, GGG, and GGU. In Mycoplasma mycoides only a single tRNAGlyUCC is sufficient to recognize all four codons. Strikingly, the two tRNAGlyUCC from both bacteria are highly similar in their sequence, but their modifications differ significantly. In order to identify the decisive differences between this tRNAs in a defined and systematic manner, we developed an in vitro approach employing synthetic tRNAs and an E. coli based recombinant translation system. The decoding capacity of a tRNA was addressed by translation of short mRNAs by a small set of synthetic tRNAs and in competition with native tRNAs by translation of a luciferase gene. Unexpectedly, tRNA modifications do not significantly modulate decoding, while the sequence of the T-stem was crucial. This systematic study gives a detailed insight on the interplay of tRNA sequence, modifications, and codon usage, which provokes different decoding concepts. Furthermore, this approach can serve as tool to advance synthetic biology and the development of novel codon-anticodon base pairs.

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# CaV1.3 L-type Ca2+ channel modulates pancreatic β-cell electrical activity and gene transcription.

Pancreatic β-cells express several high voltage-gated Ca2+ channel isoforms that mediate Ca2+ influx and are critical for insulin release, cell differentiation, and survival. RNAseq and qPCR analyses showed that CaV1.3 L-type Ca2+ channels are highly expressed in pancreatic islets of both mice and men. In humans, genetic polymorphisms leading to loss-of-function associate with increased susceptibility for diabetes. Conversely, CaV1.3 gain-of-function mutations cause hyperinsulinaemic hypoglycemia. Here we show for the first time, that CaV1.3 deletion led to a 6-fold increase in DNA damage and a 3-fold decrease in proliferation markers, resulting in a reduced β-cell mass of 14d old mice. Single cell RNAseq revealed that CaV1.3 deletion altered gene transcription suppressing cell-differentiation pathways. Functionally, CaV1.3 ablation led to ~20% reduction in β-cells Ca2+ influx accompanied by slower kinetics, rightwards shift in the voltage-dependence of activation, and impaired activity-dependent Ca2+ current facilitation. In contrast, CaV1.3 gain-of-function significantly increased β-cell Ca2+ influx at lower voltages and therefore shifted the whole-cell conductance towards hyperpolarized voltages. Additionally, action potential (AP) -clamp experiments indicated that CaV1.3 deletion led to a smaller pace-making current amplitude at resting membrane potential. Consequently, CaV1.3-/- β-cells exhibited reduced glucose-induced electrical activity characterized by delayed AP-onset, reduced AP-firing and AP-train frequency. This however, increases the synchronization of intra-islet Ca2+ wave propagation. Therefore, our data demonstrate that CaV1.3 Ca2+ channel exerts a multifactorial modulation on glucose homeostasis by controlling both gene transcription and β-cell mass as well as glucose-stimulated insulin release both at single β-cell and islet level.

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# Identifying voltage-sensor III of CaV1.1 as crucial for activation of Excitation-contraction coupling in skeletal muscle

Skeletal muscle CaV1.1 is a voltage gated calcium channel, which primarily functions as voltage sensor of excitation-contraction coupling (ECC), a process that opens RyR1 to supply the calcium essential for muscle contraction. Upon strong stimulation, CaV1.1 also elicits calcium currents (ICa) with kinetics and voltage-dependence different from those of ECC. CaV1.1 contains four separate voltage-sensing domains (VSDs), which sense changes in the membrane potential and thus activate these two functions. Previous results indicate the importance of VSD I and IV in controlling ICa but not for ECC. However, the functions of VSDs II and III remained unknown. Here we apply two different mutation strategies to alter the function of specific VSDs, and examine the effects on current gating and activation of ECC using combined patch-clamp and fluorescence calcium recordings. Neutralizing the innermost gating charge of VSD I causes an ablation of ICa, while ECC remains unchanged. Analogous mutants in VSDs II (K537A(KS)) and III (R906A(NR)) show comparable effects on ICa. Importantly, only R906A(NR) shifts the voltage necessary to activate ECC by 30 mV to more depolarizing potentials. In a second approach, we transfer an extracellular loop (IVS3-S4) of CaV1.1 from VSD IV to the analogous positions of VSDs II and III. While this had no effect on ICa, insertion of the IVS3-S5 loop in VSD III shifted the activation of ECC by 60 mV to less depolarized potentials. Together these results clearly demonstrate that VSD III of CaV1.1 is the exclusive regulator of ECC.

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# Integrative metabolomics-genomics analysis identifies key networks in a stem cell-based model of schizophrenia

Schizophrenia (SCZ) is a neuropsychiatric disorder, caused by a combination of genetic and environmental factors. Recently, metabolomic studies based on patients' biofluids and post-mortem brain specimens have revealed altered levels of distinct metabolites between healthy individuals and patients with SCZ. However, a putative link between dysregulated metabolites and distorted neurodevelopment has not been assessed and access to patient-derived material is restricted. In this study, we aim to investigate a presumed correlation between transcriptomics and metabolomics in a SCZ model, employing patient-derived induced pluripotent stem cells (iPSCs). For this purpose, iPSCs were differentiated towards cortical neurons and samples were collected longitudinally at defined developmental stages. Samples were subsequently analyzed by bulk RNA-sequencing and targeted metabolomics. The transcriptomic analysis revealed dysregulations in several extracellular matrix-related genes in the SCZ samples observed in early neurogenesis, including members of the collagen superfamily. On a metabolic level, several lipid and amino acid discrepancies were correlated to the SCZ phenotype. Additionally, by employing a novel in silico analytical approach, we correlated the transcriptome with the metabolome through the generation of integrative networks. The network comparison between SCZ and healthy controls revealed a number of consistently affected pathways in SCZ, indicating abnormalities in the cellular membrane composition, lipid homeostasis, and amino acid imbalances. Ultimately, our study suggests a novel approach of correlating in vitro metabolic and transcriptomic data obtained from a patient-derived iPSC model. This type of analysis will offer novel insights in cellular and genetic mechanisms underlying the pathogenesis of complex neuropsychiatric disorders, such as schizophrenia.

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# A Cav1.4 L-type calcium channel truncation mutation affects primarily the retinal rod pathway

In the retina, the Cav1.4 L-type calcium channels (LTCCs) are predominantly expressed at the synaptic terminals of photoreceptors in the outer plexiform layer (OPL) to support tonic glutamate release. Mutations in the CACNA1F gene, which encodes Cav1.4 channels can cause congenital stationary night blindness type 2 (CSNB2). In this study we investigated a C-terminal truncation mutation in the Cav1.4 channel (Cav1.4-RX). Heterologously expressed Cav1.4-RX channels showed calcium-dependent inactivation.

To determine the impact of the missing CTM domain in Cav1.4 channels in vivo, we investigated a mouse model carrying the Cav1.4-RX mutation functionally (multielectrode array analyses (MEA), electroretinogram (ERG)), morphologically and biochemically. Western blot analyses showed a significant decrease of Cav1.4 channel protein in Cav1.4-RX retinas. Reduced channel expression destabilized the synaptic integrity obvious from the fact that synaptic ribbons were dislocated in the outer nuclear layer (ONL) and of punctate shape. We also found dislocated photoreceptor terminals in the ONL of Cav1.4-RX mice. These changes in the retinal presynapse affected also second-order neurons, as we observed neurite sprouting of horizontal and rod bipolar cells in Cav1.4-RX retinae, comparable with previously published mouse models. However, the cone bipolar cells seemed to be largely unaffected. This phenotype was also reflected in our functional analyses: both, ex-vivo (MEA) and in-vivo (ERG) recordings.

This observation could be explained by a difference in the protein composition in rod and cone photoreceptor terminals. Further investigations will therefore also include a proteomic approach to better understand the molecular role of the distal Cav1.4 C-terminus in retinal synapses.

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# Cav1.2 requirements in delta-cells indirectly regulate the gylcemic set point in beta-cells

Assessing the response of pancreatic islet cells to glucose stimulation is important for understanding the tightly regulated release of hormones, including insulin.

Voltage-dependent calcium channels (Cav) function as key components in the signaling cascade mediating glucose induced insulin secretion (GIIS) in pancreatic beta-cells.

Consistent with a conserved function, we recently showed that mutant zebrafish embryos of the Cav1.2 isoform display hyperglycemic conditions and beta-cell hyperactivation as revealed by in vivo imaging of genetically encoded calcium indicator (GCaMP6s) expressing reporter lines. Here, we address the importance of the α1 subunit Cav1.2 in non-cell autonomous beta-cell regulation.

We demonstrated that somatostatin (sst) secreting delta-cells display a rhythmic calcium influx pattern in fasting wildtype embryos; moreover, this pattern is lost in Cav1.2 mutants. In addition, we found that delta-cell ablated embryos show increased spontaneous beta-cell activity, similar to the phenotype seen in Cav1.2 mutants. Since sst-secretion from delta-cells is known to inhibit beta-cells, our results also provide evidence for a novel requirement for Cav1.2-controled sst-release in preventing excessive beta-cell activation.

In pharmacological and genetical approaches, we demonstrate that L-type voltage-gated calcium channels in general, and Cav1.3 in particular, are providing an alternative source of Ca2+ influx in beta-cells. This is in line with our RNA-sequencing data, showing comparable expression levels of both isoforms.

Further experiments are already in progress to address cell-autonomous effects of Cav1.2 on intra-islet communication. Moreover, experiments on the electrophysiological properties of Cav1.2 mutant islet cells are ongoing, which is crucial to the understanding of the hyperglycemic phenotype.

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# The Dsc ubiquitin ligase complex mediates endosome and Golgi associated degradation (EGAD) of orphaned membrane proteins to maintain post-ER organelle integrity

To maintain cellular integrity, quality control processes detect and selectively degrade proteins that misfold, cannot integrate into protein complexes, or fail to target to the correct organelles. For membrane proteins in eukaryotic cells, three selective protein degradation pathways deal with the hydrophobic nature of their substrates. The ER-associated degradation (ERAD) pathways ubiquitinate substrates and retro-translocate them into the cytoplasm for proteasomal degradation. Once membrane proteins are exported from the ER, they can either be sorted into the lumen of lysosomes for degradation by the endosomal sorting complexes required for transport (ESCRT) or they are extracted from membranes for proteasomal degradation by the endosome and Golgi associated degradation (EGAD) pathway.

To understand how the EGAD pathway is embedded in cellular quality control networks and maintains cellular function, we conducted unbiased genetic lethality/sick screens, using saturated transposon analysis in budding yeast (SATAY). These screens identified loss of function mutations in several genes that showed negative interactions with defective EGAD. The top hit was Rer1, which returns membrane proteins (e.g.: Sec12) from the Golgi back to the ER. Loss of Rer1 and EGAD resulted in a collapse of post-ER organelle organization and caused the formation of aberrant and dysfunctional Golgi -, endosome-, and vacuolar structures, where orphaned/mis-localized proteins accumulated. These findings demonstrate for the first time that EGAD and ER retrieval pathways cooperate to control post-ER organelle organization. I will present our efforts to characterize how EGAD and ER retrieval pathways work together to maintain membrane integrity and prevent membrane stress signalling.

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# The MYC target BASP1 encodes a potential tumor suppressor

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.

Recently, we discovered that the brain acid soluble protein 1 (BASP1) is downregulated in a variety of MYC-dependent human cancer cells. Using an avian cell transformation system, we found that ectopic expression of BASP1 inhibits MYC-induced cell transformation by calmodulin (CaM) sequestration, thereby causing a decrease in MYC stability.

By using the human colon cancer cell line SW480, featured by high MYC and very low BASP1 expression levels, we aim to further investigate the tumor-suppressive properties of BASP1. Ectopic expression of BASP1 in SW480 cells leads to a decrease in MYC protein levels and significantly reduces the transformed phenotype. Using these cells, stably expressing ectopic BASP1, we currently investigate the MYC signaling network by analyzing and comparing its proteome with that of untreated SW480 cells via liquid chromatography coupled to mass spectrometry (LC-MS). We hereby identify proteins that are differentially expressed in both cell types, which should lead to the discovery of cellular processes relevant for the regulation of oncogenic MYC activities.

To further analyze the dynamics of these processes we established a conditional SW480 cell line, where BASP1 gene expression is controlled by the Tet operator enabling controlled induction of this potential tumor suppressor protein over a desired time frame.

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# Prader-Willi and Schaaf-Yang syndrome: Bridging the gap between two neurodevelopmental diseases

The Prader-Willi syndrome (PWS) and Schaaf-Yang syndrome (SYS) are two phenotypically related complex genetic, neurodevelopmental disorders. Both diseases are characterized by intellectual disability, endocrine dysfunctions, hypotonia and developmental delay. While hyperphagia and obesity are hallmark features of PWS, SYS patients show contractures and autism spectrum disorder. PWS is caused by paternal deletions at the maternally imprinted PWS critical region on human chromosome 15q11.2-q13. Recently, microdeletions encompassing the orphan snoRNA SNORD116 gene cluster have been shown to be sufficient to cause PWS. SYS originates from point mutations in the paternal copy of MAGEL2, which result in truncated and pathogenic protein variants. Given that mutations in the MAGEL2 gene or deletion of the SNORD116 gene cluster lead to highly similar pathological phenotypes we hypothesized that MAGEL2 might directly or indirectly influence the function or expression of SNORD116. Northern blot and qPCR analyses of induced pluripotent stem cells from SYS patients provide evidence for downregulation of SNORD116 and SNORD115 but not for other genes of the PWS locus. Furthermore, by a proximity-based labeling approach with an N-terminally fused TurboID biotin ligase to wild-type (MAGEL2) or frameshift-mutated (MAGEL2Q666Pfs*47) MAGEL2, we identified ANKHD1 as a putative novel interaction partner for MAGEL2. In contrast, several nuclear proteins were identified as possible interaction partners of MAGEL2Q666Pfs*47. Interestingly, ANKHD1 also interacted with SNORD116 in a biotinylated RNA pulldown assay. Our data provide evidence for a potential mechanism of MAGEL2 influencing SNORD116 abundance and function, including involvement in neuronal differentiation.

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# Exploring the impact of matrix effects on untargeted and targeted metabolomics in dried blood spot analysis

Dried blood spots have arisen to a major and convenient tool in a diverse spectrum of clinical applications, especially in the field of biomarker analysis. However, most of these applications are targeted methods, which only cover a small subset of the metabolome. Although targeted methods often have the advantage of being fast and reliable, it is required to combine several of these approaches to obtain a comprehensive overview on different metabolic pathways. In contrast, metabolomics and lipidomics approaches have the potential to strongly increase the information content in one single measurement, however, with the difficulty to integrate absolute quantification strategies. In order to allow a reliable interpretation of untargeted metabolomics datasets derived from dried blood spots it is necessary to pin down the precise analyte interactions and matrix effects.

We developed an experimental setup to quantify the recovery of metabolic biomarkers from the filter paper in comparison to the liquid sample. The analysis included two targeted methods (acylcarnitines, amino acids) and an untargeted metabolomics approach. The results showed that most acylcarnitines, as well as amino acids had a good recovery above 75%. However, the signal for specific dicarboxylic acid acylcarnitines was lost almost completely. Also untargeted metabolomics measurements revealed that there is a reduced recovery of specific metabolites. These results have to be taken into account in further studies that for example aim at analysing existing cohorts of DBS samples using untargeted approaches.

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# Protein Kinase D3: A novel regulator of T cell activation?

A robust T cell activation contributes critically to immune responses. However, uncontrolled activation is deleterious in form of autoimmune diseases. Thus, it is of great importance to understand the regulation of this process.

T cell receptor (TCR) signaling comprises activation of protein kinases C. Of note, these are implicated in activation of protein kinase D (PKD) family members. Hence, PKDs are linked to TCR signaling. There are two T cell-expressed isoforms: PKD2 and PKD3. While PKD2 is rather well understood, hitherto much less is known about PKD3's distinctive functions in T lymphocytes. Here, we tackled this gap by characterization of conventional and T-cell specific PKD3-deficient mice.

Contrary to PKD2-deficient mice, conventional PKD3-deficient ones showed a hyper-reactive T cell response upon polyclonal stimulation ex vivo accompanied by heavier spleens upon immunization in vivo. Since this putative T cell-hyper-phenotype was lost with naïve-sorted T cells, it seems to be caused indirectly by the observed skewed T cell compartment towards an effector/memory phenotype. Of note, despite strong downregulation of PKD3 upon TCR stimulation, it remained to be clarified whether this phenotype is mediated by T cell-intrinsic mechanisms. Interestingly, T cells from CD4-Cre-driven conditional knockout mice did neither phenocopy the skewed T cell compartment nor the hyper-activation upon stimulation in vitro or in vivo.

Taken together, our analyses of (T-cell specific) PKD3-deficient mice suggest that this kinase is an external regulator of T cell fate. Moreover, although strongly regulated upon TCR signaling, T cell intrinsic PKD3 seems to be dispensable for the cells' activation.

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# Serum and saliva mediated protection against novel SARS-CoV-2 Omicron BQ.1.1 and BF.7 in vaccinated individuals after BA.4/5 infection or bivalent booster vaccination

The rapid emergence and global spread of novel SARS-CoV-2 Omicron sub-variants lead to concerns about the current immune status induced by vaccination and previous infections. At the moment, the subvariants BQ.1.1 and BF.7 are on the rise and, thus, it is vital to assess the present protection in the population. In this work, we measured SARS-CoV-2 specific antibody titers in serum and saliva as well as the neutralization capacity against replication competent SARS-CoV-2 Wildtype, BA.4/5, BQ.1.1 and BF.7 in triple vaccinated individuals following infection with BA.4/5 or bivalent booster vaccination. Analysis of SARS-CoV-2 spike specific IgG and IgAs revealed significantly higher titers in the vaccinated group while IgA levels in serum and saliva remained comparable in both cohorts. Neutralization capacity in serum was high against Wildtype and BA.4/5 but drastically reduced against novel BQ.1.1 and BF.7. For salivary neutralization, only BA.4/5 convalescent individuals demonstrated a significantly higher neutralization capacity compared to vaccinated, while no differences were observed for the other tested variants between the groups. In addition, we utilized a human 3D respiratory model to assess serum and salivary protection against these variants of concern in a personalized and diagnostic approach. Testing antiviral efficacy of serum in our 3D respiratory model confirmed reduced viral infection and inflammation as well as enhanced tissue integrity in a dose dependent manner. In general, saliva offered a better protection against BA.4/5 infection compared to serum. Our findings emphasize further adaptations of COVID-19 booster vaccines which might be applied orally/intranasally to enhance protective mucosal immunity.

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# Energy metabolism and innate immunity cell signaling in tissue regeneration of Cd-exposed earthworms

The heavy metal cadmium (Cd) is an immunotoxic agent. Earthworms solely possess an innate immune system and are well-established study organisms for testing the effects of Cd detoxification. Recently published studies of earthworms revealed that Cd exposure leads to faster regeneration, while a depletion of coelomocytes, cells of the innate immune system, lowered regeneration capability. The molecular mechanisms explaining these phenomena are to date not well understood. Hence, we exposed injured and uninjured earthworms to 50 mg Cd/kg dry soil and sampled regenerated tissue once per week for three weeks to monitor stress-, immunity-, detoxification- and energy metabolism biomarkers, as well as zinc and calcium levels. Detoxification and regeneration were revealed highly energy demanding as only Cd-exposed injured earthworms show significantly increased protein levels of phosphorylated amp-activated protein kinase (pAMPK) and 70 kilodalton heat shock protein (HSP70), significantly reduced glucose levels and significantly upregulated cAMP response element-binding protein (LtCREB) gene expression, indicating a metabolic switch leading to significantly higher amounts of total protein. Gene expression of the main Cd detoxification protein metallothionein 2 (LtMT2) was significantly upregulated in Cd exposed groups, but Cd-treatment alone had no further effect on other biomarkers. Injured earthworms show significantly increased levels of Zn, Ca, as well as significantly increased gene activity of two Toll-like-receptors (LtsccTLR and LtmccTLR) and the activating transcription factor 7 (LtATF7) independent of Cd. Taken together; our data indicates that coelomocytes invade regenerative tissue to protect regeneration by detoxifying Cd site specific and to potentially enhance regeneration by immune cell signaling.

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# Characterizing neutralization profiles after bivalent COVID-19 boosting using antigenic cartography

As SARS-CoV-2 diversifies the need for further vaccine updates must be evaluated. Here, we investigated the antigenic relationship of emerging variants by comparing their neutralization in individuals with distinct SARS-CoV-2 exposure histories. We further assessed the value of antibody landscapes to distinguish between individuals with or without infection history prior to bivalent boosting.

A unique set of sera from individuals after single exposure (including BA.1, BA.2 and BA.5 convalescents) or multiple exposures by vaccination or hybrid immunity (including BA.1 or BA.4/5 bivalently boosted individuals) was tested against a variety of live SARS-CoV-2 isolates (including recent lineages such as CB, BR, CH, BQ, BF and XBB). Neutralization titers were used for antigenic cartography, a tool to blot variants according to their antigenic relationship in two dimensions on an antigenic map. Neutralization profiles of cohorts were visualized by antibody landscapes in 3D above the underlying map.

On antigenic maps, antigenically similar variants with good cross-neutralization are located close to each other, while variants with no or low cross-neutralization are more distant. We found omicron variants relatively distant to pre-omicron variants, but also distant from each other. Novel BA.2 and BA.5 sublineages showed an increased immune escape compared to initial omicron variants. Bivalent boosting improved neutralization of antigenically distant omicron variants. We further observed distinct antibody landscapes in convalescent individuals after bivalent boosting.

Our data are highly relevant for considerations on future vaccine updates. Moreover, the discrimination between previously naïve and hybrid immune individuals by antibody landscapes is important for clinical vaccine trials.

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# Impact of microbiota-derived molecules on epithelial cell signaling

Background: In a recently published study, Tanoue and colleagues identified eleven commensal bacteria from healthy human donor feces, which increased the abundance of cytotoxic CD8+ T lymphocytes in the intestine and improved efficacy of immune checkpoint inhibitors in mice subcutaneously engrafted with colon adenocarcinoma cells (Tanoue et al., 2019). It is tempting to speculate that these immune-modulatory gut bacteria produce metabolites, which stimulate immune responses. We aim at mechanistic validation of the eleven bacterial strains.

Methods: Murine colon organoids were incubated with bacterial cell-free culture supernatant (BCS) derived from the eleven effector microbes to gain transcriptomics data, allowing us to extract information about the impact of microbial metabolites on epithelial cell signaling pathways and gene expression. Apical-out organoids serve as a model system for studying the host-microbe interactions, as this model is allowing easy access to the apical cell surface, which is normally facing the lumen (Co et al., 2019).

Results: By performing Differential Gene Expression Analysis we could show that the 11-Mix BCS upregulates chemokine genes involved in recruitment and activation of neutrophils in comparison to organoids that were incubated with a 10-Mix control microbiota BCS. In line with this finding, it modulates NFkB and TNF signaling pathway activity and gene sets associated with receptor binding and activation are differentially enriched upon treatment with 11-Mix BCS as shown by Overrepresentation Analysis (ORA).

Conclusion: The 11-Mix BCS exhibits immunomodulatory bioactivity upregulating immunologically relevant pathways and the presence of bacterial ligands act as immune adjuvants affecting epithelial inflammatory responses.

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3 These authors contributed equally to this work.

# Role of the orphan nuclear receptor Nr2f6 in natural killer cell biology

Background: The orphan nuclear receptor NR2F6 suppresses pro-inflammatory cytokine transcription in T lymphocytes and acts as an immune checkpoint in cancer. NK cell-dependent metastasis formation in the B16-F10 model is significantly reduced in Nr2f6-deficient mice (Hermann-Kleiter et al., 2015). Transcription factor network analysis of human NK cells revealed an interaction of NR2F6 with the key regulators RUNX2 and BCL11B (Holmes et al., 2021). In addition, the gene expression of Nr2f6 is substantially higher in splenic NK cells compared to CD8 T cells in mice, but the functional role of NR2F6 in NK cell biology remains elusive.

Methods: We analyzed germline Nr2f6-deficient mice via flow cytometry, chromatin immunoprecipitation, in vitro cell culture experiments, and in vivo disease models to reveal phenotypic and functional consequences during NK cell development, maturation, and function in the bone marrow and various peripheral organs.

Preliminary Results: In this study, we demonstrated that NK cell frequencies in the spleen and the blood were comparable to wild-type controls, but the activating receptor NKp46 was highly upregulated in Nr2f6 deficient NK cells.

The anti-tumor capacity of Nr2f6-deficient NK cells was investigated by subcutaneous injection of the MHC-I-deficient RMA-S lymphoma cells. While control mice had to be sacrificed on day 16 at the latest due to the high tumor burden, Nr2f6-deficient mice survived significantly longer, or completely rejected the tumor (50%).

Mechanistically, two putative NR2F binding sites were predicted via TRANSFAC TF-binding analysis and preliminary ChIP analysis of the murine Ncr1 promoter suggests a direct transcriptional regulation by NR2F6.

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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 |
|---|---|---|---|
| Birstonas | Lukas | 1 | Elucidating the contribution of SrbA and AtrR in Aspergillus fumigatus triazole resistance |
| Shahbazi | Nargess | 2 | Deciphering the role of progenitor and stem cells in exocrine pancreas regeneration in zebrafish |
| Eschlböck | Alexander | 3 | iPSC-based modeling of Schaaf-Yang-Syndrome reveals increased levels of reactive oxygen species as pathological driver |
| López-Amo Calvo | Beatriz | 4 | Deciphering growth control in the developing human brain |
| Lindlbauer | Theresa | 5 | Inducible CRISPR Interference Allows for Specific Gene Repression in Human Pluripotent Stem Cells and Neuronal Progenitors |
| Sathianathan | Marc | 6 | MNX1 acts as a fate regulator in human in-vitro beta-cell differentiation |
| Oberegger | Simon | 7 | The BolA Family Protein Bol3 is Dual Localised by Alternative Translation Initiation in A. fumigatus |
| Pierson | Siebe | 8 | Phytohormones: putative signalling molecules in mycoparasitic Trichoderma species |
| Aguiar | Mario | 9 | Substrate Specificity Of Siderophore Uptake By Aspergillus fumigatus |
| Zott | Melanie | 10 | Regulatory role of Mnx1 in beta-cell differentiation and maturation |
| Caballero | Patricia | 11 | The acyltransferase SidF is involved in biosynthesis of fusarinine-type and ferrichrome-type siderophores in A. fumigatus |
| Tisch | Marcel | 12 | PATIENT-DERIVED STEM CELLS TO STUDY THE PATHOLOGY OF AUTISM SPECTRUM DISORDERS-RELATED VOLTAGE-GATED CALCIUM CHANNEL GAIN-OF-FUNCTION MUTATIONS |
| Waich | Stephanie | 13 | Novel and substrate-specific roles of the myosin co-chaperone UNC45A in trafficking and enterocyte polarity |
| Marchet | Nikolas | 14 | Alpha-arrestin mediated control of cellular nutrient uptake and its role in metabolic signalling |

## Posters

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

| Presenting Author | | # | Abstract title |
|---|---|---|---|
| Kahlhofer | Jennifer | 15 | Molecular mechanism of nutrient transporter regulation in human cells |
| Mari | Martina | 16 | SZT2 regulation of mitochondrial function and in response to oxidative stress |
| Kinz | Nadine | 17 | Defining the role of caspase-2-PIDDosome in ploidy control during heart development |
| Knell | Astrid | 18 | Sex specific immune response in chronic kidney disease patients and renal transplant recipients |
| Schapfl | Marina | 19 | Centrosomes are critical for B cell development but not function |
| Dorigatti | Ilaria | 20 | Where ether lipids metabolism meets immunology: a role for PEDS1 |
| Karbon | Gerlinde | 21 | Deregulation of the spindle assembly checkpoint triggers myelosuppression and gastrointestinal atrophy |
| Rodríguez Peiris | Maria | 22 | A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression in breast cancer |
| Timpen | Lea Emmy | 23 | Systems approaches reveal resistance mechanisms to mTOR-directed therapies in pancreatic neuroendocrine tumors and ER-positive breast cancer |
| Kummer | Denise | 24 | Modulation of lipid peroxidation by ether lipids and tetrahydrobiopterin in Crohn's disease |
| Barile | Cecilia | 25 | A systems approach to stratify breast cancer patients based on metabolism and signaling crosstalk |
| Rusu Hutu | Elena Cristina | 26 | ExonSurfer - a primer design tool for optimal qPCR results |
| Mayr | Michaela Maria | 27 | The Role of Sec16B in the Export of Proteins from the Endoplasmic Reticulum |
| Heiss | Martin | 28 | Ionic interactions between gating charges and countercharges in voltage-sensing domain I independently regulate kinetics and voltage-dependence of CaV1.1 gating |
| Riehl | Lydia | 29 | The role of neuronally expressed IL6ST in gut microbiome composition and post-operative cognitive function in a mouse model of neuropathic pain |

## Posters

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

| Presenting Author | | # | Abstract title |
|---|---|---|---|
| Tuinte | Wietske | 30 | Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation-contraction coupling |
| Hajdu | Renata | 31 | Regulation of STING trafficking from the ER |
| Török | Enikő | 32 | Identifying the STAC3/CaV1.1 interactions responsible for CaV1.1 expression in skeletal muscle |
| Halim | Victoria Christine | 33 | Paralemmin-3 – an essential constituent of the submembrane cytoskeleton of auditory hair cells |
| Zimmermann | David | 34 | Biophysical Essentials – An Open Source Software Framework for Conserved and Advanced Analysis of Patch-Clamp Recordings |
| Sanvido | Ilaria | 35 | Molecular mechanisms and physiological importance of a novel interaction between Kv channels across families |
| Jacobo Piqueras | Noelia | 36 | Increased pancreatic β-cell electrical activity reduced diabetes susceptibility in female mice |
| Juric | Viktorija | 37 | Damage and repair of membrane lipids in mitochondrial fatty acid β-oxidation disorders |
| Dury | Louisa | 38 | iPS-based disease modeling of Mucopolysaccharidosis IIIB using patient-specific neurons |
| Humer | Dominik | 39 | Targeting the orphan nuclear receptor NR2F6 in T cells primes tumors for immune checkpoint therapy |
| Framm | Johanna | 40 | Investigating potential functions of nuclear encoded intergenic mitochondrial tRNA lookalikes |
| Koch | Jakob | 41 | Unequivocal Mapping of Molecular Ether Lipid Species by LC–MS/MS in Plasmalogen-Deficient Mice |
| Haj Ahmad | Janti | 42 | Generation of monoclonal antibodies targeting murine C5aR2 |
| Abd El Halim | Hussam | 43 | The role of metabolic iron changes in macrophages upon SARS-CoV-2 infections |
| Rosam | Katharina | 44 | Intrinsic amino acid substitutions in the SDM-F5 paralogue of Mucor circinelloides are a major reason in short-tailed azole resistance |
| Jäger | Michael | 45 | Investigation of systemic and mucosal immunity of boostered and/or Omicron BA.1/BA.2 convalescent against BA.4/5 |

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|---|---|---|---|
| Danklmaier | Sarah | 46 | Establishing a workflow to compare T cell receptor sequences in blood and tumor |
| Riepler | Lydia | 47 | Development of a VSV-based therapeutic HPV16 vaccine |
| Trovato | Olga | 48 | Structural determinants of Dopamine Receptor Agonist selectivity |
| Guastadisegni | Maria | 49 | Dual activation of mu and delta opioid receptors by new oxymorphone analogues produces effective antinociception without the risks of antinociceptive tolerance and physical dependence in mice |
| Schöppe | Helge | 50 | Computational prediction of activating kinase mutations |
| Olivé Martí | Aina Leonor | 51 | Antinociceptive efficacy of selective κappa-opioid receptor agonists HS665 and HS666 in chronic inflammatory pain without inducing anxiety-like behavior in mice |
| Lechuga | Ana | 52 | Investigating and modulating κ-Opioid Receptor signaling. |
| Scherfler | Amelie | 53 | Are chlorido(diarylsalene)iron(III) complexes potent anti-breast cancer drugs due to induction of ferroptosis? |
| Ferro | Federico | 54 | VIP/VPAC1/2 receptor signaling in the central amygdala regulates stress and anxiety responses in mice |
| Bernkop-Schnürch | Astrid | 55 | Discovery of anticancer drugs: Can iron in chelated form induce ferroptosis? |
| Lanzetti | Stefano | 56 | Modulation of L-type calcium currents associates with changes of dendritic growth in hippocampal neurons |
| Zwerger | Michael | 57 | Exploring Mycosporine-like Amino Acids in Marine Algae with Feature-Based Molecular Networking (FBMN) |
| Sanchez Armengol | Eva | 58 | CHITOSAN-MALEIC ACID CONJUGATE AS POTENTIAL EXCIPIENT CANDIDATE FOR ORAL DRUG DELIVERY? |
| Geisler | Stefanie M. | 59 | Genetic deletion of Stac2 adaptor protein alters electrical activity of mouse chromaffin cells |
| Jörgensen | Arne | 60 | Biodegradable arginine based steroid-surfactants: Cationic green agents for hydrophobic ion-pairing |

## Posters

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

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| Veider | Florina | 61 | Design of nanostructured lipid carriers and solid lipid nanoparticles for enhanced cellular uptake |
| Zierer | Hannah | 62 | Tales of tails: scale patterns in the Australian gecko genus Strophurus |
| Jakobi | Milos | 63 | Optimizing CRISPR/Cas9 approaches in the tunicate Ciona intestinalis |
| ZENG | Fan | 64 | Bioadhesion in the Ascidian Ciona intestinalis |
| Achrainer | Matthias | 65 | Chitin as a structural element in the Hydra glue |
| Grüner | Kevin | 66 | Identification and characterisation of the Claudin family in Hydra vulgaris |
| Gronauer | Raphael | 67 | Integrated immuno-genomic analyses of high-grade serous ovarian cancer reveal vulnerability to combination immunotherapy |
| Tang | Xuechen | 68 | Pathogenicity prediction of voltage-gated calcium channel mutations |
| Bereuter | Leonhard | 69 | The ferroptosis-sensitizing EMT transcription factor ZEB1 orchestrates the cellular (redox)lipidome |
| Gariglio | Giacomo | 70 | Improving cancer resection with dual-modality imaging: comparison of two probes targeting the CCK2 receptor |
| Van Goubergen | Jasper | 71 | The identification of functional germline variants in 3´UTR of androgen receptor variant 7. |
| Holl | Aline-Sophie | 72 | Establishment of a HepG2 cell culture model for liver machine perfusion research |
| Zierke | Maximilian | 73 | More than sweet: 68Ga-labelled sugar trimers for functional liver imaging |
| Steinkellner | Sabrina | 74 | Investigation of NMP-related hemolysis using a precision-cut-kidney slice model |
| Zavvar | Taraneh Sadat | 75 | Preclinical evaluation and automated synthesis of [177Lu]Lu-labelled DOTA-MGS5 for application in cholecystokinin-2 receptor expressing neoplasms |
| Quilez Playan | Nayima | 76 | The role of glucocorticoids in Non-Alcoholic Fatty Liver Disease Development |

## Posters

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

| Presenting Author | | # | Abstract title |
|---|---|---|---|
| Savic | Dragana | 77 | Erk1/2-Dependent HNSCC Cell Susceptibility to Erastin-Induced Ferroptosis |
| Andolfi | Chiara | 78 | MED12 promotes prostate cancer (PCa) cell proliferation through c-MYC and AR signalling |
| Damisch | Elisabeth | 79 | Identification and characterisation of the stromal heterogeneity in prostate cancer |
| Filippi | Vera | 80 | MR-Spectroscopy: Investigating neurochemical changes in brain metabolism in migraineurs before and after CGRP-Antibody treatment – a randomized, controlled, open-label trial. |
| Kobakhidze | Nino | 81 | Behavior and cortical activation following mild hypercapnia in high-anxiety subjects |
| Berek | Klaus | 82 | Sex- and Gender-related Differences in Treatment of Multiple Sclerosis – a retrospective analysis of the Austrian National MS registry |
| Karisik | Anel | 83 | Gender Differences in Psychological Sequelae of Dyspagia after Ischemic Stroke |
| Kindl | Philipp | 84 | Sex differences in Quality of Life after COVID-19 |
| Altmann | Klaus | 85 | Gender aspects of decision-making in neurocritical care patients in the early course of the disease |
| Chietera | Paola | 86 | Nuclear lamina–dependent mechanisms in neuroplasticity |
| González Díaz | Adrián | 87 | SATB2-dependent mechanisms in human cognitive ability and neuropsychiatric disorders |
| De Cleene | Nicolas | 88 | Olfactory training in COVID-19 associated loss of smell (SMELL) |
| Garmsiri | Carolin | 89 | Role of PKN1 in neuroregeneration and neurodegeneration after an ischemic stroke |
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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 daily lives and usually, those spores are neutralized by the person's immune system. However, people with a compromised immune system have a higher risk to develop severe health problems. Out of more than 1.5 billion people affected by fungal diseases each year, in excess of 1.5 million end up in deaths. One of the deadliest fungal species, responsible for a large proportion of these deaths, is Aspergillus fumigatus. Treatment of infections caused by this pathogenic mold, generally termed aspergillosis, is limited to only four major drug classes with different cellular targets and efficacy: azoles, polyenes, echinocandins and nucleobase analogs.

First-line treatment of aspergillosis comprises azole antifungals that target sterol 14-α demethylase (Cyp51), a key enzyme in ergosterol biosynthesis. Similar to cholesterol in human cells, ergosterol stabilizes the fungal cell membrane, determines its fluidity and permeability. Inhibition of Cyp51 leads to the accumulation of toxic intermediates and depletion of ergosterol, eventually growth inhibition. Ensuring adequate activation of several ergosterol biosynthetic genes including cyp51A, in A. fumigatus two major transcription factors involved in sterol regulation as well as azole resistance represent SrbA and AtrR. Employing tunable atrR and srbA strains as well as mutated versions of these transcription factors, in this work we aimed to investigate common as well as independent functions of these proteins that play dominant roles in resistance of A. fumigatus to azoles.

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

Exocrine pancreas display an outstanding capacity for regeneration and cell fate 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 source for tissue regeneration after virtually complete removal of mature acinar cells.

To better understand this progenitor pool, we now established novel transgenic tools and FACS approaches to further characterize these cells in zebrafish.

Quantification of sectioned and dissociated healthy ptf1a:GFP/ela3l: E2Crimson labeled pancreas suggest that the proportion of Ptf1a+/Ela3l- progenitor cells reduced from 5% in larva to <0.2% in the adult exocrine pancreas. To enable efficient FACS-Sorting of these rare cells and distinguish their borders from other cells, we established ela3l:mScarlet ablation line with bright and stable fluorescence which does not form aggregates and is easier to detect. Moreover, to find out the fate of Ptf1a+ cells during pancreas development in fish we developed a Cre ERT2/lox line with 3 labeling colors called Ptf1a:CreERT2;CryaaRFP;hsp70: Cytbow. We also established Ptf1a:Casp8;CryaaRFP;Ptf1a:GFP line , in order to follow the regeneration events after ablation of even these immature Ptf1a+ cells. Our early FACS-Sorting results also confirmed the existence of this rare but conserved population of ptf1a+ only cells in healthy adult zebrafish which needs to be further optimized and compared to ablated condition.

After confirming the existence and conservancy of Ptf1a+ cells even in adult stage of zebrafish life, we can now study the molecular identity and role of these progenitor cells.

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# iPSC-based modeling of Schaaf-Yang-Syndrome reveals increased levels of reactive oxygen species as pathological driver

Schaaf-Yang Syndrome (SYS) is a neurodevelopmental disorder caused by a mutation in the paternally inherited MAGEL2 gene. Patients with SYS exhibit similar symptoms as Prader-Willi-Syndrome (PWS) patients, such as neonatal hypotonia, feeding difficulties, hypogonadism, intellectual disabilities and sleep apnea, but show also different outcomes like joint contractures, a higher prevalence of autism spectrum disorder symptoms and cognitive impairment.

The aim of this study is to better understand the underlying cellular mechanisms of SYS that lead to neurobiological dysfunction. To this end, we subjected three induced pluripotent stem cell (iPSC) lines derived from SYS patients carrying frameshift mutations in the MAGEL2 gene (c.1996dupC and c.1802delC), together with two controls, to differentiation into neural progenitor cells (NPCs) and subsequently into neurons. We observed no apparent changes between mutated and control cells regarding differentiation outcome as judged by morphology and staining against neural markers. However, we observed increased reactive oxygen species (ROS) levels by flow cytometry analysis in the mutant cell lines compared to the controls. At transcriptomic level, we analyzed candidate genes such as MAGEL2 and mammalian target of rapamycin (mTOR) by quantitative real-time PCR and observed 3-fold increase mTOR mRNA in c.1996dupC SYS cell lines. Furthermore, Western Blot analysis revealed an altered LC3-II/LC3-I ratio between mutation of MAGEL2 and control indicative of possible malfunction of autophagic flux.

These results suggest that dysfunctions in the mTOR signaling pathway may lead to increased ROS production in SYS patients via altered endosomal trafficking which cause some of the neurodevelopmental phenotypes of this disease.

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# Deciphering growth control in the developing human brain

Like many organs, development of the brain depends on the ability of tissue stem cells to symmetrically divide to expand the progenitor pool and to asymmetrically divide to produce differentiated cell types. Variability among stem cells to shift between their division modes generates heterogeneity in individual stem cell lineages' sizes. Stem cell lineages compete with and compensate deficits among each other to ensure normal brain tissue growth. However, little is known about the genetic and molecular cues that ensure proliferation control and lineage size heterogeneity in the human brain tissue context. Genetic studies in a 3D model of the developing human brain, brain organoids, help elucidate the mechanisms underlying the growth control of the human brain. Thus, loss-of-function (LOF) screens in brain organoids were performed based of the CRISPR-LIneage tracing at Cellular resolution in Heterogenous Tissue (CRISPR-LICHT) methodology. The screens targeted 195 brain tumor susceptibility genes as possible regulators of brain tissue growth. To study combinatorial effects of targeted genetic pathways, 5 screens were performed in different genetic backgrounds comprising the LOF of an additional neural gene or tumor suppressor gene: screens were performed in cells mutant for NF1, PTEN, TP53 or CDKN2A/B function, along with a wildtype control. Initial analysis of scoring genes identified several candidate genes that score uniquely in specific backgrounds as well as hits which act on multiple mutational backgrounds. These candidate genes will be further studied in new brain organoid models to validate and characterize their roles in growth control of the early human brain.

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# Inducible CRISPR Interference Allows for Specific Gene Repression in Human Pluripotent Stem Cells and Neuronal Progenitors

The targeted modulation of gene function provides deeper knowledge of most biological processes. Gene silencing by CRISPRi (CRISPR interference) is a novel tool to repress gene expression of target genes with little off-target effects. Previously, it has been shown that CRISPRi can be successfully applied in induced pluripotent stem cells (iPSCs) and iPSC-derived endoderm in a time-dependent manner. However, the inducibility of the system in iPSC-derived neuronal progenitor cells remains unknown.

Here we describe the generation of several novel monoclonal human iPSC lines with stable integration of a doxycycline (DOX)-inducible dCas9-KRAB expression cassette. Verification of the lines via genotyping PCR, RT-qPCR and dot blot showed an average 100-fold increase of dCas9-KRAB expression upon DOX treatment. Furthermore, gene repression of candidate genes ATM, ERCC6 and XRCC1 revealed a knockdown efficiency of 98% in iPSCs. Next, we differentiated the CRISPRi-iPSC lines to neuronal progenitor cells (NPCs). These exhibited positive signal for neural epithelial markers such as SOX1, Nestin, and PAX6, and maintained the capability to further differentiate into neurons. dCas9KRAB expression could be induced in NPCs yielding an average 50-fold increase compared to controls. Nonetheless, preliminary observations in NPCs indicated up to 70% knockdown efficiency only using gRNAs directed against the candidate genes.

Our results demonstrate that the CRISPRi system can be applied to human iPSCs in an inducible manner, however, application in NPCs is so far limited by reduced knockdown efficiency. We plan to include additional candidate genes into the analysis and further investigate negative selection effects on cell growth.

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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 and PBX3 as significantly upregulated genes in MNX1 mutants. HHEX is a transcription factor involved in delta cell development, whereas the role of PBX3 is not clearly defined in the development of hormonal cells. We currently aim to analyze PBX3 and HHEX by a functional lentiviral knockout during differentiation to the beta-like stage. Detailed analysis of these data will be presented as well as planned experiments such as Single-cell-RNA-seq of MNX1-/- and ChIP-seq of MNX1 binding partners.

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# The BolA Family Protein Bol3 is Dual Localised by Alternative Translation Initiation in A. fumigatus

The biosynthesis of FeS requires complex biosynthetic machineries: mitochondrial core FeS assembly machinery generates [2Fe-2S] clusters, representing precursors of mitochondrial and cytosolic [4Fe-4S] clusters. In Aspergillus fumigatus, mitochondrial [2Fe-2S] biosynthesis and the cytosolic/nuclear glutaredoxin GrxD have been shown to be essential for iron sensing. Most eukaryotes possess genes encoding BolA homologs with and without mitochondrial targeting sequences (MTS). In contrast, both A. fumigatus homologs possess putative MTS, suggesting the lack of cytosolic/nuclear versions. However, closer inspection of Bol3 protein sequence revealed a methionine residue, located downstream of the MTS and highly conserved in various Aspergillus species. Proteomic analyses identified a Bol3 peptide indicating that this methionine is derived from alternative translational initiation. Generation and phenotyping of different bol3 mutant strains lacking the Bol3-encoding gene (Δbol3), the putative cytosolic/nuclear Bol3 (bol3M41L) or the mitochondrial Bol3 (bol3M1L, bol3Δ38) revealed different phenotypes, supporting a dual-localisation. The most pronounced phenotype – a growth defect under iron limitation – was caused by the loss of the cytosolic Bol3. Fluorescence microscopy confirmed dual localisation of Venus-tagged Bol3 protein versions in mitochondria and the cytosol/nucleus. Purification of C-terminally Venus-tagged Bol3 proteins followed by nLC-MS/MS analysis revealed peptides, confirming different Bol3 proteins derived from alternative translational initiation, followed by proteolytic processing. Interestingly, this analysis indicated that mitochondria and the cytosol/nucleus contain the very same Bol3 protein discriminated only by N-terminal acetylation of the cytosolic/nuclear form. Mutation of the initial Kozak sequence demonstrated that increased translation initiation at the first AUG decreases translational initiation at the downstream AUG.

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# Phytohormones: putative signalling molecules in mycoparasitic Trichoderma species

The fungal genus Trichoderma contains a vast array of species that are well known for their high opportunistic potential and adaptability to various ecological niches. The ability of many Trichoderma species to both colonize the rhizosphere and antagonize plant pathogenic fungi has led to their use in biological pest control for several decades.

Phytohormones, which are signalling molecules known for controlling various aspects of plant growth and development, are produced by both plants and microorganisms, including fungi that interact with plants, both in a beneficial and harmful manner.

In the present study, we characterized and compared the phytohormone production profiles of three Trichoderma species through UHPLC MS/MS analysis. To this end, Trichoderma atroviride, Trichoderma virens and Trichoderma asperellum were cultivated on solid medium in the presence or absence of a plant host and the production of secreted phytohormones was assessed. All three Trichoderma species produced two auxins, indole-3-acetic acid (IAA) and oxidised indole-3-acetic acid (oxIAA), as well as salicylic acid. Notably, the presence of the plant host only had minor effects on the quantity of phytohormones produced by the Trichoderma spp. These data indicate that the phytohormones produced by Trichoderma spp. could play a role in the physiology of the fungus, regardless of their role during plant interactions. The analysis of phytohormones in these Trichoderma spp. is the basis for currently ongoing experiments that aim at further elucidating the functions of phytohormones in the physiology of filamentous fungi.

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# Substrate Specificity Of Siderophore Uptake By Aspergillus fumigatus

The opportunistic human pathogen Aspergillus fumigatus employs two high-affinity uptake systems for the micronutrient iron: reductive iron assimilation (RIA) and siderophore-mediated iron acquisition. This mold produces two fusarinine-type (fusarinine C and triacetylfusarinine C (TAFC)) and two ferrichrome-type siderophores (ferricrocin and hydroxyferricrocin) for acquisition and storage of iron. Siderophores have been shown to play a crucial role in virulence of several fungal pathogens and to have high potential as biomarker for imaging of fungal infections. Moreover, the siderophore transporter (SIT) Sit1 was found to mediate uptake of the novel antifungal drug VL-2397. However, siderophore uptake in filamentous fungi is poorly characterized.

To enable characterization of siderophore uptake in A. fumigatus by growth studies, SITs mutants (Sit1, Sit2, MirB, MirD and MirC) were studied and we demonstrated that (i) Sit1 and Sit2 have overlapping and unique substrate specificities with respect to different ferrichrome-type siderophores, e.g., utilization of ferrirhodin and ferrirubin depends exclusively on Sit2, use of ferrichrome A depends mainly on Sit1, and utilization of ferrichrome, ferricrocin, and ferrichrysin is mediated by both transporters; (ii) both Sit1 and Sit2 mediate weak use of the coprogen-type siderophores; (iii) Sit1 transports the bacterial ferrioxamine-type xenosiderophores; (iv) MirB transports TAFC; (v) MirD transports fusarinine C; (vi) MirB but not MirD was crucial for virulence in a murine aspergillosis model; vii) lack of MirC causes a growth defect under iron limitation that cannot be cured by siderophore supplementation, which questions a role in siderophore metabolism in line with MirC localization to the vacuolar membrane.

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# Regulatory role of Mnx1 in beta-cell differentiation and maturation

The neonatal Diabetes factor Mnx1 is a key regulator of fate-determination and –maintenance of insulin producing beta-cells. Despite its essential role in beta-cell formation, Mnx1 function at molecular levels is still widely unknown.

To gain a better understanding of Mnx1 function we generated different mnx1-mutant and -transgenic zebrafish lines and started exploring molecular activities by a combination of RNA-sequencing and detailed expression approaches.

Our studies revealed the presence of a previously missed delta-cell sub-population in zebrafish and they showed that in mnx1 mutants the majority of beta-cell-precursors trans-differentiate into this novel delta-like cell type.

In addition, we found that adult mnx1 mutants display a loss of mono-hormonal beta- and delta1-cells while the beta/delta1 hybrid cells became the dominant cell type. The whole pancreas seemed to be strongly increased in size in the mutants which mainly consists of ductal like structures shown by histological analysis. Analyzing earlier stages revealed that zebrafish larvae suffer from sever hyperglycemia and developmental issues the first couple of weeks but recover after 4 wpf. This correlates with a severe islet hyperplasia we could observe starting at this time window and a strong increase in hybrid cells. We reason that the ectopic cell mass likely reflects secondary consequences of cellular mechanisms that aim to compensate the hyperglycemia caused by the lack of insulin. Whether the ectopic cells originate by migration from duct progenitor cell or in addition by proliferation of already differentiated cells is center of currently ongoing experiments.

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# The acyltransferase SidF is involved in biosynthesis of fusarinine-type and ferrichrome-type siderophores in A. fumigatus

The opportunistic human pathogen Aspergillus fumigatus employs two high-affinity uptake mechanisms for iron: reductive iron assimilation (RIA) and siderophore mediated iron acquisition (SIA), which has been shown to be crucial for its virulence. This mold species produces fusarinine-type siderophores such as triacetylfusarinine C (TAFC) and ferrichrome-type siderophores such as ferricrocin (FC). The first committed enzymatic step for all siderophores is hydroxylation of ornithine catalyzed by SidA. Subsequently, the pathways for synthesis of fusarinine- and ferrichrome-type siderophores split. For fusarinine-type siderophores an anhydromevalonyl group is linked to hydroxyornithine mediated exclusively by the transacylase SidF, while for ferrichrome-type siderophores an acetyl group is linked by the transacylase SidL and a yet unknown enzyme. Both SidF and SidL belong to the GNAT protein family, showing similarity only in the C-terminal half. SidF is localized in peroxisomes and the encoding gene is induced by iron starvation, while SidL is a cytosolic enzyme and expression of the encoding gene is largely iron-independent.

Here we found that simultaneous inactivation of both SidF and SidL abrogates biosynthesis of both fusarinine- and ferrichrome-type siderophores. In line, the ΔsidFΔsidL double mutant phenocopies the ΔsidA mutant. Our studies also revealed an interdependence of fusarinine- and ferrichrome-type siderophores as inactivation of SidF blocked biosynthesis of TAFC but increased FC production. Moreover, we demonstrate that truncation of either the GNAT-motif containing C-terminal half showing similarity to SidL or the N-terminal half blocks all SidF functions.

Taken together, this study suggests that SidF is the so far unknown enzyme catalysing acetylation of hydroxyornithine.

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# PATIENT-DERIVED STEM CELLS TO STUDY THE PATHOLOGY OF AUTISM SPECTRUM DISORDERS-RELATED VOLTAGE-GATED CALCIUM CHANNEL GAIN-OF-FUNCTION MUTATIONS

Voltage-gated calcium channels (VGCCs) are highly expressed in the human brain and are involved in many physiological processes. Increasing evidence suggests that VGCC are key modulators of early neurodevelopment. VGCC mutations, as observed in the Cav1.3 encoding CACNA1D gene, are associated to neurological pathologies, including Autism Spectrum Disorders (ASD). One such mutation affects the Cav1.3 L271 residue. Electrophysiological studies overexpressing Cav1.3 L271H in HEK cells, indicate that this mutation lowers the voltage dependency of channel activation and inactivation, thereby inducing channel gain-of-function. However, currently no functional studies on how this mutation affects early neurodevelopment or the physiology of disease-relevant human neurons are available.

Here, we describe the generation of an induced pluripotent stem cell (iPSC)-line, carrying the heterozygous Cav1.3 L271H mutation, from patient material. An in vitro disease model including multiple stages of neurodevelopment, comprising neural progenitor cells (NPC) and human (midbrain) neurons, was established using this iPSC line. Functional analysis indicates alterations in calcium signaling and electrical activity affecting the resting membrane potential, firing frequency and action potential shape of the mutated NPCs and neurons. Three-dimensional cell culture systems of early human neurodevelopment revealed structural alterations, indicating a deficit in self-organization of the mutated cells. Furthermore, transcriptomic analysis highlights the upregulation of genes, which have previously been associated to ASD and other neurodevelopmental disorders.

Overall, this study will broaden our understanding of the role Cav1.3 channels play during neurodevelopment and how such gain-of-function mutations contribute to CACNA1D channelopathies, thereby paving the way for novel therapeutic strategies for affected individuals.

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## Novel and substrate-specific roles of the myosin co-chaperone UNC45A in trafficking and enterocyte polarity

Severe congenital enteropathies comprise a group of rare diseases caused by gene mutations primarily affecting intestinal epithelial cell function. The characterization of mutations and their effects on cell biological and physiological aspects provides a better understanding of disease-associated mechanisms.

We have identified mutations in MYO5B as one major cause for the microvillus inclusion disease (MVID) that presents with severe syndromic diarrhea in neonates. MVID is characterized by impaired intracellular trafficking with defective apical transport and enterocyte polarization. Recently, bi-allelic mutations in the myosin co-chaperone UNC45A were associated with an MVID-like enteropathy. A loss of UNC45A function leads to aggregation of its substrate MYO5B, thereby provoking impaired MYO5B-dependent apical trafficking in patient enterocytes.

UNC45A provides chaperone function towards different classes of myosin. This prompted us to investigate whether other myosins (e.g., class II and I) are affected by UNC45A deficiency and how this would contribute to disease. We also present here a particular case of a patient with severe MVID-like symptoms underlying a UNC45A missense mutation. The mutated UNC45A maintains chaperone activity and shows a distinct disease-causing mechanism. We demonstrated that either loss or mutation of UNC45A affects various myosin-dependent processes such as intracellular cargo transport, organization of the cytoskeleton, and epithelial polarization. Cellular morphology, recycling processes and epithelial barrier integrity were analyzed in our established CaCo2 cell models, patient derived tissue and fibroblasts by means of different methods. Together, this study contributes to a broader understanding of the complex physiological and pathological roles of UNC45A in an MVID-like enteropathy.

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

Uptake of nutrients is essential for cellular growth, proliferation and survival. Amino acid transporters (AAT) are solute carriers located on the plasma membrane (PM). The abundance of different AATs at the PM determines the quality and quantity of amino acid import into the cytoplasm, which plays a major role in cellular metabolism and in the regulation of cell growth and proliferation. Remarkably, cells entering quiescence selectively downregulated a subset of AAT by endocytosis and lysosomal degradation. The underlying molecular mechanisms are only partially understood, but appear to involve ubiquitin ligase adaptors of the a-arrestins family.

We have now identified two a-arrestins ARRDC2 and TXNIP (Jennifer Kahlhofer; MS in preparation) that selectively target two AAT for endocytosis and lysosomal degradation, only as cells enter quiescence.

In this project we address how the activity of these a-arrestins is regulated, how the activated a-arrestins target their cognate AATs for endocytic degradation and how other a-arrestins contribute to endosomal degradation of AATs and ultimately how these processes contributes to metabolic adaption as cells enter and exit quiescence.

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# Molecular mechanism of nutrient transporter regulation in human cells

Proliferating cells increase nutrient acquisition to fuel anabolic processes for biomass formation. Conversely, differentiated or quiescent cells adjust their nutrient uptake to maintain metabolic homeostasis for survival. A key strategy by which cells reconfigure nutrient uptake across the plasma membrane is the selective addition or removal nutrient transporters. How cells control nutrient uptake to increase biomass or to preserve homeostasis is not understood.

Our results show that, in non-cancerous cell lines, exit from cell cycle and entry into quiescence correlated with the selective endocytic downregulation and lysosomal degradation of the glutamine transporter SLC1A5/ASCT2 and the neutral amino acid transporter SLC7A5/LAT1. Upon re-entry into the cell cycle, SLC1A5 and SLC7A5 protein levels increased again. The protein levels of several other transporters were not regulated under these conditions. Based on these results we hypothesize that the selective regulation of nutrient transporter abundance is directly linked to cell growth and proliferation. To analyze the contribution of SLC1A5 and SLC7A5 to these processes, we generated CRISPR/Cas9-mediated knockout cell lines. While SLC1A5 and SLC7A5 were essential for efficient exit from quiescence and re-entry into the cell cycle, they were not required for entry into quiescence. Moreover, we could identify two alpha-arrestins, which seems to be involved in the selective regulation of either SLC1A5 or SLC7A5. Currently we are characterizing the underlying molecular mechanisms.

Our results will provide a better molecular understanding of how proliferating and quiescent cells control their nutrient transporter repertoire to adjust nutrient uptake accordingly.

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# SZT2 regulation of mitochondrial function and in response to oxidative stress

Seizure-Threshold-2 (SZT2) protein is a negative regulator of the amino-acids-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 patients with DEE-18. Recently, we identified more than thousand proteins in the interactome of SZT2 and some of them are involved in mitochondria metabolism and neurologic diseases. In 2018, Uittenbogaard and colleagues investigated the impact on mitochondrial metabolism in fibroblasts from one patient carrying heterozygous SZT2 variants. They showed that OXPHOs pathway was impaired in patient's fibroblasts and that the mitochondria presented an elongated shape and abnormal cristae morphology.

Our aims are to characterize SZT2 regulation of mitochondrial activity and investigate its involvement in response to oxidative stress.

I was able to reproduce the data from Uittenbogaard et al. in our HEK293 Flp-in-TRex cells and show that mitochondrial activity is significantly restricted in SZT2-KO cells. Moreover, I found that re-expression of SZT2 in KO cells can rescue the mitochondrial function in a dose-dependent manner. I also investigated the growth of SZT2-WT and -KO cells and observed that in absence of SZT2 the cells grow slower. As for mitochondrial activity, the cell growth capacity is restored when SZT2 is re-expressed in KO cells. Finally, I showed that upon prolonged amino acids starvation, SZT2-KO cells reduce mTORC1 activation. This novel finding opens the unexpected possibility that SZT2-KO cells might use other/additional mechanisms to sense the absence of amino acids.

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# Defining the role of caspase-2-PIDDosome in ploidy control during heart development

The family of caspases is best known for its involvement in inflammation and cell death. However, Caspase-2 was recently implicated as a key regulator of cellular maturation and differentiation processes. One terminal differentiated organ in our body is the heart, which is predominantly characterized by post-mitotic polyploid cardiomyocytes (CM). Polyploid cells with extra centrosomes cause the activation of the caspase-2-PIDDosome, a multiprotein complex, consisting of PIDD1, the dual adaptor protein RAIDD and caspase-2. Its activation leads to stabilization of p53 to restrict further proliferation of the polyploid cells. Since caspase2-PIDDosome function in heart remains elusive, the overall goal of this project is to unveil the role of the caspase2-PIDDosome in CM polyploidization.

Analyzing mouse models lacking PIDDosome components (Pidd1, Raidd and Caspase-2), as well as a cardiac specific Caspase-2 knockout, revealed a ploidy increase in adult murine CM nuclei. By analyzing mouse postnatal stages, the polyploidization phase of cardiomyocytes was identified to be controlled by the caspase-2-PIDDosomestarting from postnatal day 7. Previous studies have demonstrated that PIDD1 must be tethered to the mother centriole by ANKRD26 in order to activate the caspase2-PIDDosome pathway. Preliminary research using Ankrd26 knockout mice showed that, similar to Pidd1, Raidd, and Casp2 knockout mice, Ankrd26 deficiency increases the number of tetraploid CM nuclei. Interestingly, ploidy is not significantly elevated in p53 knockout mice compared to wild-type animals, suggesting that the PIDDosome regulates CM ploidy in a p53-independent manner. These findings will be explored for their potential to improve cardiac regeneration.

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# Sex specific immune response in chronic kidney disease patients and renal transplant recipients

Infections caused by respiratory pathogens such as SARS-CoV-2, influenza viruses and Streptococcus pneumoniae can lead to severe respiratory injury and life-threatening disease. Pre-diseased persons in particular are at increased risk shown by higher morbidity and mortality rate. To protect these persons from severe courses of Covid-19, the flu and pneumococcal severe disease vaccines have been developed. Nevertheless, the effectivity of vaccine-induced immune reactions (humoral and cellular) is influenced by several factors. It is already known that the immune reponse of men and women differs, also regarding the response to vaccines. Our study investigates the immune reaction of nephrologic pre-diseased individuals with chronid kidney disease (CKD) and renal transplant recipiets (KTR) to SARS-CoV-2 mRNA vaccine (two doses of Moderna). Serum and PBMCs at the day of vaccination and 3 month later was analysed for specific SARS-CoV-2 S1 antibodies, neutralising antibody titres against different SARS-CoV-2 variants and for virus-specific T-cell response via IFNgamma ELISpot assay. Statistical analyses will be directed to the question whether sex differences between men an women exist.

A Knell 1

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# Centrosomes are critical for B cell development but not function

Defects in centrosome duplication can lead to a variety of human diseases, including cancer. As such centrosome numbers are tightly regulated. Too little as well as too many centrosomes activate p53 by the mitotic surveillance- or the PIDDosome pathway, respectively, to limit the growth of cells at risk to lose genome integrity. Here, we observed that proliferating B cell progenitors frequently present extra centrioles, a phenomenon no longer seen in mature B cells, suggesting cell clearance.

Consistently, centriole overduplication induces BCL2-dependent apoptosis, yet, this response was independent of p53 or PIDD1. In contrast, B cell specific centriole loss arrests development at the pro B cell stage and promotes BCL2 regulated apoptosis in a strictly p53-dependent manner. Remarkably, B cell development and function can be rescued by co-deletion of USP28, a major component of the mitotic surveillance pathway.

Together, these results demonstrate a previously unappreciated presence of extra centrioles in developing B cells that may aid oncogenesis at this developmental stage. Moreover, centrosomes are necessary for normal B cell development but, surprisingly, not humoral immune responses.

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# Where ether lipids metabolism meets immunology: a role for PEDS1

Plasmalogens are a subclass of ether lipids of particular relevance for the organization and stability of biological membranes. The synthesis of plasmalogens starts in peroxisomes and is finalized at endoplasmic reticulum, where the enzyme plasmanylethanolamine desaturase (PEDS1) is responsible for the insertion of the characteristic vinyl ether double bond. The gene for PEDS1 was recently described by our laboratory, where a mouse model lacking PEDS1 is available. Defects in enzymes involved in ether lipid metabolism in mouse models also recapitulate the most frequently observed human phenotypes. Among these, osteo- and adipogenesis as well as neurological and behavioral disorders are observed. Furthermore, fertility dysfunctions and a peculiar ocular phenotype are reported.

Moreover, only recently the role of plasmalogens in the context of inflammation and immune response has started to gather interest and data from the literature report significant alterations in the hematopoietic system in mice lacking PEDS1.

Hence, we performed a first exploratory analysis of the hematologic phenotype in PEDS1 deficient mice, and could detect abnormalities in mean platelet volume, hematocrit and hemoglobin content. Therefore, it is now the aim of this ongoing project to conduct a complete immunologic analysis, evaluating lymphoid and myeloid lineage cellular subset development both in young and late adult mice. Our preliminary data already provide interesting insights into the impact of PEDS1 deficiency on the hematological system and with the further investigations we aim to uncover a putative molecular regulatory role for this metabolic enzyme in the frame of the immune system.

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# Deregulation of the spindle assembly checkpoint triggers myelosuppression and gastrointestinal atrophy

Interference with microtubule dynamics activates the spindle assembly checkpoint (SAC) to prevent erroneous chromosome segregation. Activating the SAC induces mitotic arrest due to inhibiting the anaphase-promoting complex (APC) by the mitotic checkpoint complex (MCC). The MCC component MAD2 neutralises the critical APC cofactor, CDC20, leading to extended mitotic arrest. In cancer cell lines, a prolonged mitotic arrest can promote apoptosis that depends on distinct pro-apoptotic BCL2 family members, including BIM/BCL2L11, BID and NOXA/PMAIP and that can be blocked by BCL2 overexpression. However, the relevance of mitochondrial apoptosis in response to SAC perturbation in vivo is unknown.

Using a mouse model allowing conditional MAD2 overexpression, we learned that SAC deficiency is associated with severe bone marrow aplasia and intestinal atrophy, causing premature lethality in MAD2 transgenic animals. While chronic SAC activation triggered transient myelosuppression, gastrointestinal atrophy was causal for the early lethality observed in MAD2-transgenic mice. Remarkably, co-deletion of pro-apoptotic Bim, but neither Bid, Puma/Bbc3 or Noxa, sufficed to prevent gastrointestinal syndrome but failed to rescue hematopoietic defects in response to MAD2 overexpression. Our study identifies the BH3-only protein BIM as most critical for apoptosis induction in response to impaired SAC function in the gastrointestinal tract.

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# A stress granule protein integrates metabolic signals and controls lysosomal TSC recruitment and mTORC1 suppression in breast cancer

The tuberous sclerosis protein (TSC) complex acts as a relay for anabolic signaling and as a tumor suppressor. These functions are mediated via the inhibition of the metabolic master regulator mTORC1 (mechanistic target of rapamycin complex 1) at its central signaling platform – the lysosomes. We recently discovered that the stress granule protein G3BP1 (Ras GTPase-activating protein-binding protein 1) anchors the TSC complex to lysosomes needed for the suppression of mTORC1 by nutritional signals [1]. Using biochemical approaches we found that the G3BP1-TSC axis mediates metabolic signals to mTORC1, suggesting a novel mode of TSC-mediated nutrient sensing. The G3BP1-TSC tumor suppressor axis is highly preserved in breast cancer patients. Therefore, targeting this axis could increase the efficacy of current therapies.

[1] Prentzell MT, Rehbein U, Cadena Sandoval M, De Meulemeester A-S, Baumeister R, Brohée L, et al. G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling. Cell. 2021;184(3):655-74.e27.

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# Systems approaches reveal resistance mechanisms to mTOR-directed therapies in pancreatic neuroendocrine tumors and ER-positive breast cancer

The kinase network converging on mTOR (mammalian/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, neuronal and hereditary disorders. Toward disease mechanism-driven personalized therapies, we develop systems approaches to predict metabolic control by kinase networks. Through detailed modeling, 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. We propose and prioritize combinatorial drug therapies to improve therapy response to mTOR inhibitors in pancreatic neuroendocrine tumors (panNET) and estrogen receptor positive breast cancer.

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# Modulation of lipid peroxidation by ether lipids and tetrahydrobiopterin in Crohn's disease

The existing variety of lipids in mammalian cells is high and their functions and interactions are still not well enough understood1. One lipid subclass comprises the ether lipids, which are divided into plasmanyl and plasmenyl species. It was recently shown that metabolic enzymes as plasmanylethanolamine desaturase (PEDS1) and alkylglycerol monooxygenase (AGMO), as well as its cofactor tetrahydrobiopterin (BH4) together with the rate-liming biosynthetic enzyme GTP-cyclohydrolase 1 (GCH1) have an impact on the lipid peroxidation of membrane lipid-contained polyunsaturated fatty acids (PUFAs)2,3. Lipid peroxidation is a hallmark of ferroptosis, an iron-dependent regulated cell death4. It becomes activated upon a deficiency or reduced activity of the antioxidative cyst(e)ine-glutathione-GPX4 axis, which inhibits the accumulation of toxic lipid peroxides5. Its compromised activity in intestinal epithelial cells and an increased intake of PUFAs, excessed in Western diet, trigger the development and course of inflammatory Crohn’s disease in the gut6,7.

Our laboratory has a longstanding expertise in both BH4/GCH1 as well as ether lipids and their metabolic routes and this puts us in the unique position to study the impact of AGMO, PEDS1 and GCH1/BH4 in lipid peroxidation of PUFA side chains in models of the intestinal epithelium, where all these enzymes are robustly expressed. I will present the rationale underlying our investigation, and present our model systems, which are available or are currently established. Ultimately, we want to better understand the influence of ether lipids and BH4/GCH1 on ferroptosis and elucidate possible interactions of these entangled metabolic routes on this form of cell death.

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# A systems approach to stratify breast cancer patients based on metabolism and signaling crosstalk

According to the World Health Organization (WHO), breast cancer has claimed the life of over 685.000 people worldwide in 2020. 80% of the patients suffer from estrogen receptor (ER)-positive tumors and are treated with endocrine therapies (ET) targeting the ER. As the ER is a signaling molecule, research and clinical trials on therapy resistance focus so far mainly on the crosstalk of the ER with other oncogenic signaling networks. For this reason, many compounds inhibiting signaling kinases are in clinical use and trials. However, the success of these interventions remains limited.

Only little is known about the contribution of tumor metabolism to endocrine therapy resistance mechanisms. The MESI-STRAT consortium aims to identify marker metabolites measurable in body fluids that predict relapse and guide targeted interventions at early onset. Therefore, we explore the interaction of amino acid metabolism with signaling networks. To reach this goal, we have developed a protocol to measure both metabolites and signaling dynamics in ER-positive breast cancer cell lines and patient derived organoid systems under ET. Using these data, we generated ordinary differential equation (ODE)-based computational dynamic models to simulate the behaviour of breast cancer cells under ET treatment and to discover possible resistance mechanisms. We will present our most recent data and simulations, focusing on crosstalk between amino acid metabolism and signaling under ET.

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# ExonSurfer - a primer design tool for optimal qPCR results

Transcript-specific primer design is key for accurately quantifying splice variants via quantitative real-time polymerase chain reaction (qPCR) and to mitigate DNA or hnRNA contamination during reverse transcription experiments. However, this type of primer design is complex and can often result in failure. Here, we present ExonSurfer, a web-tool that combines all the steps of transcript-specific primer design, such as target selection excluding other transcripts and avoiding common polymorphisms, as well as specificity and self-complementarity verification. By default, ExonSurfer designs multiple possible primer pairs, both spanning and flanking exon junctions, in order to verify the specificity using BLAST and filter the primer pairs accordingly. The user can define different primer parameters, such as melting temperature, primer GC content or amplicon length, in order to best suit their experiments. Additionally, ExonSurfer also allows for the detection of all protein coding transcripts of a gene, trying to place the primers in the most conserved regions of the different splice variants. ExonSurfer offers the research community a unique tool that incorporates target selection, thus enabling highly accurate and efficient primer design for a wide range of qPCR applications.

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# The Role of Sec16B in the Export of Proteins from the Endoplasmic Reticulum

The transport of proteins from the endoplasmic reticulum (ER) to the Golgi apparatus is mediated by the coat protein II (COPII) complex and its accessory proteins. Cargos that cannot bind to COPII directly can either bind to so called cargo receptors, which mediate their concentration into COPII carriers, or use a non-concentrative export mechanism called "bulk flow", which relies on fluid export from the ER.

The protein Sec16 was suggested to play a role in organizing ER exit sites (ERES) and the COPII complex. In mammalian cells, two Sec16 paralogs (A&B) were described, but only Sec16A was characterized closely, while the function of Sec16B remains enigmatic.

In this thesis, I attempt to define a function for Sec16B in the context of the two different ER-export mechanisms. I found that overexpressed GFP-Sec16B colocalizes with Sec16A at ERES. Its colocalization rates with different COPII proteins are similar to Sec16A. My results also suggest a trend that ERES with higher cargo levels localize more closely to the Golgi, which is more pronounced for Sec16A-positive than for Sec16B-positive ERES. Finally, I found that Sec16B depletion delays the transport of the bulk flow cargo ssGFP, but not the receptor dependent cargo α1-antitrypsin. Sec16A depletion affects both cargos.

Therefore, I suggest that Sec16B might be a regulator of bulk flow. Sec16A is necessary for both transport types, indicating that Sec16B function in protein trafficking is linked to Sec16A. High Sec16A-Sec16B colocalization rates furthermore suggest that there are no separate ERES for receptor-mediated transport or bulk flow.

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# Ionic interactions between gating charges and countercharges in voltage-sensing domain I independently regulate kinetics and voltage-dependence of CaV1.1 gating

Voltage-gated calcium channels control a variety of processes in excitable cells. However, the exact mechanisms regulating kinetics and voltage-dependence of channel activation are not fully understood. Voltage-gated activation is determined by four distinct voltage-sensing domains (VSD I-IV) coupled to a common pore. Each VSD consists of four transmembrane helices (S1-S4) with S4 containing four to five gating charges. Upon membrane depolarization, consecutive interactions of these gating charges with negatively charged countercharges are believed to facilitate an upward movement of the S4 helices, leading to channel activation and pore opening. Previous studies linked naturally occurring mutations of the innermost gating charge R4 (R174W) and its negative countercharge (E100) in CaV1.1 to muscle disease. To investigate the contribution of VSD I and the roles of its gating- and countercharges in channel gating, we combined structure-guided site-directed mutagenesis with patch-clamp analysis in dysgenic myotubes (CaV1.1-null). As E100 in helix S2 together with D126 in helix S3 form a highly conserved charge-transfer center, we also included this second negative countercharge (D126) in our analysis. While mutation of R174A resulted in a strong right-shift of voltage dependence and slowing of activation kinetics, charge neutralizing mutations of the countercharges E100 and D126 both lead to a left-shift of voltage-dependence. The double mutant of both countercharges resulted in slowing of activation kinetics. Our findings indicate that VSD I substantially contributes to the regulation of both kinetics and voltage-dependence of activation, but that the molecular mechanisms governing the two gating properties are functionally separate.

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# The role of neuronally expressed IL6ST in gut microbiome composition and post-operative cognitive function in a mouse model of neuropathic pain

Increasing evidence links alterations of the gut microbiota with cognitive disorders and inflammation-related microbiome alterations might be causally involved in the development of post-operative cognitive deficits (POCD). Interleukin 6 (IL-6), its receptor (IL-6R) and the IL-6 signal transducer gp130 (IL-6ST) are well known regulators of innate immunity but also affect neuron morphology and function. Therefore, we aimed to identify the alterations in cognitive performance, gut motility and feces composition in a transgenic mouse model with a conditional depletion of gp130 in neurons expressing the nociceptor specific ion channel Nav1.8 (SNS-gp130-/-) and littermate controls.

Mice of both sexes and two age groups were subjected to the spared nerve injury model (SNI) for neuropathic pain. In order to assess cognitive performance at baseline and 14 days after SNI induction we performed the marble burying, open field and novel object recognition tests. Gut motility was assessed by quantification of fecal boli and weight. Feces were collected and subject to 16S sequencing to identify microbiota strains.

Our preliminary data suggest that SNI did not cause significant overall cognitive deficits dependent on the IL6ST depletion and no significant changes in gut motility were observed. However, 16S analysis revealed sex related changes in fecal microbiome composition. Therefore, we will further compare behavioral signatures between female and male young and old mice and investigate transcriptomic signatures of relevant brain regions to explore sex-dependent differences in gut to brain communication.

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# Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation-contraction coupling

The adaptor protein STAC3 was reported to be essential for skeletal muscle EC coupling and exerts three distinct functions. It facilitates membrane expression of CaV1.1. It is crucial for CaV1.1 function as the voltage sensor of EC coupling. Lastly, it is essential for the conformational coupling between CaV1.1 and RyR1. Previously, two distinct STAC3/CaV1.1 interactions were identified: the one between the SH3-1 domain of STAC3 and the II-III intracellular loop of CaV1.1, and the one between the C1-linker region of STAC3 and the proximal C-terminus of CaV1.1. To determine which interaction is important for each function, two STAC3 fragments, each containing the domain responsible for one interaction, were reconstituted in a double CaV1.1/STAC3 KO skeletal muscle cell line. Electrophysiological recordings revealed that the STAC3 C1-linker fragment expression rescued CaV1.1 charge movement and calcium currents. However, the calcium release from the SR was severely reduced. Conversely, reconstitution of only the STAC3-SH3s domains did not rescue any function. Simultaneous reconstitution of both fragments also did not fully rescue EC coupling, suggesting that the isolated SH3 domains interact with low affinity. To increase the local concentration, we linked the SH3 domains to the CaVβ1a subunit. This fragment alone rescued minimal EC coupling, but no calcium currents. However, when co-expressed with the other STAC3 fragment, full currents and EC coupling were reconstituted. Altogether, these results demonstrate that the C1-linker/C-terminus interaction is responsible for STAC3 targeting to the EC coupling machinery and CaV1.1 functional expression, while the low-affinity SH3s/II-III loop interaction merely enhances EC coupling.

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# Regulation of STING trafficking from the ER

STING is an endoplasmic reticulum (ER)-associated transmembrane protein, that when activated, mediates production of type I interferon and pro-inflammatory cytokines. It is currently assumed that STING must leave the ER in order to be activate. This assumption is supported by the observation that constitutively active STING mutant (284M) resides permanently in the Golgi. The aim of the project is to identify the core ER export machinery that regulates STING trafficking out of the ER and to test the conjuncture that exiting the ER is a prerequisite for activation. Sec24 is the subunit of the COPII coat that interacts with the cargo protein and mammals have four isoforms. We performed knockdown experiments for all Sec24 in cells expressing the Golgi localized constitutively active 284M STING mutant. Our results suggest that Sec24A and Sec24C might play a significant role in STING selection into COPII vesicles. Although silencing of Sec24A&C resulted in the redistribution of 284M-STING to the ER, the downstream signaling of this mutant was not affected. To identify further regulators of STING trafficking, we investigated by mass spectrometry partners interacting with STING in the ER and the Golgi. Surprisingly, the most consistent partner interacting with STING was Exportin-1, which has not been previously implicated in ER-Golgi trafficking. We validated the interaction using co-immunoprecipitation and showed that inhibiting XPO1 has a negative effect on ER-to-Golgi trafficking. Overall, these findings might unfold additional information about the molecular mechanism and cellular function underlying STING activation and signaling.

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# Identifying the STAC3/CaV1.1 interactions responsible for CaV1.1 expression in skeletal muscle

The adaptor protein STAC3 was shown to be crucial for the membrane and functional expression of the voltage sensor of EC coupling CaV1.1. Two distinct STAC3/CaV1.1 interactions have been identified: one between the II-III loop of CaV1.1 and the SH3-1 domain of STAC3 and a second between the proximal C-terminus of CaV1.1 channels and the linker region of STAC3. Here, we investigated whether a single interaction is responsible for the CaV1.1 membrane and functional expression or if both synergistically contribute. To this end, we generated two STAC3 fragments, STAC3-NT, consisting of the N-terminal part, including the c1 domain and the linker region; and STAC3-CT, consisting of only the SH3 domains. Expression of either one of these fragments, in a newly-generated STAC3 KO skeletal muscle cell line promoted CaV1.1 membrane expression, but at a reduced level compared to full length STAC3, suggesting that both interactions contribute to CaV1.1 membrane expression. However, only expression of STAC3-NT supported CaV1.1 functional expression, indicating that the STAC3 interaction with the proximal C-terminus is solely responsible for CaV1.1 function in muscle cells. In addition, we analysed the ability of each STAC3 fragment to colocalize with CaV1.1 in the triads of myotubes. While the STAC3-NT fragment is still able to incorporate in CaV1.1 clusters, albeit with a 50% reduction in the Pearson´s coefficient compared to full length STAC3, the STAC3-CT fragment remained diffusely localized in the cytoplasm. Experiments are planned to establish the importance of each fragment for EC coupling.

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

The exquisite sensitivity of mammalian hearing relies on the electromotility of the cochlear outer hair cells (OHCs), which enables active mechanical sound amplification. 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 a 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 seemed to decrease 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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# Biophysical Essentials – An Open Source Software Framework for Conserved and Advanced Analysis of Patch-Clamp Recordings

Patch-Clamp recordings allow for in depth electrophysiological characterization of single cells, their general biophysical properties as well as characteristics of voltage- and ligand-gated ionic currents. Different acquisition modes, such as whole-cell patch-clamp, in the current or voltage clamp mode as well as capacitance measurements or single channel recordings from cultured cells as well as acute slices are routinely performed for these purposes. Nevertheless, multipurpose transparent and adaptable software tools to perform reproducible state-of-the-art analysis of multiple experiment types and to account for larger sets of experimental data are currently unavailable.

We therefore developed Biophysical Essentials (BPE), an open source software platform for reliable analysis of recordings in the different modes. BPE was optimized to provide a complete workflow from data acquisition, preprocessing, visualization and normalization of single recordings up to stacked calculations and statistics of multiple experiments. Additionally, photo documentation and object recognition of the cells during the experiment was included. With more than 60 000 lines of code, BPE was used to integrate various data analysis functions considering different file formats from different recordings systems, such as Heka and Axon. Therefore, for data storage we chose an in-process SQL OLAP DuckDB database, which was also conceived to serve as a central storage point for patch clamp recordings from different experiments and experimenters within a research group.

Further research will aim to establish machine and deep learning models on the collected data to strengthen the differentiation between biological variety and functional alteration.

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# Molecular mechanisms and physiological importance of a novel interaction between Kv channels across families

Background: Voltage-gated K+ channels (Kv) allow K+ flux over the plasma membrane and are formed by a tetramer of four related family members. Four of the Kv families (i.e., Kv5, Kv6, Kv8 and Kv9), termed silent Kv (KvS), are electrically silent when assembled as homomers. Interestingly, KvS co-assemble with Kv2 subunits, leading to properties different from homomeric Kv2 channels. This constitutes the only yet known example for heteromerization of subunits across Kv families. As Kv7 subunits exhibit a higher sequence homology to KvS than to all other families, an interaction of Kv7 with KvS is probable.

Hypothesis: Kv7 and KvS subunits co-assemble into functional channels (i.e., with all subunits contributing to the channel's pore), leading to modified channel properties in different excitable cells types.

Methods: Using a combination of molecular, biological, biochemical, and electrophysiological techniques, we will (1) determine (co-)expression of different Kv and KvS subunits in different tissues using RT-qPCR and RNAscope ISH, (2) investigate excitability and network differences in KvS knockout mice using single-cell patch-clamp and multielectrode-array recordings, and (3) probe physiological relevance of KvS subunits by applying sensory and cognitive behavioral tests in KvS knockout mice.

Discussion: This project will deliver significant insights into the mechanisms underlying a novel interaction between members of different Kv channel families, challenging a central theorem on the formation of functional Kv channels. It will help to develop new treatment options against KvS-dependent pathologies by evaluating the therapeutic potential for repurposing specific channel agonists or gene-therapeutic strategies.

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# INCREASED PANCREATIC β-CELL ELECTRICAL ACTIVITY REDUCES DIABETES SUSCEPTIBILITY IN FEMALE MICE

In humans, the incidence of Type 2 Diabetes Mellitus (T2DM) in premenopausal women is higher compared to men, phenotype also recapitulated by many rodent models. The

T2DM etiology involves impaired or total lack of insulin release from pancreatic β-cells. High voltage-gated Ca2+ channels (HVCCs) are multi-subunit protein complexes responsible

for β-cell electrical activity and insulin vesicle exocytosis. Previously, we showed that genetic deletion of α2δ-1 HVCC auxiliary subunit equally reduced Ca2+ influx by 60% in β-cells of both males and females but caused diabetes only in males because female pancreatic islets released significantly more insulin (Mastrolia et al., 2017). Functional characterization shows that both male and female β-cells display a similar glucose-induced increase in the membrane potential under all stimulatory glucose levelsconsistent with similar HVCC Ca2+ influx and KATP, Kv, BK and SK K+ effluxes. The glucose induced intracellular Ca2+ transients are also similar between sexes consistent with identical Ca2+-induced Ca2+-release and intracellular store Ca2+ content. However, females have a higher percentage of β-cells that respond to glucose stimulation with increased electrical activity. Additionally, female β-cells displayed a higher frequency of glucose-induced AP-trains (@10 mM, female β-cells 2.2±0.5 AP-trains/min, males 0.41±0.1, p

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# Damage and repair of membrane lipids in mitochondrial fatty acid β-oxidation disorders

The constant repair of damaged lipids in biological membranes is a vital process for cellular integrity and function. Many metabolic diseases result in pathological alterations of the membrane lipid composition contributing substantially to the patients' symptoms. This also holds true for different inborn errors of mitochondrial membrane lipid metabolism, like very long-chain acyl-CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (VLCADD and LCHADD). Some studies report a reconfiguration of lipids in these diseases and membrane damage is still largely uncharacterized and a mechanistic link to the functional changes is missing. The detection and quantification of damaged membrane lipids from different sources presents a challenge due to the inherent complexities involved. Especially the amount of distinct lipid species creates a major hurdle when aiming to identify and quantify them with the help of liquid chromatography - tandem mass spectrometry. Those challenges of lipid analysis are even amplified for oxidized lipid species that are typically low abundant due to their very short half-life in living cells. We will characterize membrane lipid damage in inherited mitochondrial diseases using two-dimensional liquid chromatography-mass spectrometry in a lipid-class specific manner. With this methodology, we utilize the merits of hydrophilic interaction liquid chromatography in a fractionation approach to reduce sample complexity in the first dimension, followed by reversed-phase liquid chromatography for a detailed characterization of the membrane lipid state in the second dimension. Combined with subcellular fractionations, this makes it possible to specifically investigate disease-related damage to mitochondrial membrane lipids in VLCADD and LCHADD patient derived fibroblasts.

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# iPS-based disease modeling of Mucopolysaccharidosis IIIB using patient-specific neurons

Mucopolysaccharydosis IIIB (MPS IIIB) is a degenerative, pediatric lysosomal storage disease characterized by neurological symptoms including visual impairment, progressive dementia and motor function disorders. MPS IIIB is an autosomal recessive disease caused by deficiency in the NAGLU gene resulting in lysosomal accumulation of glycosaminoglycans. Currently, there is no effective treatment or cure for this disease. Therefore, patient-specific disease models are important to enhance further understanding of disease mechanisms and the development of therapy approaches.

Thus, we aim to establish a cell-based disease model for MPS IIIB using NPCs, neurons and RPE derived from patient iPSCs of a homozygous MPS IIIB donor (Pro358Leu). First, iPSCs were characterized by flow cytometry and immunocytochemistry. We found high expression of pluripotency associated markers SOX2, OCT4, SSEA4, and TRA1-60 and demonstrated the differentiation into the three germ layers. Subsequently, iPSCs were differentiated into NPCs and cortical neurons. Differentiated NPCs and neurons were assessed by immunocytochemistry using TUJ1, Nestin, SOX1, and FOXG1, indicating forebrain-type identity. Finally, we found that patient-derived fibroblasts, iPSCs, NPCs, and neurons exhibits disease-specific molecular phenotypes, such as increase in lysosomes examined by LysoTracker and Lamp1/2 staining. In addition, we differentiated RPE cells to assess the disease phenotype.

Future work will include the generation of isogenic lines using CRISPR/Cas9 as well as further analysis by electrophysiology and bulk RNA sequencing to better understand the effects of NAGLU-deficiency MPS IIIB iPSC-based cellular models. Moreover, these in vitro models can be used to test the therapeutic effects of enzyme replacement therapy.

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# Targeting the orphan nuclear receptor NR2F6 in T cells primes tumors for immune checkpoint therapy

Nuclear receptor subfamily 2 group F member 6 (NR2F6) is an orphan nuclear receptor and has been proposed as an alternative cancer immune checkpoint in the effector T cell compartment (Hermann-Kleiter et al., 2015, Klepsch et al., 2018). In previous studies, we could show that germline ablation of NR2F6 leads to a hyperactive phenotype in-vitro and superior tumor rejection in-vivo. To assess its suitability for immune checkpoint therapy (ICT), it requires acute T-cell restricted depletion. Here, we established a method for efficient CRISPR/Cas9-mediated knock-out in primary murine T cells, isolated from Cas9 transgenic mice, with synthetically synthesized Nr2f6 single guide RNA (sgRNA).

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# Investigating potential functions of nuclear encoded intergenic mitochondrial tRNA lookalikes

The presence of mitochondrial DNA sequences in the nuclear genome (numtDNA) has previously been determined. Among numtDNA sequences, our research revealed more than 700 homologs of mitochondrial tRNA genes, designated as mitochondrial tRNA lookalikes (MTLs), in the human genome. MTLs exhibit sequence and structural conservation, indicating an acquired function of MTLs within cells. We recently showed that intronic MTLs, designated as nuclear intronic mitochondrial tRNAs (nimtRNAs), act as splicing regulatory elements (SREs). The function of MTLs located in intergenic regions, designated as intergenic nuclear mitochondrial tRNAs (inter-nmtRNAs), remains elusive.

By analyzing RNAseq data of established cell lines, (focusing on unique mapping reads with a cutoff of 200 nucleotides) we found initial evidence the expression of several inter-nmtRNAs. To determine the function of inter-nmtRNAs we performed transient transfections of human and murine cells with plasmid-encoded pol III promoter-driven inter-nmtRNAs. Interestingly, several inter-nmtRNAs were shown to be partially processed resulting in a tRNA transcript containing the 5'-leader sequence, while others appear as fully processed transcripts.

By investigating the subcellular localization of inter-nmtRNAs, we aim to elucidate their biological relevance. Further research will also investigate whether mutations within nuclear encoded nimtRNAs or inter-nmtRNAs might be involved in the etiology of human diseases, since a number of mitochondrial mtRNAs cause various muscle and brain diseases in humans. Hence, our approach will not only identify novel functional ncRNA genes in the human genome but will also generate important knowledge on evolution of the human and other eukaryal genomes.

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# Unequivocal Mapping of Molecular Ether Lipid Species by LC–MS/MS in Plasmalogen-Deficient Mice

This study investigated the use of LC–MS/MS to unambiguously map molecular ether lipid species in plasmalogen-deficient mice. To this end, a targeted lipidomic method was developed to quantify and identify plasmalogen-deficient mice by the relative abundance of molecular ether lipids. The results showed that LC–MS/MS enabled the unequivocal identification of molecular ether lipid species in plasmalogen-deficient mice compared to wild-type mice. This method provides a useful tool for the investigation of molecular ether lipid species in plasmalogen-deficient mice, and could be extended to other types of lipid-mediated diseases.

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# Generation of monoclonal antibodies targeting murine C5aR2

Background

C5aR2 is an enigmatic receptor binding C5a and C5adesArg. It serves as a decoy receptor for C5aR1, acts synergistically with C5aR1, or exerts functions independent of C5aR1. Previously, we have generated tdTomato-C5aR2fl/fl reporter mice to track and cell-specifically delete C5aR2 (Karsten et-al. J.Immunol-2017). However, mAbs that specifically bind C5aR2 are still lacking. Here, we aim to close this gap. We designed an immunization strategy to generate mAbs targeting C5aR2.

Methods

Three C57BL/6 C5ar2-/- mice were immunized with HEK293-transfected cells expressing murine (m)C5aR2. The antibody response was tested with BSA-coupled peptides from the extracellular domains of mC5aR2 by ELISA. Further, immune sera were tested for mC5aR2 reactivity with Ly6G+ primary bone-marrow cells (pBMCs) from wildtype mice by flow cytometry.

Results

We observed a strong serum response towards the mC5aR2-derived N-terminal peptide (Np) and Ly6G+ pBMCs. After fusion, we identified two priority groups of clones that: (i) reacted strongly with Ly6G+ pPBMCs and Np; (ii) reacted strongly with Ly6G+ pPBMCs but not Np (Priority group I); or (iii) reacted strongly with Np but only moderately with Ly6G+ pBMCs (Priority group II). Work is in progress to characterize these clones.

Conclusions

Our results identify the N-Terminus as immunodominant region of mC5aR2 which is also accessible in the naïve mC5aR2 as evidenced by the strong reactivity of some antibodies with Ly6G+ pBMCs. Additionally, epitopes outside the N-terminus contributed to immune response resulting in mAbs that also reacted with naïve mC5aR2. Future experiments will determine their viability for tracking and functional inhibition of mC5aR2.

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# The role of metabolic iron changes in macrophages upon SARS-CoV-2 infections

We examined the impact of SARS-CoV-2 infections on metabolic changes in iron within macrophages. Iron is a crucial nutrient for the immune system, and dysfunction of iron metabolism can lead to severe illnesses such as macrophage activation syndrome, a severe inflammatory systemic abnormality with lethal potential. SARS-CoV-2 affects iron metabolism and has an impact on the severity of the illness in COVID-19 patients. To address this question, we employed methods to investigate the impact on iron metabolism of primary macrophages isolated from healthy donors. Furthermore, we aimed to investigate the influence of COVID-19 medication on iron metabolism. Therefore, we examined the effect of Sotrovimab, Cilgavimab, and Tixagevimab, monoclonal antibodies that are utilized for the treatment of severe COVID-19 infections. The two variants of concern Delta (B1.617.2) and BA.4/5 isolated from patient samples were compared. We conducted gene expression analyses to identify changes in genes associated with iron metabolism. We used plaque assays and virus PCR to quantify the neutralization by macrophages and the viral load in upon phagocytosis. Our results reveal that SARS-CoV-2 infections can cause changes in iron metabolism within macrophages. We found that the gene expression of key regulators in Delta infections was stronger compared to BA 4/5. However, the ability of macrophages to neutralize and phagocytose the virus was similar in both cases. We also found that treatment of the virus with monoclonal antibodies had no impact on the macrophages. Our findings indicate that SARS-CoV-2 infections can lead to alterations in iron metabolism within macrophages.

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# Intrinsic amino acid substitutions in the SDM-F5 paralogue of Mucor circinelloides are a major reason in short-tailed azole resistance

Mucor circinelloides is a major causative agent of mucormycosis. Intrinsic resistance to short-tailed azoles (e.g.voriconazole) limits treatment options to amphotericin B, posaconazole, and isavuconazole. The amino acid substitutions (AA) Y129F, V293A within the ligand-binding-pocket of Mucor circinelloides (Mc) sterol-14-α-demethylase (SDM) paralog F5 are suspected of causing this resistance. Subsequently, we aim to prove the impact of the AA changes on drug resistance, using a heterologous Saccharomyces cerevisiae model.

McSDM paralogues (SDM-F1, SDM-F5) were overexpressed with/without their cognate cytochrome-P450-reductase (CPR) at PDR5 and PDR15 loci in a hypersensitive model. AA changes were reverted in the SDM-F5 gene (F129Y, A293V, F129Y & A293V) to study the effect on drug binding. The susceptibility profiles were assessed by EUCAST. Strains were characterized using growth kinetics, SDS-PAGE, and western blots. The ergosterol pathway response to azole exposure was quantified using GC-MS.

Recombinant protein expression did not impact growth rates compared to the parental strain and gave comparable (SDM-F5: 98%) or lower levels (SDM-F1: 74%) than the control overexpressed ScERG11. According to resistance profiles, SDM-F1 and SDM-F5 mutants presented susceptible phenotypes for voriconazole (0.016-0.06 mg/L) and posaconazole (0.008-0.125 mg/L). SDM-F5 showed comparable MICs for posaconazole (0.06 mg/L) but significantly higher values for voriconazole (4 mg/L). According ergosterol content, voriconazole treatment was more effective on SDM-F1.

The heterologous McSDM isoforms were functional and advantageous in the inhibition of azole efficacy on ergosterol biosynthesis, especially for SDM-F5. MIC profiles support these findings. Summarizing, the heterologous model identifies SDM-F5 as primary reason for intrinsic short-tailed azole in M. circinelloides.

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# Investigation of systemic and mucosal immunity of boostered and/or Omicron BA.1/BA.2 convalescent against BA.4/5

Background: The SARS-CoV-2 pandemic has been extremely challenging for the global population by rapid transmission and several mutations as found in SARS-CoV-2 Omicron BA.4/5 causing recurring break-through infections. Since numerous studies focusing on systemic immune response, we here conducted combined investigations on systemic and mucosal immunity, which has been barely evaluated yet.

Methods: Within this cohort study we determined humoral immune responses and virus neutralization with serum and saliva samples of 92 vaccinated and/or BA.1/BA.2-convalescent individuals. We therefore evaluated SARS-CoV-2 Spike-specific IgG/IgA titers as well as neutralizing activity against replication competent SARS-CoV-2 wildtype and BA.4/5 variant.

Results: Although high serum IgG/IgA and neutralization titers against SARS-CoV-2 wildtype were observed in particular for vaccinated and BA.1/2 convalescent groups. No sufficient neutralization against BA.4/5 was detected for vaccinated or BA.1 convalescent individuals. In contrast, vaccinated and BA.2-convalescent patients demonstrated the best serum neutralization against BA.4/5. Despite comparable salivary IgA levels for individuals with previous Omicron infection, vaccinated and BA.2-convalescent group demonstrated highest salivary neutralization levels against wildtype virus. However, this advantageous neutralizing effect was not observed against BA.4/5.

Conclusions: Overall, we could demonstrate that for vaccinated individuals in particular in combination with an infection strong systemic humoral immune response could be induced, indicating an effective prevention of severe or critical COVID-19. However, detected levels of salivary neutralization might not be sufficient enough to prevent infection. These findings emphasize a change of present vaccination strategies to oral or intranasal approaches for a higher induction of mucosal immunity.

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# Establishing a workflow to compare T cell receptor sequences in blood and tumor

In recent years, T cell receptor (TCR) sequencing has established its usefulness in immuno-monitoring of novel immunotherapies. A diverse TCR repertoire has been shown to serve as prognostic biomarker for several cancer types and changes in clonality and diversity of TCR show prognostic biomarker potential for immunotherapies. So far, TCR sequencing in clinical trials is most commonly applied as bulk sequencing of tumor tissue (bulk TCRseq). However, state-of-the art single cell RNA and TCR sequencing (sc TCRseq) offers a much deeper immune repertoire analysis with a higher level of complexity, as it allows exact pairing of the TCR alpha and beta chains and provides additional information such as protein and gene expression of single cells. The aim of this study is to find out whether the TCR sequences derived from tumor and blood using sc RNA and TCRseq (BD Rhapsody system) is compatible with the bulk sequence data from a small tumor biopsy. Therefore, we collected biopsies from head and neck squamous cell carcinoma (HNCC) patients and analyzed the overlap of TCR sequences among different samples and different sequencing approaches. First data indicate that we have an overlap of TCR sequences comparing tumor (sc TCRseq) and tumor (bulk TCRseq) as well as blood (sc TCRseq) and tumor (bulk TCRseq). Taken together, we show that sc TCR sequencing in tumor and blood yields TCR sequences that overlap in part with bulk sequencing while providing a wealth of additional information.

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# Development of a VSV-based therapeutic HPV16 vaccine

High-risk human papillomavirus infections (HPV) can induce various oral/anogenital cancers, being responsible for >95% of cervix carcinomas. Approved vaccines act in a preventive, but not in a therapeutic manner. Therefore, we aim to develop a therapeutic vaccine against high-risk HPV type 16 based on the chimeric vector VSV-GP that elicits a potent cytotoxic CD8 T cell response targeting infected/cancerous cells. Since VSV-GP replication results in a transcriptional gradient of viral genes, we inserted a HPV16-derived antigen cassette at first (HPVp1) or fifth (HPVp5) position within the viral genome and characterized both constructs. For HPVp1, we observed increased antigen expression in vitro and slight viral attenuation compared to HPVp5. Upon immunization of mice the HPV-specific T cell response was stronger for HPVp1, while the CTL response against the vector backbone was weaker, being potentially favorable for repeated administrations. Preventively administered, we observed superior protection from tumor challenge for HPVp1. Therapeutically, tumors regressed using each vaccine, but relapsed in most animals. We demonstrated that neither the loss of E7 copies nor mutations of the immunodominant E7 epitope in TC-1 cells isolated from relapsing tumors are reasons for the recurrence. Overall, inserting the antigen cassette at first position was superior to fifth, however low viral titers caused by the attenuation could be limiting for production of high titer stocks for in vivo studies. Therefore, we currently analyze additional vector configurations to optimize replication fitness versus antigen expression. We demonstrated in our experiments that VSV-GP is a promising candidate as therapeutic vaccine for HPV-infections.

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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. Following activation, they 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 a prominent early death of dopaminergic neurons in substantia nigra pars compacta. Available therapies for PD acting on DRs include the D2-like selective agonists ropinirole and pramipexole. The structural determinants which are responsible for this D2-like DR selectivity have not yet been investigated in detail. 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 investigate DRs signaling cascades through 16 Galpha-proteins (collectively named the transducerome) using bioluminescence resonance energy transfer (BRET) biosensors. Computational analyses will be performed to identify critical residues responsible for ligand selectivity. Subsequent site-directed mutagenesis allows us to experimentally evaluate the changes of binding properties between the different selected agonists and between the mutated and wild-type receptors.

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# Dual activation of mu and delta opioid receptors by new oxymorphone analogues produces effective antinociception without the risks of antinociceptive tolerance and physical dependence in mice

Opioids are highly effective painkillers. The most clinically used opioid analgesics (morphine, oxymorphone, fentanyl) are agonists to the mu opioid receptor, also responsible for the development of severe side effects. Currently, intensive research focuses on innovative strategies to mitigate deleterious side effects such as analgesic tolerance, physical dependence and addictive potential. In this study, we report on in vitro and in vivo profiles of two new oxymorphone analogues that emerge as bifunctional mu/delta opioid receptor agonists. Radioligand binding studies showed the new derivatives to display very high affinities to the mu, delta and kappa opioid receptors in rodent brain. In the [35S]GTPγS functional assay, they were very potent and full agonists to the human mu and delta opioid receptors and partial agonists to the human kappa opioid receptor. In mice, both oxymorphone analogues were highly effective as antinociceptives in models of acute thermal nociception (tail-flick test) and inflammatory pain (the formalin test) after subcutaneous (s.c.) administration. Their antinociceptive effect was significantly reversed by selective mu and delta, but not by the kappa opioid antagonist, demonstrating the involvement of mu and delta opioid receptors to the antinociceptive action. Chronic s.c. treatment of mice did not lead to the development of antinociceptive tolerance. The potential for physical dependence was determined using the naloxone-precipitated withdrawal, with none of the new oxymorphone analogues inducing withdrawal syndrome. In conclusion, dual activation of the MOR and DOR by the new oxymorphone analogues produces effective antinociceptive effects without the opioid-mediated risks of antinociceptive tolerance and physical dependence.

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# Computational prediction of activating kinase mutations

Aberrant kinase signalling is an important cause of cancer and these proteins are thus a major drug target in cancer therapy. Kinases undergo significant conformational changes during their switch from inactive to active states. Amino acid mutations, such as the EGFR L858R mutation, shift the equilibrium to an active state and are oncogenic. Likewise, mutations which shift the equilibrium from a drug-bound - and thus inhibited – kinase conformation towards an active state confer resistance to drug treatment. However, such mutations are often only identified and characterized once they have emerged in the clinic.

We have developed an in-silico workflow to identify these activating/resistance amino acid mutations, which is based on calculating the stability of different protein conformations. In addition, we use mutational signatures to predict which mutations are most likely to occur in patients.

We have retrospectively validated our workflow using mutation data from patient samples and confirmed that a large number of mutations occurring in patients were found by our workflow. In addition, newly identified and currently uncharacterized patient mutations will be tested in-vitro to further validate our workflow.

This allows us to identify so far uncharacterized oncogenic kinase mutations and, importantly, to design new anticancer drugs in a prospective way.

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# Antinociceptive efficacy of selective κappa-opioid receptor agonists HS665 and HS666 in chronic inflammatory pain without inducing anxiety-like behavior in mice

Effective chronic pain treatment remains an unmet medical need. Targeting the kappa-opioid receptor (KOR) represents a promising strategy, as the KOR does not produce dependence, euphoria or leads to respiratory suppression. However, it induces dysphoria, sedation, anxiety and psychotomimesis. Our group has developed two diphenethylamines, HS665 and HS666, as G protein-biased highly selective KOR agonists. HS665 (full agonist) and HS666 (partial agonist) displayed potent antinociceptive effects in mouse models of acute and visceral pain, with reduced KOR liabilities (aversion and sedation/motor impairment). In this study, we report on the antinociceptive activity of HS665 and HS666 in a mouse model of chronic inflammatory pain as well as their liabilities on anxiety-like behavior and spontaneous locomotor activity in mice after subcutaneous (s.c.) administration. Additionally, in vitro functional KOR activity in mouse striatum was investigated. Both HS665 and HS666 efficiently reversed thermal hyperalgesia (the Hargreaves test) in mice with Complete Freund's Adjuvant-induced paw inflammation in a time- and dose-dependent manner, with a long duration of action. The antihyperalgesic effect was reversed by the selective KOR antagonist nor-binaltorphimine demonstrating a KOR-dependent mechanism of action. Particularly, both compounds showed no anxiogenic behavior and no alteration in spontaneous locomotion in the elevated-plus maze test. In the [35S]GTPγS binding assay, HS665 and HS666 stimulated G protein signaling in striatal membranes from wild-type mice, but not in striatum of KOR-knock-out mice. In summary, we show that HS665 and HS666 are effective antinociceptives in experimental chronic inflammatory pain with a favorable benefit/side effect ratio.

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# Investigating and modulating κ-Opioid Receptor signaling.

The κ-Oppioid receptor (OPRK) belongs to the family of G-protein coupled receptors (GPCRs). OPRK is mainly localized at axon terminals in different brain regions and is involved in e.g., nociception, consciousness, or mood.

GPCRs mediate downstream signaling via G proteins (transducers), which consist of one of 16 different Gα subunits with specific Gβ and Gγ proteins. Depending on the Gα subtype (i.e. Gs, Gi/o, Gq/11 and G12/13), GPCR activation either leads to cell inhibition or excitation.

In this project we aim to elucidate the coupling preference of OPRK and investigate the molecular determinants involved in OPRK Gα subunit selectivity.

We employed the recently published TRUPATH assay, based on Bioluminiscence Resonance Energy Transfer (BRET), to analyse the OPRK transducerome. Subsequent computational analyses identified important residues responsible for OPRK Gα selectivity and suggested mutations modifying this preference. Quantification of the modified receptors confirmed two amino acid (aa) positions where mutations prevent OPRK Gα12 signaling and also diminish binding to Gα15. Moreover, one of them interestingly even allows GαS interaction, in stark contrast to OPRK wildtype. Two other aa positions which diminish Gα12 and eliminate Gα15 coupling were also identified.

A detailed understanding of critical aa will allow us to further decipher the OPRK – G-protein interactions and may even help to predict coupling of human orphan GPCRs, involved in different diseases.

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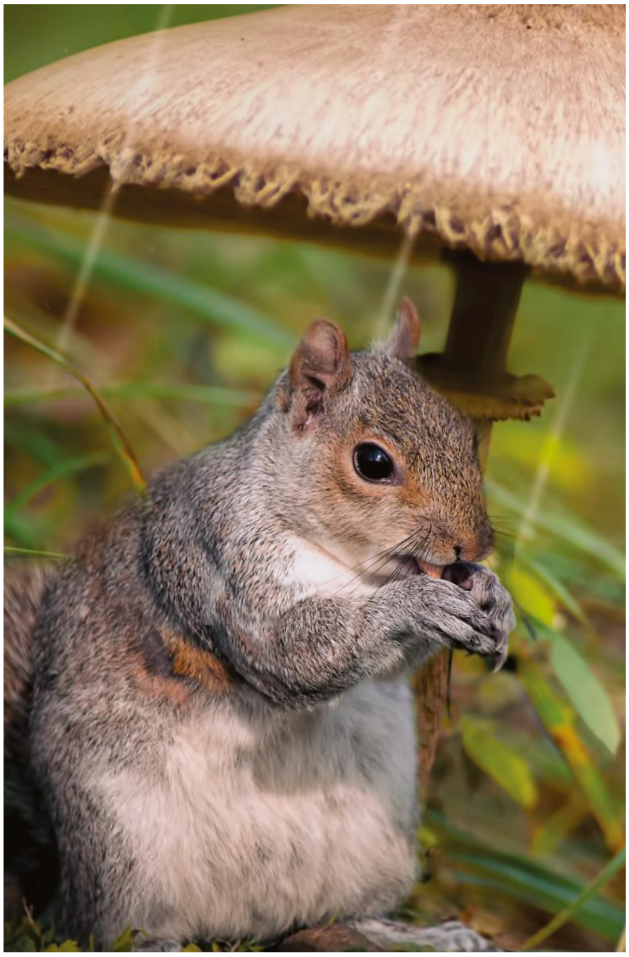

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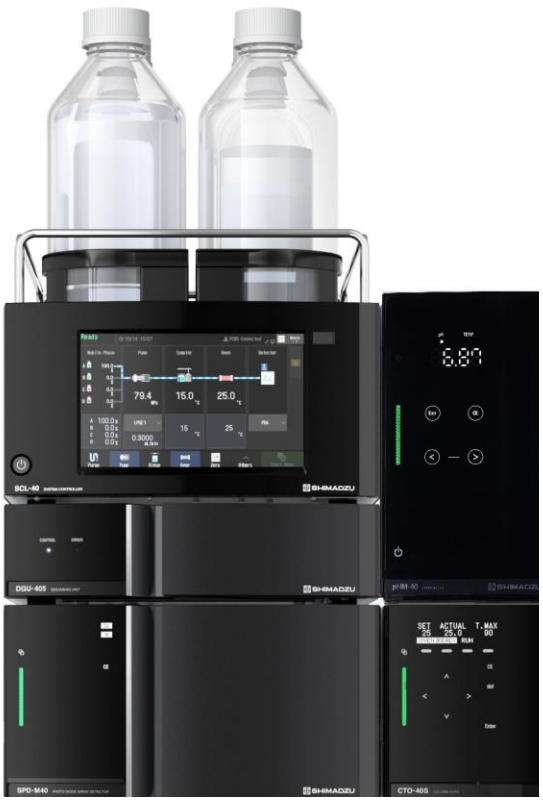

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# Are chlorido(diarylsalene)iron(III) complexes potent anti-breast cancer drugs due to induction of ferroptosis?

Cisplatin-based chemotherapy is widely used for the treatment of various carcinomas, even though primary or acquired resistance or side effects frequently occur. To circumvent these disadvantages complexes with other metals than platin such as iron and cobalt are currently under investigation as potential anticancer drugs.

In this project we analyzed the antitumor activity of 12 chlorido[diarylsalen]iron(III)complexes differing in the configuration of the diaryl part (d,l, meso) and the position of fluorine or a methoxy group on the respective diaryl residue on breast cancer (MDA-MB-231) and acute myeloid leukemia (HL-60) cells. Metabolic activity was determined by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the mode of cell death was analyzed by inhibitor experiments using ferrostatin-1 and necrostatin-1.

The complexes in the d,l configurations inhibited antimetabolic activity at lower concentrations than the respective meso complexes and the substitution with fluorine was higher effective than that with a methoxy group. The most potent compound was d,l 2F, which reduced the metabolic activity of MDA-MB-231 cells to 20% at 100 nM. Interestingly, the compounds exclusively induced ferroptosis of MDA-MB-231 cells, whereas ferroptosis and necroptosis were observed for HL-60 cells. These findings suggest that ferroptosis is an interesting approach in the treatment of breast cancer and chlorido(diarylsalene)iron(III) complexes specifically target this mode of death.

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# VIP/VPAC1/2 receptor signaling in the central amygdala regulates stress and anxiety responses in mice

Vasoactive intestinal polypeptide (VIP) is a 28-amino-acid long peptide expressed in the peripheral and central nervous system (CNS). In particular, within the CNS, VIP and its cognate VPAC receptors are found in many areas implicated in stress and anxiety regulation such as the amygdala complex. However, the exact role of VIP in this function is not fully understood. Consequently, our main focus was to investigate the effects of intracerebral VIP administration in areas with high VIP receptor expression such as the central amygdala (CeA) on anxiety- and stress-related behaviors of rodents. Thus, C57Bl6/J male mice were administered either with VIP, VIP(6-28) (a VIPAC1 and 2 receptor antagonist) or vehicle (artificial cerebrospinal fluid) and tested in different behavioral tests for the assessment of stress and anxiety. We found that VIP-injected animals show a reduction of time spent in aversive zones of the elevated plus-maze, a reduction of the self-care in the splash test and a decrease in active coping or escape-oriented behavior in the forced swim test, compared to vehicle-injected controls. Overall these findings suggest an anxiogenic-like effect of VIP after acute administration into the CeA. Conversely, administration of VIP(6-28) into the CeA increased the time and frequency of entries into the aversive area of the light-dark box test and augmented active coping or escape-oriented behavior in the forced swim test suggesting an anxiolytic-like effect and improved stress coping. Taken together, these data highlight the CeA as a crucial site for VIP signaling to mediate stress and anxiety functions.

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# Discovery of anticancer drugs: Can iron in chelated form induce ferroptosis?

Ferroptosis is an iron dependent form of cell death. However, it has not yet been shown, if it is also induced by chelated iron. Therefore, we investigated the impact of an iron (III) bis Schiff base complex on various parameters of ferroptosis.

Biological activity of the complex was determined on A2780cis, a cisplatin-resistant ovarian cancer cell line and the nonmalignant fibroblastic cell line SV80. Proliferation and metabolic activity were determined by the ability of the compound to inhibit [3H]-thymidine incorporation and a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively. Ferroptosis was analyzed by inhibitor experiments, lipid and mitochondrial reactive oxygen species (ROS) induction as well as analysis of the mitochondrial membrane potential and the redox potential.

The anticancer effect of the iron (III) bis Schiff base complex was proven by the dose-dependent inhibition of the proliferation and the metabolic activity of A2780cis cells and lacking activity on SV80 cells at lower concentrations, whereby the complex was 10-fold more effective than cisplatin. The binding of the compound to apotransferrin indicated a carrier-mediated transport. As this iron (III) bis Schiff base complex is redox-active it can generally be involved in the generation of ROS. Indeed, lipid ROS was detected 8 and 12 hours after compound addition and after 24 hours a reduced mitochondrial membrane potential and induction of mitochondrial ROS were observed. The cell-death inducing effect was abrogated by concomitant addition of the ferroptosis inhibitor ferrostatin-1.

These findings indicate that ferroptosis can also be induced by chelated iron.

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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 underpinning the promotion or inhibition of dendritic growth. However, how L-VGCCs implement signaling specificity is unknown. We hypothesize that modulation of ICaL is critical to trigger distinct processes controlling dendritic growth. Murine hippocampal neurons were transfected with soluble eGFP (DIV4), treated for 48h with L-VGCC agonists and antagonists (DIV5-7), and processed for Sholl-analysis (DIV7). We found that dihydropyridine blockers suppressed dendritic growth, but this result was abolished in neurons overexpressing a dihydropyridine-insensitive CaV1.2 mutant. Thus, ICaL through the CaV1.2 isoform is crucial for dendritic development. Channel agonists BayK-8644 and FPL 64176 both increased ICaL density. FPL 64176 but not BayK-8644 slowed ICaL inactivation. Intriguingly, FPL 64176 enhanced dendritic growth while BayK-8644 had no effect. Consistent with the notion that CamKII signaling restricts dendritic development, immunostaining experiments showed that Bay-K 8644 boosted activated CamKII levels (pCamKII), unlike FPL 64176. To test whether slower channel inactivation is involved in dendritic growth promotion we overexpressed STAC2-HA, which suppresses calcium channel inactivation while increasing ICaL density. Under these experimental conditions, dendritic growth was enhanced while maintaining basal levels of pCamKII. Furthermore, FPL 64176 but not Bay-K 8644 reduced membrane-expressed CaV1.2, suggesting that fewer available channels may constrain pCamKII and facilitate dendritic growth. Our data provide evidence that calcium entry through CaV1.2 is necessary for controlling dendritic growth and that regulation of L-VGCC kinetics may be essential for modulating CaV1.2 membrane levels to restrict CaMKII signaling, and regulating dendritic tree development.

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# Exploring Mycosporine-like Amino Acids in Marine Algae with Feature-Based Molecular Networking (FBMN)

State-of-the-art separation (UHPLC) hyphenated to high-resolution tandem mass spectrometry (HRMS2) has become an irreplaceable tool in analytical chemistry, paving the way to sophisticated chemoinformatics. Raw MS data, subjected to pretreatment and spectral organization, can subsequently be implemented in the Global Natural Products Social (GNPS) workflow, whereby a FBMN is created and visualized in the software Cytoscape. By matching the obtained spectra against open source and in-house libraries and adding further informational layers (UV-response, retention time, etc.), an informed network facilitates the characterization of known as well as novel compounds even at first glance. A set of 33 crude algal extracts was explored focusing on highly relevant bioactive metabolites, mycosporine-like amino acids (MAAs). As these compounds possess pronounced absorption in the UV-A and -B range, they represent very potent natural sunscreens, highlighting their potential application in cosmeceutical formulations. Via the development of an UHPLC-HRMS2 FBMN workflow both the dereplication of known MAAs, as well as the claim of novel structures were enabled, thereby also identifying promising targets for the subsequent isolation of unknown compounds. FBMN can also serve as a taxonomic tool: Specific clusters were found not only on the phylum level, which enabled easy differentiation of lichen and green algae, but also species-specific features were identified, e.g. as shown for different Bostrychia specimen. Our study outlines an intriguing approach for the comprehensive and comprehensible visualization of the entire metabolomic space that can be conveniently and successfully integrated in phytochemical research.

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# CHITOSAN-MALEIC ACID CONJUGATE AS POTENTIAL EXCIPIENT CANDIDATE FOR ORAL DRUG DELIVERY?

Aim: To develop and evaluate chitosan-maleic acid conjugate. Methods: Maleic anhydride was attached to chitosan backbone via amide bond formation resulting in chitosan-maleic acid. After characterization of the product via 1H-NMR (nuclear magnetic resonance), ATR-FTIR (infrared spectroscopy) and 2,4,6-Trinitrobenzenesulfonic acid (TNBS) assay, examination of mucoadhesion assessment was carried out. Results: The conjugate presented 44.91% modification and no toxicity could be observed after one day of incubation. Mucoadhesive properties exhibited 40.97-fold, 13.31-fold and 9.07-fold increase in elastic modulus, dynamic viscosity and viscous modulus, respectively. Moreover, detachment time was increased in 44.44-fold. Conclusions: Chitosan-maleic acid demonstrated enhanced in mucoadhesive properties resulting in biocompatibility. Therefore, potent candidates as polymeric excipients for oral drug delivery could be developed over corresponding chitosan.

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# Genetic deletion of Stac2 adaptor protein alters electrical activity of mouse chromaffin cells

Voltage-gated L-type Ca2+ channels (LTCCs) regulate action potential (AP) firing and catecholamine release in mouse chromaffin cells (MCCs). Src homology 3 and cysteine rich domain adaptor proteins have recently been identified as novel regulators of neuronal LTCCs expression and biophysical properties. Upon overexpression in cultured hippocampal neurons Stac2 abolished LTCC Ca2+ dependent inactivation via an allosteric inhibition of calmodulin binding. Additionally, in Drosophila, deletion of the homologous DStac gene resulted in deficient LTCC Ca2+ transients and reduced neuropeptide release. Here we investigated the effect of Stac2 genetic ablation on neuronal LTCC function and electrical activity in MCCs. Constitutive deletion of Stac2 does not affect the MCCs resting membrane potential or spontaneous firing frequency. However, the AP depolarization threshold was significantly reduced in Stac2-/- compared to WT (WT = -35.6 ± 1.2 mV, Stac2-/- = -40.6 ± 0.6 mV; ** $P < 0.01$). Additionally, step current injection elicited an electrical activity with higher initial AP firing frequency in Stac2-/- compared to WT that led to earlier depolarization block. Surprisingly, our data indicate that Stac2 deletion does not alter whole-cell Ca2+ current amplitude or inactivation kinetics but significantly shifts the voltage-dependence of activation to more hyperpolarized potentials (V0.5: WT = -5.95 ± 1.65 mV, Stac2-/- = -15.25 ± 2.05 mV; * $P < 0.05$). We are currently investigating possible effects on other ionic conductances governing MCC excitability and the role of Stac2 on catecholamine vesicle exocytosis. Support: TWF F.18863, LFU 2021-CHEM-8 to SMG; FWF P31434, P36053, DOC30-B30 to PT; P33776 to MC.

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# Biodegradable arginine based steroid-surfactants: Cationic green agents for hydrophobic ion-pairing

The aim of this study was to evaluate the safety and efficacy for hydrophobic ion-pairing of surfactants based on arginine (Arg). The prepared Arg-cholesteryl ester (ACE) and Arg-diosgenyl ester (ADE) were characterized regarding solubility, pKa, critical micellar concentration (CMC), biodegradability as well as membrane- and aquatic toxicity using DOTAP as reference. The ability for hydrophobic ion-pairing was evaluated and the lipophilicity of formed complexes was determined.

NMR, FT-IR and MS confirmed successful synthesis of Arg-surfactants. The slightly soluble single-charged Arg-surfactants (pH < pKa3 (ACE = 10.42 ± 0.52; ADE = 10.38 ± 0.27)) showed CMCs of 27.17 µM for ACE and 35.67 µM for ADE. CMCs of the sparingly soluble double-charged species (pH < pKa2 (ACE = 5.30 ± 0.20; ADE = 5.55 ± 0.06)) were determined at concentrations of ≥ 250 µM for ACE and ≥ 850 µM for ADE. The enzymatic- and environmental biodegradability was proven by an entire cleavage of Arg-surfactants within 24 h, whereas DOTAP remained stable. Arg-surfactants exhibited lower membrane- (> 2-fold) and aquatic toxicity (> 15-fold) than DOTAP. The complexes formed with Arg-surfactants and insulin showed higher lipophilicity than the DOTAP-complex. According to these results, Arg-surfactants might be a promising safe tool for the delivery of peptide drugs.

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# Design of nanostructured lipid carriers and solid lipid nanoparticles for enhanced cellular uptake

In this study PEG-free and zeta potential changing lipid-based nanocarriers providing enhanced cellular uptake were developed. Nanostructured lipid carriers (NLC), consisting of paraffin wax, caprylic/ capric triglyceride, cetyltrimethylammoniumchloride and either soy lecithin or polyglycerol-4 laurate and solid lipid nanoparticles (SLN) with the same composition but without the liquid lipid content were developed. All formulations exposed a positive surface charge and were then coated with the polyphosphate Graham's salt. Phosphate release from these formulations was evaluated by incubation with intestinal alkaline phosphatase as well as on a Caco-2 monolayer and zeta potentials were measured. Additionally, cellular uptake studies were performed.

Within 5 h, a remarkable amount of phosphate was released from all formulations incubated with intestinal alkaline phosphatase. Enzymatically induced phosphate release with intestinal alkaline phosphatase led to a zeta potential shift up to Δ 26 mV. Results of phosphate release and zeta potential change were confirmed on Caco-2 cells. Cellular uptake studies on Caco-2 cells showed an up to 5.6-times higher uptake compared to cells with inhibited phosphatase. According to these results, polyphosphate coating is a powerful tool to obtain lipid-based nanocarriers for enhanced cellular uptake.

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# Tales of tails: scale patterns in the Australian gecko genus Strophurus

The genus Strophurus comprises 22 Australian gecko species. This genus is very unique due to its exceptional defense mechanism, which consists of glands that are distributed over the whole length of the tail. These defensive glands release a sticky and smelly secretion by rupturing the skin at a defined position above the gland in between certain scales. These scales, which surround the rupturing position, show a clear difference in size and shape compared to the surrounding scales of the tail. In earlier studies, scale patterns of different parts of the animal body, like the snout, were used as characteristics for species differentiation and determination in geckos. The aim of the study was to see, if the scale pattern around the glandular rupture zones varies along the tail or between different individuals and if it is possible to distinguish species by these patterns. Therefore, we reconstructed and compared the "rupture zone scale pattern" of eight Strophurus species using dry, moulted skins. The comparison demonstrates that the scale pattern around the rupture zone is species specific. We illustrate, that it is possible to clearly identify the species by their unique scale pattern and that none of the investigated species share the same pattern. To further validate our results we want to include life observations and museum material. This work should facilitate the clear determination of Strophurus in museum collections as well as for private breeders.

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# Optimizing CRISPR/Cas9 approaches in the tunicate Ciona intestinalis

CRISPR/Cas9 is a powerful tool for genetic engineering and knockout approaches in living cells and organisms. Our model organism Ciona intestinalis belongs to the tunicates, closest sister group of vertebrates, has an invariant cell lineage, a compact genome and allows for electroporation mediated transgenesis to efficiently analyze cellular processes in vivo. However, due to a mosaic expression of electroporated constructs it is critical to optimize the introduced CRISPR tools. Here we present an enhanced CRISPR/Cas9 protocol for in silico, in vitro and in vivo applications.

In gRNA design, an alignment of target C. intestinalis sequences to the reference genome of C. robusta and prior sequencing of the genomic region of interest, allowed us to circumvent subspecies polymorphisms. Screening for efficient sgRNAs fitting multiple Ciona genomes will generate a database suitable for the entire Ciona community. Establshing in vitro validation of gRNAs is thought to reduce the amount of in vivo experimentation. Single animal genotyping is implemented for targeted sequencing of the knockout region from affected phenotypes. Furthermore, using a Cas9::geminin fusion achieved a higher cutting efficiency by a suggested synchronization with the cell cycle repair machineries. Finally, next generation sequencing (NGS) is currently introduced towards a peak shift verification of the sgRNA efficiency in vivo. While this method is established for Illumina sequencing our Oxford Nanopore sequencing offers high precision at much lower cost. Overall, these improvements should be readily applicable to most CRISPR approaches.

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# Bioadhesion in the Ascidian Ciona intestinalis

Biological underwater adhesion of marine organisms has inspired the first biomimetic glues but synthetic adhesives perform poorly in marine environments and a deeper molecular understanding of natural adhesives is required. Tunicates (ascidians) are one of the major marine adhesive organisms. Ascidian larval attachment initiates ascidian adhesion and as we previously showed is based on adhesive proteins and their modified carbohydrates (1). We also determined the adhesive properties of C. intestinalis larvae (2) and elucidated the required cellular components within the larval adhesive papillae (1).

Currently, for the study of larval adhesion, single-cell transcriptomics data (3) from the Ciona community help to allocate adhesive-relevant transcripts to adhesion-associated cell types. Consequently, the comparison of redefined papillae transcriptomes and adhesive prints proteomes guides the analyses of adhesive candidates. We are investigating several adhesive candidates by their cis-regulatory reporter expression in papillar cells. Furthermore, a series of functional assays via CRISPR/Cas9 is ongoing for selected papillae-expressed adhesive candidates.

This study contributes to the effort of finding green tissue compatible glues under physiological wet conditions and biofouling relevant formulas for shipping and aquaculture.

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# Chitin as a structural element in the Hydra glue

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. In several species (e.g. flatworms, limpets, sea urchins and sea stars), very large multi-modular proteins with highly conserved domains, like van Willebrand factors, were identified as major component of the glue. Surprisingly, no according analogues can be found in the cell-type-specific transcriptome or glue proteome of the ancestral fresh water cnidarian Hydra sp.. However, a lectin screen revealed an abundance of N-acetylglucosamine in the secreted Hydra glue (called footprint). This might indicate the presence of chitin and indeed, chitinase-treatment leads to a loss of the lectin signal. Chitin is a common biopolymer, which has been described to be involved in the attachment of barnacles. These findings lead to our work hypothesis that in the Hydra glue, 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 inhibitor treatments. Furthermore, we knock-down potential chitin binding proteins and enzymes that presumably modify the glue proteins (p.e. peroxidases). In conclusion, we start to unravel Hydra glue composition, with emphasis on the role of chitin and associated proteins. Our results could potentially lead to a new application for chitin as a component of biomimetic glues, which function under aquatic conditions, are non-toxic and sustainable.

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# Identification and characterisation of the Claudin family in Hydra vulgaris

Claudins are a major component of Septate/Tight Junctions and ensure epithelial integrity and para-cellular transport of ions. A previous study hinted at a vertebrate-independent diversification of Claudins in cnidarians. In order to get a complete image of the diversity of the claudin gene family in Hydra vulgaris, we searched the Hydra Genome Browser for candidates. Based on their sequence, hydrophobicity and domains, we identified 33 putative claudin orthologs. Their 3D-structure shows a similar folding pattern and interaction of specific amino acids in the extracellular loops as previously demonstrated in mammalians and can therefore be considered conserved. Furthermore, their expression patterns showed a unique combination of Claudins for every cell type. A phylogenetic tree including all known human and Hydra claudins, and claudins of other metazoans shows a clear separate diversification of Claudins in vertebrates. Genetic knockdown of Claudin-1, a rather broadly expressed paralog, led to defects in epithelial integrity and osmoregulation, and to a strong inhibition of head and foot regeneration. In conclusion, we were able to demonstrate a potentially complex and diverse construction of the apical junctions in Hydra vulgaris and show independent diversification of the claudin gene family in distantly related phyla.

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# Integrated immuno-genomic analyses of high-grade serous ovarian cancer reveal vulnerability to combination immunotherapy

Cancer immunotherapy has shown limited therapeutic efficacy in high-grade serous ovarian cancer (HGSOC). To overcome these limitations, a number of clinical trials combining immunotherapies with PARP inhibitors are underway. Hence there is an urgent need to identify predictive biomarkers. We performed multi-omics analyses of the TCGA HGSOC cohort (n=226) and based on genomic instability defined BRCAness. Using machine learning we could identify a gene expression signature determining BRCAness. TMB and neoantigen load was significantly different ($p<0.001$) between BRCAness and non-BRCAness samples, whereas immune characteristics, analyzed based on gene expression data, showed only a moderate positive association with BRCAness (rho=0.16, p=0.018). However, in vitro data in a BRCA1-/- cell line (UWB1.289) treated with the PARPi Olaparib showed interferon type I response and cGAS-STING pathway activation. To identify potential responders to combination immunotherapy we divided BRCAness tumors into a group with both an infiltrated tumor-immune phenotype and immune reactive molecular subtype and a group including all other combinations. Although CD8+ T cells were more abundant in the immune group ($p<0.001$) overall survival (HR=0.81, 95%-CI: 0.42-1.60, p=0.55) was not different. This could be due to observed higher CD8+ Tcell exhaustion ($p<0.001$), and an increased immune suppressive environment including Tregs ($p<0.001$), MDSCs ($p<0.001$), and M2 macrophages ($p<0.001$). Although this environment could be adverse for immunotherapy a recent clinical study indicate for some responders T cell exhaustion and macrophage infiltration. Accordingly, we propose a score to predict response to combination therapy that indicates BRCAness with a favorable balance between activated and suppressive immune environment.

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# Pathogenicity prediction of voltage-gated calcium channel mutations

Voltage-gated calcium channels (VGCC) translate membrane depolarizations into calcium influx. Despite their roles in key procedures such as neuron firing, muscle contraction and hormone secretions shown by previous studies, structural and functional impacts of individual residues remain unclear. Here we use cutting-edge methods to predict phenotypes of VGCC mutations.

Dysfunctions of different VGCC isoforms can manifest in various diseases according to locations and mechanisms of the dysfunctions. Despite their diversity in phenotypes, mutations of distinct channels subtypes can share similar perturbations on structure and functioning of channels. We investigate the joint picture of various subtypes to improve our understanding of individual point mutations. With structural models and pioneering analyses, we provide to roadmap to unveil effects of mutations throughout the VGCC. Our results help forecasting structural changes and functional outcome of VGCC mutations and better control of their pathogenic effects.

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# The ferroptosis-sensitizing EMT transcription factor ZEB1 orchestrates the cellular (redox)lipidome

Epithelial-to-mesenchymal transition (EMT) is coordinated by transcription factors like ZEB1 and hijacked by therapy-resistant cancer cells to enhance stemness and plasticity. Cancer cells undergoing EMT interestingly gain sensitivity towards ferroptosis, which offers high potential to fight aggressive and persistent cancer. We ascribed this enhanced ferroptosis susceptibility to a metabolic shift from monounsaturated to polyunsaturated fatty acids in membrane phospholipids that become susceptible to peroxidation. Here, we report on the global changes in the lipidome of triple negative breast cancer cells by ZEB1. Our targeted approach addresses i) major membrane phospholipid classes (phosphatidylcholines, phosphatidylethanolamines and phosphatidylinositols) ii) neutral lipids (triacylglycerides and diacylglycerides), and iii) free fatty acids. Moreover, we explore the redox lipidome of cells exposed to pro-inflammatory stimuli or ferroptosis inducers, thereby focusing on (per)oxidized phospholipid species with one to three oxygens incorporated and oxylipins with pro-inflammatory, immunomodulatory and tumor-regulating properties. We conclude that the regulation of the metabololipidome by ZEB1 in relation to ferroptosis is not limited to an increase in the PUFA/MUFA ratio but extends to diverse lipid species, whose contribution to the ZEB1-mediated ferroptosis vulnerability is enigmatic.

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# Improving cancer resection with dual-modality imaging: comparison of two probes targeting the CCK2 receptor

Introduction:

Preoperative sensitive imaging and intraoperative real-time surgical guidance improve the outcomes of tumour resection.Both applications are achievable with dual-modality probes which combine positron emission tomography (PET) with fluorescence imaging (FI) capabilities in the same molecule.

Objectives:

The study compares two PET/FI probes targeting the CCK2 receptor. In both cases, the same fluorescent dye and two units of Minigastrin targeting sequence are coupled to different chelators as central scaffold.

Methods:

The multifunctional cyclic chelators TRAP-Pr and Fusarinine C (FSC) were selected along with the Sulfo-cyanine5.5 fluorophore and the PEG4-minigastrin5 peptide sequence. The synthetic strategy is based on a first derivatization of the chelator to enable the conjugation of the other components by CuAAC click reaction and concluded with the removal of the coordinated metal. The compounds are initially characterized in vitro regarding their 68Ga radiolabelling, lipophilicity, protein binding and stability properties, continuing with binding studies with CCK2R expressing cell lines. Eventually, targeting and imaging properties are investigated in animal tumour models with biodistribution, PET and optical imaging studies.

Results:

Two novel PET/FI agents were synthesized: SulfoCy5.5-TRAP(PEG4MGS5)2 and SulfoCy5.5-FSC(PEG4MGS5)2. Quantitative radiolabelling along with high molar activity were achieved for both compounds along with specific CCK2R mediated cell internalization. Further preclinical investigations aimed to directly compare the two compounds are currently ongoing.

Conclusion:

The synthetic approach chosen allowed to prepare two PET/FI agents with promising in vitro properties. Following studies will provide data about the differences and applicability of the two prepared compounds.

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# The identification of functional germline variants in 3´UTR of androgen receptor variant 7.

Therapies targeting the Androgen Receptor (AR) axis are the current gold standard for the treatment of advanced prostate cancer. Nevertheless, patients eventually progress on these therapies. The upregulation of constitutively active AR variants (AR-Vs) is one of the mechanisms of resistance. Clinically, AR-V7 is the most relevant. This isoform results from the inclusion of a cryptic exon at the C-terminal end. Thus, AR-V7 mRNA also possesses a distinct 3´ untranslated region (UTR) compared to the full-length AR, suggesting a differential regulation. Here, we set out to better characterize the regulatory role of AR-V7´s 3´UTR by analyzing the contribution of a single nucleotide polymorphism (SNP, rs5918762, minor allele frequency=0.32) in alternative splicing. Bioinformatic analysis predicted multiple binding sites for SRSF9 (spliceosome component) in the AR-V7 3´UTR of which one is affected by the allele status of rs5918762. SRSF9 expression correlates with AR-V7 expression. Additionally, SRSF9´s involvement in AR-V7 splicing was confirmed by CLIP and loss-of function experiments. Further mapping of SRSF9 binding to AR-V7 3´UTR should reveal the exact binding. AR-V7 minigene assays show an allele-specific involvement of rs5918762 in AR-V7 mRNA expression. Moreover, the addition of pan-CLK inhibitor Cirtuvivint in the minigene assay, which inhibits the SR-protein family, also had a similar outcome. Taken together, these results suggest an involvement of the common SNP rs5918762 in AR-V7 splicing mediated by SRSF9 and therefore could help strengthen the specificity of AR-V7 as a therapy-guiding biomarker in advanced prostate cancer.

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# Establishment of a HepG2 cell culture model for liver machine perfusion research

Ex vivo machine perfusion, especially normothermic machine perfusion (NMP), improves liver graft preservation and long-term NMP may provide a platform for organ regeneration and recovery in the future. A major obstacle when performing long-term NMP is the potential toxic effects of the aging perfusate. Understanding the molecular mechanisms leading to graft damage by such factors is crucial. Since whole organ NMP is rather expensive, time-consuming and requires ethical approvement, accompanying small-scale in vitro models are demanded. Thus, we aimed to establish a cell culture model to study liver NMP using HepG2 cells.

Methods

A cell culture medium was reconstituted based on the perfusate used for NMP of livers. HepG2 cells were either cultivated overnight in this reconstituted perfusate or in supplemented DMEM as a control. Cell viability was assessed by determining intracellular adenosine triphosphate (ATP) levels. Leakage of lactate dehydrogenase (LDH) into the cell supernatant served as marker for cytotoxicity. MTS assay was applied for measuring metabolic activity of the cells. Mitochondrial respiration was assessed using high-resolution respirometry.

Results

Reconstituted perfusate had no detrimental effect on the viability of HepG2 cells compared to the control, although a significant decrease in metabolic activity ($p=0.001$) and in oxidative phosphorylation ($p=0.0020$) was observed. In line with this, LDH levels increased ($p=0.009$). Nevertheless, metabolic activity and mitochondrial respiration remained sufficiently high to study cytotoxic effect in context of machine perfusion.

Conclusion

The successful cultivation of HepG2 cells in reconstituted perfusate allows the establishment of HepG2 cells as an accompanying model for liver NMP research.

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# More than sweet: 68Ga-labelled sugar trimers for functional liver imaging

The Asialoglycoprotein receptor (ASGPR) is selectively expressed on functional hepatocytes in high numbers making it an excellent target for liver specific drug design. Here we compare [68Ga]Ga-NODAGA-TriGalactan (1) and [68Ga]Ga-TRAP-Galactan (2), two small molecule-based galactose trimers as PET imaging agents for this receptor.

The design of both radiopharmaceuticals follows data from Khorev et al. (Bioorg Med Chem 2008) indicating high binding of trimeric galactose structures. Radiosynthesis of (1) was accomplished within 10 min in a 1 M acetate buffer (pH 5) at 56°C, whereas for (2) a 5 M HEPES-buffer (pH 5.8) at 95°C was used. In vitro evaluation included octanol/buffer distribution (logD), protein binding, and metabolic stability studies in human blood serum. For biodistribution experiments, healthy BALB/c mice were dissected 10, 30 and 60 min p.i. and accumulation of the tracer was measured (n=3). Additional pharmacologic studies will be carried out in a PET/MR scanner.

Synthesis of the precursors could be achieved in seven (1) or four steps (2), respectively, and radiolabelling resulted in high (>95%) radiochemical purity. LogD values (-3.7 (1); -4.3 (2)) demonstrated high hydrophilicity of both tracers. Both compounds showed high metabolic stability in human blood serum and low plasma protein binding. Biodistribution data revealed high liver uptake (32% iD/g (1) vs. 30% iD/g (2), 30 min p.i.) and excellent target/background ratios. Small animal imaging data are currently being generated.

Further studies in a murine disease model have to demonstrate the full potential of these tracers and might help identifying the superior ligand design concept.

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# Investigation of NMP-related hemolysis using a precision-cut-kidney slice model

Background: Normothermic machine perfusion (NMP) is a promising preservation method as an alternative to static cold storage (SCS) for kidney transplantation. However, a major concern with prolonged NMP is hemolysis in the perfusate. In this setting, the extent of organ damage caused by free hemoglobin is currently unknown. Focused hemolytic studies during NMP may face major financial, time, and ethical hurdles, thus, in vitro models, such as precision-cut-kidney slices (PCKS), are required to mimic the situation of an NMP.

Methods: Punch biopsies (∅ 8 mm) were obtained from porcine and human kidneys. Using a tissue slicer (Alabama R&D), PCKS with a thickness of 300 µm were prepared and cultivated for 48 hours in (1) control medium (A. DMEM), (2) reconstituted perfusate (modeling control perfusate conditions during NMP) or (3) hemolytic perfusate (with 500 mg/dL of free hemoglobin). Viability was determined using an ATP assay and real-time-confocal microscopy. LDH was quantified in the supernatant and metabolic activity was assessed via an MTS assay. Mitochondrial respiration was assessed by high resolution respirometry.

Results: Porcine and human PCKS were cultivated under different conditions. Viability and metabolic activity of PCKS remained stable for 24 hours among all groups. Increased levels of free hemoglobin led to decreased viability.

Conclusion: PCKS were successfully cultivated for up to 24 hours and a hemolytic condition reflecting NMP was established. PCKS were proven a suitable in vitro model for studying the effects of NMP. Different conditions (present during NMP) can be applied to study isolated mechanisms.

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# Preclinical evaluation and automated synthesis of [177Lu]Lu-labelled DOTA-MGS5 for application in cholecystokinin-2 receptor expressing neoplasms

DOTA-MGS5 is a novel minigastrin (MG) analogue with improved tumour-to-kidney ratio and optimised in-vivo stability which can be considered as a promising candidate for targeting cholecystokinin-2 receptor (CCK2R) expressing neoplasms. With the goal of the clinical translation of [177Lu]Lu-DOTA-MGS5 for peptide receptor radionuclide therapy (PRRT), we performed specific preclinical testing and established the automated synthesis process.

A431-CCK2R cells and AR42J cells expressing CCK2R were used to investigate the receptor-specific cell internalization of [177Lu]Lu-DOTA-MGS5. Biodistribution studies in A431-CCK2R xenografted female BALB/c nude mice were performed up to 7 days post-injection. The radiolabelling process was validated using a Modular-Lab PharmTracer synthesis module, DOTA-MGS5 in GMP quality and a no-carrier added [177Lu]LuCl3.

[177Lu]Lu-DOTA-MGS5 showed a high cell uptake of ~60% in A431-cells and of ~50% in AR42J cells 4h after incubation. The radiolabelled peptide demonstrated a promising tumour targeting potential with a low non-specific accumulation of radioactivity in most of the tissues and high tumour retention over time (~30% IA/g one day after injection). A somewhat prolonged retention of radioactivity was observed in CCK2R-expressing stomach. Using the cassette-based preparation [177Lu]Lu-DOTA-MGS5 was prepared in a standardized way and with high radiochemical purity of >95%.

The performed preclinical testing and automation of synthesis support the clinical translation of [177Lu]Lu-DOTA-MGS5 in patients with advanced MTC and other CCK2R expressing tumours.

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# The role of glucocorticoids in Non-Alcoholic Fatty Liver Disease Development

NAFLD is a spectrum of diseases that ranges from simple liver steatosis, to lobular inflammation (NASH), and that can finally lead to irreversible fibrosis, or cirrhosis. NAFLD is estimated to affect one in four individuals globally, and it is the leading cause of chronic liver disease. Several studies indicate that glucocorticoids (GC) play a role in NAFLD development, but the results remain highly controversial. GC, commonly called, stress-hormones, and one common anti-inflammatory, act by binding to the glucocorticoid receptor (GR), a nuclear transcription factor that controls a wide variety of physiological functions, one of them being metabolism.

In this first year of my PhD project, I have studied the hypersensitivity of the GR in a mouse model of liver steatosis and in steatotic human hepatic cells treated with free-fatty acid. On the other hand, we plan to collect the livers of steatotic mice, in different periods, to evaluate their GR hypersensitivity. Finally, test the different cell-specific mechanisms by which the GR can be contributing to the development of the NAFLD.

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# Erk1/2-Dependent HNSCC Cell Susceptibility to Erastin-Induced Ferroptosis

Unfavorable clinical outcomes mean that cancer researchers must attempt to develop novel therapeutic strategies to overcome therapeutic resistance in patients with head and neck squamous cell carcinoma (HNSCC). Recently, ferroptosis was shown to be a promising pathway possessing druggable targets, such as xCT (SLC7A11). Unfortunately, little is known about the molecular mechanisms underlying the susceptibility of HNSCC cells to ferroptosis. The goal of this study was to determine whether HNSCC cells with activated Erk1/2 are vulnerable to ferroptosis induction. Our results have shown that xCT (SLC7A11) was overexpressed in malignant tissues obtained from the patients with HNSCC, whereas normal mucosa demonstrated weak expression of the protein. In order to investigate the role of Erk1/2 in the decrease in cell viability caused by erastin, xCT-overexpressing FaDu and SCC25 HNSCC cells were used. The ravoxertinib-dependent inhibition of Erk1/2 signaling led to the decrease in erastin efficacy due to the effect on ROS production and the upregulation of ROS scavengers SOD1 and SOD2, resulting in repressed lipid peroxidation. Therefore, it was concluded that the erastin-dependent activation of ferroptosis seems to be a promising approach which can be further developed as an additional strategy for the treatment of HNSCC. As ferroptosis induction via erastin is strongly dependent on the expression of Erk1/2, this MAP kinase can be considered as a predictor for cancer cells' response to erastin.

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

Prostate cancer (PCa) relies on the androgen receptor (AR) signaling for its growth, which is therefore targeted by the androgen deprivation therapy (ADT). In spite of its therapeutical efficacy, the disease is ultimately progressing in the castration-resistant prostate cancer (CRPC).

The Mediator Complex, involved in the modulation of gene expression, has been associated with PCa. Its MED12 subunit is upregulated in metastatic CRPC in comparison to the localized disease. MED12 was also preliminarily associated with PCa cell proliferation. Therefore, we aim at studying the involvement of MED12 in PCa-driver signalling pathways exploring its potential as a therapeutic target.

Concordantly to previous data, MED12 gene amplification is increased in metastatic PCa tissues (SU2C) than in primary PCa tissues (TCGA). We analysed the dependency of PCa cell line growth on MED12 in two independent CRISPR- and RNAi-screens (DepMap portal), highlighting stronger dependency on MED12 expression in AR+ cell lines compared to AR- cell lines. However, the knockdown of MED12 expression in PC3 (AR-) and 22Rv1 (AR+) significantly decreased both cell proliferation and the protein expression of the PCa-driver c-MYC by around 40%. Through the RNA-sequencing and pathway analysis of MED12-knockdowned 22Rv1 cells, we confirmed the significant MED12 positive association with c-MYC and AR signalling. Preliminary data also showed that MED12 knockdown is consistently downregulating AR protein expression, paving the way to further studies.

Finally, we aim at inhibiting the kinase module of the mediator complex to assess if MED12 promotes PCa as a component of the complex or independently from it.

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# Identification and characterisation of the stromal heterogeneity in prostate cancer

Prostate cancer (PCa) is the second most common cancer in men worldwide and in its primary, localised form often curable. However, a subset relapses which is then treated with androgen deprivation therapy. Despite initial response, most patients progress to lethal castration-resistant prostate cancer (CRPC). Consequently, there is a necessity for new therapeutic strategies that either prevent the development of or target this final stage of PCa.

Since stromal fibroblasts and mural cells of the tumour microenvironment (TME) are well established as key contributors to the development, progression and therapy resistance of PCa, there is increasing interest in targeting the stromal component. However, the TME displays functional heterogeneity with some types of cancer-associated stromal cells (CASCs) mediating either tumour-supportive or –restrictive properties. In view of this functional heterogeneity, a better characterisation of CASC subtypes is needed to specifically target cancer-promoting CASCs.

We therefore aim to identify, isolate and characterise the CASC subtypes present in the PCa microenvironment. Based on scRNAseq data from digested PCa punches, we identified stromal cell clusters of interest, which display prognostic gene signatures. Further analysis determined distinctive markers that label the respective CASC subtypes in patient tissues via immunohistochemistry and in-situ-hybridisation. Additionally, a cell surface marker panel was developed which distinguishes different CASC populations in digested PCa punches and allows for isolation via flow cytometry-based sorting.

These marker panels will enable better characterisation of the prostate TME at the cellular level and evaluation of the contribution of distinct CASC subtypes to PCa pathophysiology and therapy resistance.

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# MR-Spectroscopy: Investigating neurochemical changes in brain metabolism in migraineurs before and after CGRP-Antibody treatment – a randomized, controlled, open-label trial.

Background: Imaging techniques have revealed important aspects of the underlying pathophysiological mechanisms of migraine, suggesting abnormal energy metabolism and increased cerebral hyperexcitability as triggers for a migraine attack. Since only a small percentage of monoclonal CGRP antibodies crosses the blood-brain barrier, their main site of action outside the blood-brain barrier is discussed. It is uncertain whether they lead to central effects through their action outside the blood-brain barrier or exert direct central effects.

Objective: To investigate whether neurochemical, structural, and functional changes in the migraine brain are associated with CGRP-antibody treatment.

Methods: This prospective, randomised, controlled, open-label study will enrol 38 patients diagnosed with episodic migraine (w/o aura) according to ICHD-3 criteria. All participants will undergo an initially stratified 1H-, 31P- MR-Spectroscopy and resting-state fMRI interictally. Half of the participants (n=19) will receive CGRP mAB treatment (Fremanezumab 225mg monthly) after the first scan for three months according to local standard guidelines for CGRP mAB treatment. MR-spectroscopy and resting-state fMRI will be repeated after the treatment. Controls will be measured in an identical setting at the same time points but without CGRP mAB treatment.

Future aspects: Investigating the effects of CGRP mAB on the metabolism and its association to functional connectivity in the migraine brain provides in-depth knowledge about the mechanism of action of the CGRP antibody and permits individualized treatment.

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# Behavior and cortical activation following mild hypercapnia in high-anxiety subjects

Patients with dysregulated anxiety processing often display aberrancies in sensing the internal state of the body. Interoceptive pathways can be activated by mild hypercapnia causing homeostatic disturbances of the body and eliciting anxiety across species. Physiological responses to CO2 inhalation are elevated in subjects with high-trait anxiety compared to normal anxiety subjects. Although altered interoception is increasingly recognized as an important component of anxiety-related disorders, its underlying neural mechanisms remain insufficiently understood. Here, we aimed to elucidate whether differences in trait anxiety levels determine the engagement of the anxiety network in response to CO2 challenge. Mice bred for high (HAB) or normal (NAB) anxiety-related behavior were exposed to CO2-enriched (10%) air for 10min and neuronal activation was assessed by mapping the expression of the immediate early genes c-Fos and Zif26. Relative to NAB mice, HABs showed significant anxiety-like behavior during CO2 exposure. On the contrary, it rather promoted active coping strategies including rearing and jumping in NABs. The preliminary findings showed that the number of c-Fos-positive cells was generally increased in the agranular insula, a brain area known to mediate interoceptive stimuli, following CO2 exposure as compared with control air condition in NABs, and the contrary was observed in HABs. Taken together, the present findings indicate increased CO2 sensitivity in individuals with high-trait anxiety. The differential engagement of the insular cortex for the processing of interoceptive signals in high-trait anxiety may represent a potential biomarker for stratifying patient subgroups to optimize individualized therapeutic interventions.

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# Sex- and Gender-related Differences in Treatment of Multiple Sclerosis – a retrospective analysis of the Austrian National MS registry

Introduction: Multiple Sclerosis (MS) is a neurological disease predominantly affecting young women. An early initiation and – if necessary – escalation of a disease modifying therapy (DMT) plays a crucial role in preventing future disability.

Objectives:To investigate whether the time to DMT escalation differs between female and male MS patients in Austria.

Methods: In this retrospective, national observational study, we collected demographic, clinical and radiological data from the Austrian Multiple Sclerosis Treatment (AMST) registry. Participation at the AMST is mandatory for reimbursement of MS therapies in Austria. Eligible patients for inclusion were diagnosed with relapsing-remitting MS and aged at least 18 years at the time of start of DMT, with a minimum of 2 consecutive visits. Eligible patients were categorized as starting high-efficacy DMT (hDMT), or moderate-efficacy DMT (mDMT).

Results: 4451 patients were included in this study with a median of 10 (5-18) visits over an observation period of 3.2 (1.4-5.8) years. At baseline, 2901 patients received hDMT, while 1550 patients received mDMT. Frequency of mDMT and hDMT were similarly distributed between women and men. 172 patients receiving mDMT at baseline were escalated to hDMT, while 152 patients with initial hDMT were deescalated to mDMT. Multivariable Cox regression analysis identified a longer time to DMT escalation in female than in male, while no difference was found for the time to de-escalation.

Discussion: Our data suggest that female MS patients witness a delay in regard of DMT escalation in relation to males which may lead to higher risk for future disability.

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# Gender Differences in Psychological Sequelae of Dyspagia after Ischemic Stroke

Background and aims: This study examines the relationship between gender and dysphagia after ischemic stroke, including psychological consequences.

Methods: Within the STROKE-CARD Registry study (NCT04582825) from 2020 to 2022 dysphagia was diagnosed within clinical routine by standard swallowing examinations by speech therapists. SINGER Independency Index assessed swallowing issues at discharge and after 3 months. Affective symptoms were recorded after 3 months based on Hospital Anxiety (HADS-A) and Depression (HADS-D) Scale and Beck Depression Inventory (BDI).

Results: Of 648 patients, including 36.6% women and 63.4% men, 19.3% had dysphagia at baseline. At hospital discharge and 3-month follow-up, 13.5% and 6.8% reported ongoing swallowing issues, respectively. The longer the swallowing problem persisted, the higher were the scores in HADS-A, HADS-D, and BDI. In linear regression analysis adjusting for age, sex and functional disability at 3 months HADS-D ($p = < 0.001$) and BDI ($p = 0.007$), but not HADS-A ($p = 0.090$) scores at 3 months were significantly higher in patients with persistent dysphagia compared to those without initial dysphagia or who recovered until follow-up. There was no difference in mean scores between the sexes on HADS-A, HADS-D and BDI, however, men had a higher incidence of reporting being irritable more quickly (66.7% vs. 27.8%; $p = 0.009$). In addition, women were significantly more likely to receive antidepressants after 3 months (64.5% vs. 40.4%; $p = 0.030$).

Conclusions: Post-stroke dysphagia has impact on depressive symptoms 3 months after ischemic stroke with poor gender-specific differences.

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# Sex differences in Quality of Life after COVID-19

Background and underlying question:

Many of the almost 700 million COVID-19 patients do not only suffer from symptoms during the acute phase of the illness, but also for weeks and months afterwards. One possible long-term impact of an infection can affect the quality of life. The registration of persistence and course over time of such an impairment is therefore needed, together with an investigation for specific risk factors and differences between the sexes.

Methods:

Quality of life was assessed 12 months after COVID-19 infection, using the 36-item Short Form Health Survey Version 2 (SF-36v2). This questionnaire consists of 36 items grouped into 8 subscales (including vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health). The subscales are summarized into a physical and mental component summary. Each scale and component summary is scored with 0 to 100 points; 0 points indicate greatest possible impairment, whereas 100 points indicate no health restrictions. The presence of impairment is determined with 40 points or less.

Results:

Data from 57 patients (22 female, 35 male) was assessed 12 months after infection. 8.8% of the patients reported an impairment in the physical and 21.1% in the mental domain of the SF-36v2. Hereby, women reported impairments in 9.1% and 22.7% respectively men 8.6% and 20.0% in the physical and mental domain.

Conclusions:

In a fifth of the female and male patients one year after COVID-19 infection impairment in quality of life remains.

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# Gender aspects of decision-making in neurocritical care patients in the early course of the disease

Objectives: Early decision-making is a crucial step in the trajectory of neurocritically ill patients. Scarce data exist on gender-specific differences in satisfaction with care and the decision-making process.

Methods: In this single center survey, family satisfaction with decision making and care of patients admitted to the neurointensive care unit (NICU; Department of Neurology, Medical University of Innsbruck) was assessed using the FS-ICU questionnaire (current version FS-ICU 24 R) within 72 hours of admission to the NICU. The FS-ICU consists of two subsections assessing family satisfaction with care and decision making; three summary scores (satisfaction with care, decision-making and a total score ranging from 0 to 100 with higher scores indicating greater satisfaction) can be calculated. One family member, who was engaged in the decision-making process and provided consent, was recruited to participate in the survey. During the COVID-19 pandemic, one visitor was allowed per day. In the setting of palliative care, visiting restrictions were lifted.

Results: Between December 2021 and July 2022, 301 patients were admitted to the local NICU. Relatives of 131 patients met all inclusion criteria, of which 112 relatives participated in the survey. While 45 out of 112 patients (40.2%) were male, 84 out of 112 relatives were female (75%). Satisfaction with care (94/100 (IQR 76-97)) and decision-making (83/100 (IQR 72-95)) was high with no gender-specific differences.

Conclusion: Seventy-five percent of decision-makers for neurocritical care patients were female. No gender-specific differences in satisfaction with care or decision-making were found.

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# Nuclear lamina–dependent mechanisms in neuroplasticity

The Nuclear lamina (NL) is a filamentous meshwork of A- and B-type lamins attached to the nuclear envelope via interactions with the inner nuclear membrane (INM) proteins. Emerging evidence from non-neuronal cell types implicates interactions between NL and chromatin in the regulation of both spatial genome organization and gene expression. A comprehensive understanding is currently lacking whether these mechanisms also operate in neurons and, if so, which proteins serve as neuronal chromatin-NL 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 and activity-dependent gene expression programs in cortical neurons.

Here, we aim to characterize the influence of LEMD2-SATB2 complex on lamina-associated domain (LAD) dynamics, 3D genome organization and regulation of gene expression in pyramidal neurons upon pharmacological synaptic activation. We use CUT&RUN to map Lamin A/C and Lamin B1 genomic interactions in primary cortical neurons derived from Lemd2 and Satb2 cKO vs floxed mice, and we employ bulk RNA-seq and Hi-C to compare their transcriptome profiles and correlate gene expression changes with differences in LAD and 3D genome architecture.

This analysis will provide deeper insights into the LAD dynamics/3D genome folding upon neuronal activation and the role of chromatin-NL tethers, such as LEMD2 and SATB2, in these processes. Furthermore, mapping of GWAS neuropsychiatric disease risk loci to activity-dependent LADs can provide new mechanistic hypotheses and broaden our understanding of the pathogenesis of these complex disorders.

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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 aim to generate human induced pluripotent stem cell (hiPSC)-derived SATB2 gain- and loss-of-function cellular models to gain insights into human SATB2-dependent disease mechanisms in SAS and schizophrenia.

To this aim, we will employ established protocols for differentiation of hiPSCs into neuronal progenitor cells and into cortical glutamatergic neurons by Neurogenin 2 overexpression. To generate SATB2 gain-of-function model, neurons will be further transduced with a lentivirus, mediating ectopic expression of SATB2. The two populations of excitatory pyramidal neurons will be subjected to a combination of functional genomics and proteomics assays. These analyses will uncover effects of SATB2 on multiple genomic modalities in human pyramidal neurons and allow direct comparison between mouse and human SATB2-dependent chromatin interactions. We will test the hypothesis that SATB2-directed 3D genome folding as a mechanism of transcriptional control in cortical neurons has evolved between the two species as a correlate of the evolution of 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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# Olfactory training in COVID-19 associated loss of smell (SMELL)

Olfactory dysfunction (OD) is a common symptom of SARS-CoV-2 infection, responsible for the COVID-19 pandemic, and is increasingly recognized as a persisting post-infectious complication. This could be a result of damage to the sensory olfactory epithelium or by direct neurotrophic effects on the olfactory receptors residing in the neuroepithelium. Potential treatment strategies aim for the neural plasticity of the olfactory system and its potential for recovery.

In this RCT, 100 individuals with COVID-19 related persisting OD (>3 months post-infection) will be included. Data regarding impact on daily life and health, OD-related mood and Quality-of-Life (QoL) will be collected, together with objective OD measurements using the Sniffin' sticks test (identification and discrimination). After randomization, the training cohort will perform olfactory training (OT) twice a day with a 4-odor training set for 12 weeks. The other cohort will not train for the first 12 weeks, making it possible to evaluate the natural history of OD.

Olfactory functioning plays an important role in social relationships and is often impaired during and after COVID-19. However, there are currently no proven therapeutic options for affected individuals. Nevertheless, previous studies have shown olfactory training to be an effective therapeutic option for individuals with post-viral or post-traumatic OD.

By proving the efficacy of OT after COVID-19, we offer a simple treatment option for affected individuals with persistent OD. Even more, we hope to show a correlation between OD and its impact on daily functioning and QoL, thereby stressing the importance for therapeutic options.

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# Role of PKN1 in neuroregeneration and neurodegeneration after an ischemic stroke

The brain's outstanding energy consumption is largely attributed to the complex processes of neuronal transmission. Hypoxia or hypoglycemia result in maintenance failures of basal energy levels, which within only a few minutes of exposure can lead to synaptic loss, cognitive impairment and eventually renders the brain vulnerable to damage caused by an energy deficit.

Protein kinase N, PKN1 is a serine/threonine protein kinase, activated by binding to Rho proteins and involved in processes that concern the regulation of intermediate filaments of the actin cytoskeleton, cell migration as well as transcription regulation. We have previously shown that PKN acts as a critical gatekeeper of the AKT pro-survival-pathway during brain development.

This project aims to illuminate the role of PKN1 after ischemic stroke. Both wildtype and PKN1-/- Hippocampal brain slices are exposed to oxygen-glucose deprivation and reperfusion. We provide biochemical and functional evidence that PKN1-/- is protective after ischemia. We further discuss affected downstream pathways and metabolic changes.

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# Pain in people with multiple system atrophy: a systematic review

Introduction: People with multiple system atrophy (MSA) often complain about pain. Its prevalence, characteristics and risk factors remain however poorly characterized to date.

Methods: To estimate pain prevalence and to collect information on its characteristics, risk factors and treatment strategies in people with MSA, the PubMED, Cochrane and Web of Science databases were systematically screened for papers published in English until September 2022, by using the following keywords: "pain" AND "multiple system atrophy" OR "MSA", olivopontocerebellar atrophy" OR "OPCA", "striatonigral degeneration" OR "SND", "Shy Drager", "atypical parkinsonism". Additional papers were retrieved by reference cross-check. Papers reporting information on pain prevalence in people with possible or probable MSA were included.

Results: Seven-hundred records were identified, 16 were included in the qualitative synthesis and 14 (7 cross-sectional, 1 retrospective, 6 prospective) were retained for final analysis, pooling data from 1319 individuals with MSA. Among them, 60% (n=797) reported pain, with prevalence of pain ranging from 40% to 88% across studies. Pain was reported by 67% of people with MSA-Parkinson (MSA-p) and by 47% of MSA-cerebellar phenotype (MSA-c) ($p<0.001$). According to two studies, 50% of the individuals with MSA had received any kind of pain-specific treatment. We found a high heterogeneity in pain prevalence, classification and assessment tools across studies.

Conclusions: Pain is a frequent, but still under-recognized and undertreated feature of MSA. A better characterization of pain prevalence, characteristics and predisposing factors in people with MSA will help tailoring its assessment and management.

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# THE ROLE OF PROTEIN KINASE 1 IN RETINAL DEVELOPMENT AND REGENERATION

The retina is a multilayered specialized neural tissue lining the inside of the eye of most vertebrates and is essential to the processing of visual stimuli. The vertebrate retina consists of ten distinct layers with defined cell types. Retinal ganglion cells (RGCs) which receive visual stimuli provided by the photoreceptors, transmit this information via their axons, which form the optic nerve, to higher brain areas. RGC degeneration is among the leading global causes of incurable vision loss and occurs after retinal/optic neuropathies or glaucoma. In order to develop therapies to prevent vision loss or to regenerate RGCs and the optic nerve, a full understanding of the pathways involved in RGC differentiation, axon growth and pathfinding is necessary. Previous studies have shown that the serine/threonine protein kinase N1 (PKN1) plays an important role in axonal growth and presynaptic maturation of cerebral granule neurons. PKN1 inhibits AKT phosphorylation, leading to a reduction of the transcription factor NeuroD2, which is essential for correct cerebellar synapse formation during development. Knock-Out of PKN1 leads to elevated levels of activated AKT/NeuroD2, which results in enhanced axonal outgrowth at the expanse of presynaptic maturation. While PKN1 is expressed in the retina and RGCs, its role in retinal development is still unknown. In this study, we analyzed if PKN1-mediated AKT/NeuroD2 suppression is also observed in retinal development and if this pathway is important for correct RGC axonal pathfinding and synaptic maturation in vivo.

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# Sex differences in falls and fractures following Stroke or TIA after 3 and 12 months in the STROKE-CARD REGISTRY

Background / Methology:

Falls and fractures are a frequent problem in post-stroke care leading to a considerable morbidity.

Since December 2020 information on patients with high-risk TIA or ischemic stroke treated at the University Hospital Innsbruck - Austria is collected at baseline, 3 and 12 months in the STROKE-CARD REGISTRY (NCT04582825). Fractures and falls are assessed prospectively in a physician guided interview.

Results:

912 patients have been included in the STROKE CARD REGISTRY so far, 36,6% women (N=331) and 63,7% men (n= 581), 89,4% (n=815) with ischemic stroke and 10,6% (n=97) with high risk TIA. When compared to men, women were slightly older (mean(SD) 73,1(±13,5) vs. 70,5(±13,0) years, P=0,005). There was no difference in functional recovery at discharge (mRS (median(IQR)) 2(2) vs. 2(2), P=0,679), after three months (mRS 1(2) vs. 1(2), P=0,718), and after twelve months (mRS 1(2) vs. 1(2), P=0,094), neither in neurological recovery (NIHSS) at these time points (results no shown here). 157 patients reported at least one fall. Although the majority of the study population are men (63,7% vs. 36,6% women), women suffered 44,6% of all falls.

Conclusion:

Falls and fractures are a major complication after Stroke or TIA. The increased risk of falls in women is unclear at this point and merits further research. In general, the risk of falls increases with age. Patients with higher age (especially women) have to be protected from falls. More insights in causes of fractures and bone structure changes after stroke (post stroke osteopathy) are needed.

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# Einfluss des interhospitalen Transportes auf das Outcome bei Epiduralhämatomen

## Einleitung

Kraniale Epiduralhämatome (EDH) sind eine neurochirurgische Akutpathologie, welche typischerweise eine schnelle operative Behandlung bedürfen. Das Outcome ist unter anderem abhängig von der Zeit zwischen Trauma und OP, initialem GCS und Alter zusammen. Ziel dieser Analyse war es, den potentiellen Einfluss eines interhospitalen Transportes auf das Zeitintervall bis zur Behandlung und auf das Outcome zu untersuchen.

## Methoden

Alle Patienten, die zwischen 2009 – 2019 an einem EDH operativ versorgt wurden, wurden retrospektiv analysiert. Folgende Variablen wurden analysiert: Zeitintervall Trauma-OP, Distanz vom Unfallort nach Innsbruck, initialer GCS, Alter und primärer oder sekundärer Transport sowie Outcome (GOS).

## Ergebnisse

142 Patienten (106 Frauen, 36 Frauen) wurden in dem zehnjährigen Zeitraum an einem EDH operiert. 56% der Patienten wurden direkt an unser Zentrum transferiert und präsentierten sich mit einem medianen initialen GCS von 13. 44% Patienten wurden zuerst in ein peripheres Krankenhaus eingeliefert und wiesen einen medianen initialen GCS von 11 auf. Sekundär transferierte Patienten wurden durchschnittlich 10 Stunden nach Trauma operiert, die primär transferierten Patienten nach 16 Stunden. Es zeigte sich kein signifikanter Outcomeunterschied.

## Schlussfolgerung

Das Zeitintervall zwischen Trauma und Operation ist beim EDH (überraschend) lang, so dass wohl nur eine Minderheit einer unmittelbaren Versorgung bedarf. Sekundär transferierte Patienten in Tirol werden rascher operiert als primär eingelieferte und der interhospitale Transport hat keinen Einfluss auf das Outcome von Patienten mit EDH.

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# Sex differences and cardiovascular risk assessement in a preliminary cohort of Multiple Sclerosis (MSVasc Study)

Objective: The aim of this cohort study is to evaluate the incidence of cardiovascular disease and the risk factors in a population of 100 individuals with multiple sclerosis (MS).

Methods: Participants are recruited from a specialized MS clinic and are followed for a period of 20 years. Data on demographics, medical history, and cardiovascular risk factors (Hypertension, cholesterol, blood sugar, smoking behavior and various lab values) are collected at baseline and follow-ups. Participants are then monitored for the development of cardiovascular disease, including hypertension, coronary artery disease, and stroke. The incidence of cardiovascular disease is calculated and compared to age- and sex-matched controls from the general population.

The preliminary results of this study show the estimated incidence of cardiovascular disease in individuals with multiple sclerosis by evaluating this cohort of now included 74 patients with MS to understand the risk factors associated with cardiovascular disease and potential sex differences in this population.

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# Congitive Impairment in the Stroke Card Trial

Introduction:

Cognitive abilities after stroke play a key role in the reintegration of patients into everyday life.

Overall, a prevalence of 20% to 80% has been reported for cognitive impairment after stroke.

Methods:

This is an analysis of the development of Montreal Cognitive Assessment scores of patients with acute ischemic stroke from the intervention group of the STROKE-CARD trial (ClinicalTrials.gov ID NCT02156778), a prospective block-randomized open interventional trial, and its subsequent observational study, the STROKE-CARD Long-term Follow-up trial (ClinicalTrials.gov ID NCT04205006). All patients included in this analysis were treated at the study center in Innsbruck and underwent a Montreal Cognitive Assessment (MoCA) 3 months, 12 months, and 3 to 6 years after the index ischemic event. Patients with recurrent cardiovascular events were excluded. MoCA scores from 18 - 25 points were graded as mild cognitive impairment, from 10 - 17 as moderate cognitive impairment and scores < 10 were graded as severe cognitive impairment. Statistical analysis was conducted with IBM® SPSS®

Results:

Of the 1438 eligible patients, 169 underwent MoCA testing at the 3-months, 12-months, and at the long-term follow-up visit. Study population characteristics are shown in the Table. MoCA scores decreased almost linear from the 30-39 to the 80-89 age group.

Discussion:

Our results are in line with a steady decrease of the mean MoCA score from the youngest to the oldest age group.

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# Paraneoplastic anti-SRP antibody positive necrotizing myopathy associated with lymphoma. Case report and literature review.

We report a remarkable case of idiopathic inflammatory myopathy (IIM) associated with anaplastic large cell lymphoma (ACLC). This 26-year-old female patient presented with symptoms of bilateral proximal muscle weakness and myalgia, highly elevated levels of creatinine kinase (CK), as well as seropositive anti-SRP antibodies. Tumor screening by FDG-PET/CT detected an enlarged axillary lymph node with high FDG uptake, which was resected with the histologic result of an ACLC, CD30 +, ALK +. A literature review of cases of paraneoplastic immune-mediated necrotizing myopathy (IMNM) resulted in 16 cases mainly associated with gastrointestinal, lung or breast cancer, while only one patient was diagnosed with an acute myeloid leukemia (AML). One of these 16 patients showed anti-SRP antibodies, five patients anti-HMGCR antibodies whereas the majority of these patients were diagnosed with seronegative IMNM.

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# Neurological manifestations in wild-type transthyretin amyloidosis

Background: Transthyretin amyloidosis (ATTR) is a rare progressive condition characterized by extracellular deposition of transthyretin in organs and tissues, causing a wide spectrum of symptoms, depending on the location of the transthyretin aggregates. ATTR can result from mutations in the TTR gene, causing depositions of abnormal transthyretin (hereditary form, ATTR-v), or by deposition of misfolded non-mutated transthyretin (wild-type transthyretin amyloidosis, ATTR-wt). Neurological manifestations, such as large and small fiber, and autonomic neuropathy are part of the clinical spectrum in ATTR-v. Based on the pathophysiology of ATTR, these neurological complications might also be common in ATTR-wt. Better understanding of neurological manifestations in ATTR-wt may affect clinical management.

Method: 30 ATTR-wt patients with cardial involvement and 30 age and sex-matched controls will be included. Both treatment-naïve and treatment-experienced patients are included. Assessments will include i) nerve conduction studies and clinical neurological examinations to assess poly- and mononeuropathy ii) cardiovascular autonomic function testing, quantitative sudomotor axon reflex testing and Composite Autonomic Symptom Score-31 to measure autonomic nervous function, iii) quantitative sensory testing and skin biopsies in the lower extremity to study small nerve fiber function iv) questionnaires (Norfolk Quality of Life-Diabetic Neuropathy and Chalder Fatigue Scale). These assessments will be repeated during a 1-year follow-up visit to evaluate disease progression.

Discussion: Our aim is to provide a comprehensive clinical and electrophysiological characterization of neurological and autonomic complications in patients with ATTR-wt. The frequency and severity of these complications and their progression over one year are studied.

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# Alpha-Synuclein Seed Amplification Assays in the Diagnosis of Synucleinopathies using Cerebrospinal Fluid – a Systematic Review and Meta-Analysis

Background: Real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) have been developed to detect already minute amounts of amyloidogenic proteins via amplification techniques. In recent years, these assays have been adapted to detect alpha-synuclein (alphaSyn) aggregates in the cerebrospinal fluid (CSF) and other source materials of patients with synucleinopathies.

Objectives: The aim of this systematic review and meta-analysis was to evaluate the diagnostic accuracy of alphaSyn seed amplification assays (alphaSyn-SAAs), including RT-QuIC and PMCA, using CSF as source material to differentiate synucleinopathies from controls. Controls not only included healthy controls but also patients with other neurodegenerative diseases.

Methods: The electronic MEDLINE database PubMed was searched for relevant articles published until June 30, 2022.

Results: Twenty-seven studies were eligible for systematic review according to the predefined inclusion criteria, of which twenty-two were included in the final analysis. Overall, 1,855 patients with synucleinopathies and 1,378 non-synucleinopathies as control subjects were included. The pooled sensitivity and specificity to differentiate synucleinopathies from controls using alphaSyn-SAA were 0.88 (95% CI, 0.82 – 0.93) and 0.95 (95% CI, 0.92 – 0.97), respectively. Evaluating the diagnostic value of RT-QuIC to detect alphaSyn aggregates in the CSF of patients with MSA the pooled sensitivity decreased to 0.30 (95%CI, 0.11 – 0.59).

Conclusions: Our study demonstrated a high diagnostic performance of RT-QuIC and PMCA to detect synucleinopathies. The results for the diagnosis of MSA specifically were less robust using RT-QuIC as alphaSyn-SAA.

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# CGRP and other neuropeptides in acute and subacute stroke

Stroke is the second leading cause of death from non-communicable diseases and is the most frequent cause of permanent disability worldwide. As Calcitonin Gene-Related Peptide (CGRP) is known as one of the most potent vasodilative neuropeptides and its role in migraine was found to be substantial, CGRP blockage is emerging in preventive treatment of migraine. As many studies suggest a possible correlation between stroke and CGRP, a rodent model of CGRP blockage showed more severe stroke in female and male mice.

The aim of this study is to collect data of CGRP and other neuropeptide levels in acute and post-acute ischemic stroke patients. Data work-up to establish a bio-databank of CGRP and other neuropeptide levels in stroke patients will be performed.

This study will be set up as a prospective, monocentric cross sectional study enrolling patients (n=80) with ischemic or haemorrhagic stroke. All patients enrolled will undergo three to four consecutive blood collections out of the cubital vein. The first blood sample will be taken in the emergency room immediately after stroke diagnosis. In follow-up, one blood sample will be taken on the following day of stroke and three months after stroke as part of the STROKE-CARD standard of care visit. Patients eligible for thrombectomy will undergo a fourth blood collection out of the affected cerebral artery while thrombectomy. All collection of blood samples will be performed via standard of care blood sample collection in stroke diagnosis and in follow-up.

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# Artificially aged brain organoids through Progerin overexpression reveal specific patterns of transcriptome and methylome changes

In vitro models derived from induced pluripotent stem cells (iPSCs) lack age-related signatures due to epigenetic reprogramming. Therefore, recapitulating late-onset human diseases and physiological processes such as brain ageing is restricted. iPSC-derived neurons overexpressing Progerin, a protein associated with premature ageing, transiently develop cellular ageing hallmarks.

However, the impact of Progerin-mediated premature ageing in a complex 3D brain organoid model remains unknown.

Here we report an engineered human iPSCs line overexpressing Progerin in a Doxycycline (Dox)-inducible manner that can give rise to artificially aged brain organoids. Transgenic iPSCs produced morphologically homogeneous organoids for up to three months without apparent changes in cellular composition. We found Dox-inducible Progerin overexpression to yield various hallmarks of ageing. We observed a 43% loss of H3K9me3-marked heterochromatin, and a 2-fold increase of dsDNA breaks judged by γH2Ax. 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 showing, among others, roles in exocytosis and synaptic signalling (VAT1, RAP1B, HSPA8). Notably, a proportion is identical to age-related genes reported in human post-mortem tissue. Additionally, methylome analysis showed clear clustering of Progerin-induced organoids versus controls and overlaps with transcriptional changes.

Our results demonstrate that Progerin overexpression is a proxy for the induction of age signatures in neuronal lineages in brain organoids. We anticipate that our system will be helpful to model neurodegenerative phenotypes and could be exploited to develop in vitro models of ageing in different cell types.

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# Effects of Ischemia on the Migratory Capacity of Microglia Along Collagen Microcontact Prints on Organotypic Mouse Cortex Brain Slices

Ischemic stroke is a severe insult in the brain causing cell death, inflammation, and activation of microglia. Microglia are the immune cells of the brain and play a role in any inflammatory process during neurodegeneration. Microglia are round ameboid and migrate to the lesion site, where they differentiate into ramified forms and activated phagocytic microglia. The aim of the present study is to explore the migratory capacity of microglia after ischemic insults. Organotypic brain slices of the mouse cortex (300 µm) were prepared. In order to study migration, the slices were connected to collagen-loaded microcontact prints (with or without monocyte chemoattractant protein-1, MCP-1) on the membranes. Slices were stimulated with lipopolysaccharide (LPS) for maximal microglial activation. Ischemic insults were simulated with oxygen-glucose deprivation (OGD) and acidosis (pH 6.5) for 3 days. After 3 weeks in culture, slices were fixed and immunohistochemically stained for the microglial markers Iba1, CD11b and macrophage-like antigen. Our data show that Iba1+ microglia migrated along the microcontact prints, differentiate and phagocyte 1.0 µm fluorescent microbeads. LPS significantly enhanced the number of round ameboid migrating microglia, while OGD and acidosis enhanced the number of ramified activated microglia. The effect was not visible on slices without any µCP and was most potent in µCP with MCP-1. We conclude that OGD and acidosis activate ramification and exhibit a similar mechanism, while LPS only activates round ameboid microglia. Collagen-loaded microcontact prints connected to mouse brain slices are a potent method to study activation and migration of microglia ex vivo.

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# The Use of Augmented Reality as an Educational Tool in Minimally Invasive Transforaminal Lumbar Interbody Fusion

Objective: One of the major challenges in training neurosurgical and orthopedic residents the technique for minimally invasive transforaminal lumbar interbody fusion is the lack of visualization of surgical landmarks (pedicle, pars, lamina). Augmented Reality (AR) is an emerging technology, which superimposes digital images onto the real-world environment. The purpose of this study is to assess the utility, accuracy, efficiency, and precision of AR-guided MIS-TLIF and to determine its impact in spine surgery training.

Methods: At two centers, twelve neurosurgical residents performed a one-level MIS-TLIF on a high-fidelity lumbar spine simulation model with and without AR projection into the microscope. For the MIS-TLIF procedures with AR, surgical landmarks were highlighted in different colors on preoperative image data. These landmarks were visualized in the spinal navigation application on the navigation monitor and in the microscope in order to confirm the relevant anatomy. All procedures were recorded for evaluation and time measurements. Post-procedural surveys (NASA task load index) were given to the residents.

Results: 12 residents were included in this prospective, multi-center, randomized-controlled trial. AR-guided procedures had a consistent impact on resident anatomical orientation and workload experience. Procedures performed without AR had a significantly higher mental demand ($p=0.003$) than with AR. Residents reported to a significantly higher rate that it was harder work for them to accomplish their level of performance without AR ($p=0.019$).

Conclusion: AR can bring a meaningful value in MIS teaching and training in order to confirm relevant anatomy in situations where the surgeon will have less direct visual access.

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# Spreading of P301S aggregated tau investigated in organotypic mouse brain slice cultures

Background: Neurofibrillary tangles composed of hyperphosphorylated tau protein aggregates are a key neuropathological feature of Alzheimer's disease (AD). Tau pathology extends throughout the brain in a prion-like fashion through connected brain regions. However, the details of the underlying mechanisms are incompletely understood. The present study aims to examine the spreading of P301S aggregated tau, a mutation that is implicated in tauopathies, using organotypic slice cultures.

Methods: Coronal hippocampal organotypic brain slices (170 µm) were prepared from postnatal (day 8–10) C57BL6 wild-type mice. Collagen hydrogels loaded with P301S aggregated tau were applied to slices and the spread of tau was assessed by immunohistochemistry after 8 weeks in culture.

Results: Collagen hydrogels prove to be an effective protein delivery system subject to natural degradation in 14 days and they release tau proteins up to 8 weeks. Slices with un- and hyperphosphorylated P301S aggregated tau demonstrate significant spreading to the ventral parts of the hippocampal slices compared to empty collagen hydrogels after 8 weeks. Moreover, the spread of P301S aggregated tau occurs in a time-dependent manner, which is interrupted when the neuroanatomical pathways are lesioned.

Conclusions: We illustrate that the spreading of tau can be investigated in organotypic

slice cultures using collagen hydrogels to achieve a localized application and slow release of tau proteins. P301S aggregated tau significantly spreads to the ventral areas of the slices, suggesting that the disease-relevant aggregated tau form possesses spreading potential. Thus, the results offer a novel experimental approach to investigate tau pathology.

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# The mediating role of resilience and extraversion on psychological distress during the COVID-19 pandemic

Background: Imaging techniques have revealed important aspects of the underlying pathophysiological mechanisms of migraine, suggesting abnormal energy metabolism and increased cerebral hyperexcitability as triggers for a migraine attack. Since only a small percentage of monoclonal CGRP antibodies crosses the blood-brain barrier, their main site of action outside the blood-brain barrier is discussed. It is uncertain whether they lead to central effects through their action outside the blood-brain barrier or exert direct central effects.

Objective: To investigate whether neurochemical, structural, and functional changes in the migraine brain are associated with CGRP-antibody treatment.

Methods: This prospective, randomised, controlled, open-label study will enrol 38 patients diagnosed with episodic migraine (w/o aura) according to ICHD-3 criteria. All participants will undergo an initially stratified 1H-, 31P- MR-Spectroscopy and resting-state fMRI interictally. Half of the participants (n=19) will receive CGRP mAB treatment (Fremanezumab 225mg monthly) after the first scan for three months according to local standard guidelines for CGRP mAB treatment. MR-spectroscopy and resting-state fMRI will be repeated after the treatment. Controls will be measured in an identical setting at the same time points but without CGRP mAB treatment.

Future aspects: Investigating the effects of CGRP mAB on the metabolism and its association to functional connectivity in the migraine brain provides in-depth knowledge about the mechanism of action of the CGRP antibody and permits individualized treatment.

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# Sex specific aspects of clonal hematopoiesis and inflammation in a large stroke cohort

Introduction: Acquisition of spontaneous somatic mutations in a well defined set of genes (mainly DNMT3A, TET2 and ASXL1) leads to clonal expansion of myeloid cells (Clonal hematopoiesis of indeterminate potential/CHIP), which drives cardiovascular disease via a genetically imprinted pro-inflammatory phenotype. How sex influences systemic inflammation and clinical outcome in patients with ischemic stroke in the context of CHIP is unknown.

Methods: NGS and flowcytometry were performed using whole-blood from ischemic stroke patients. Cytokines were measured in plasma.

Results: Preliminary results for 247 patients were available for analysis (32.8% women, n = 81). Men and women had similar rates of somatic variants (75% vs 73%, p=0.8), with the most frequent mutations in DNMT3A (men 27% vs women 37%), TET2 (12% vs 17%) and ASXL1 (6.8% vs 1.2%). Men with CHIP presented with higher rates of stroke from large-artery atherosclerosis (24% vs 10%), while women with CHIP had higher rates of ESUS (23% vs 2.4%, p=0.037), with no difference in stroke severity as measured by mRS (p=0.6) or NIHSS (p=0.6). We found no differences in plasma levels of CRP, IL-1β, IL-6, IL-8 or TNFa. Men had a higher Systemic Inflammatory Response Index (SIRI) (2.34 vs 1.29, p=0.006) and a lower Lymphocyte-to-Monocyte-Ratio (LMR, 2.22 vs 3.19, p <0.001. After 12-month, there was no difference in survival (p=0.98), recurring cerebral events (p=0.33) or cardiac events (p=0.3).

Conclusion: We identified clinical features (stroke etiology) as well as features of systemic inflammation (SIRI, LMR) differing between men and women with CHIP in stroke.

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# Sex disparities in Cystic Fibrosis: closer look at pulmonary function and inflammatory markers in nasal lavage samples

Rationale: Epidemiologic studies have demonstrated worse outcomes in women with cystic fibrosis (CF), thus defining the term "CF Gender Gap". Exact causes of this disparity are not fully understood and are part of numerous investigations. A correlation between pulmonary exacerbation and biofilm modulating effect of estradiol level with cycle dependence in women with CF has been described. Data on inflammatory markers regarding sex differences, especially in nasal lavage (NL), are scarce.

This is a subgroup analysis of the INFLAM-CF Study. During screening visit, pulmonary function values and laboratory analyses were collected and extraction of NL was performed.

Results: 22 patients any age group were included in the study (11 male, 11 female). There is a significant difference in lung function clearance (LCI) and lung CT with better results in male participants. Likewise, there is a significant difference in inflammatory markers in serum. Analysis of NL samples did not show any statistical significance.

Conclusion: Our results regarding pulmonary function parameters confirm previous published data, showing that men with CF have better LCI, ppFEV and CT score values. Elevated pro-inflammatory cytokines such as IL10, IL6 and TNF alpha in serum emphasize the increased inflammatory processes in women compared to men. These cytokines could be used as markers in further studies of sex differences. Analysis of cytokines in NL show no differences in men and women. However, it must be kept in mind that this is a small population and further studies, especially regarding inflammatory lung biomarkers, are needed.

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# The role of dendritic cells in tumor-targeted therapy mediated anti-tumor immunity

Half of the melanoma patients carry a specific point mutation affecting BRAF, which constitutively activates the MAPK pathway. Targeted therapy using inhibitors specific for mutant BRAF (BRAFi) elicts high response rates in melanoma patients. However, patients frequently relapse due to the development of therapy resistance.

Tumor-targeted therapy modulates the tumor immune microenvironment. However, the functional role of dendritic cells (DC) in anti-tumor responses by BRAFi remains elusive. As DC are crucial to initiate T and NK cell responses, we want to address which DC subsets are involved in immune modulation during treatment.

In order to understand the complexity of these cells, we designed a multicolor flow cytometry panel to clearly discriminate the DC compartment from other myeloid cells. We can show that treatment with BRAFi decreases immunosuppressive myeloid cells in the tumor microenvironment. Furthermore, this targeted therapy recruits activated DC to the inflammatory tumor milieu, which subsequently migrate in an increased frequency to the tumor-draining lymph nodes. In addition, by using Zbtb46-GFP mice, a DC-specific reporter mouse strain, we identified a CD64+ DC population in tumor-draining lymph nodes. We are currently investigating the function of this cell population in more detail to gain insights about the relevance in anti-tumor immunity, e.g. antigen presentation and cytokine production.

The detailed phenotypcial and functional characterization of tumor-infiltrating DC as well as migratory DC subsets in the draining lymph nodes will give us valuable insights whether alterations in DC function contribute to resistance development. With this knowledge novel combinatorial therapies can be developed.

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# Does aberrant glutamate metabolism in melanoma affect dendritic cell function?

Tumor immunity is negatively regulated by metabolites in the tumor tissue (TME). Metabolic reprogramming impacts activation and maturation of dendritic cells (DC). We work on the transgenic melanoma mouse model tg(Grm1)EPv, which spontaneously develops melanoma due to an overexpression of the metabotropic glutamate receptor 1 in melanocytes. This aberrant glutamate metabolism might affect immune cell function in the TME. We investigate metabolic changes in progressing melanoma focusing on glutamate metabolism and its possible effects on tumor-infiltrating DC and T cell responses.

Screenings of the tg(Grm1)EPv mice for metabolic changes at different stages of disease showed decreased glutamate levels with tumor progression.

To confirm these findings and extend analyses to other metabolites, we are screening tg(Grm1)EPv mice for metabolic changes with LC-MS technology. There appears to be a decrease in most TCA cycle and glycolysis metabolites during tumor-early and tumor-advanced stages. The lactate:pyruvate increases during tumor progression. This increase might indicate a disruption of the respiratory chain that we are planning to investigate with high-resolution respirometry.

We are performing detailed analyses of myeloid subsets in tumors and draining lymph nodes during tumor progression with multi-color flow cytometry. An initial investigation of DC precursors showed possible alterations in the bone marrow of tumor-bearing mice.

Further investigations will focus on alterations in the DC function and differentiation.

Acquired knowledge can benefit the design of novel therapeutic strategies for cancer patients involving potential modifications of tumor glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might improve response rates in cancer patients.

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# Changes of Glutathione excretion during normothermic machine perfusion of livers and potential influencing transplant factors

Background: Normothermic machine perfusion (NMP) is a diagnostic tool for liver assessment prior to transplantation. Recent literature supports the importance of bile composition during liver NMP. Glutathione (GSH) is known to be one of the major determinants of bile flow. It is known that biliary GSH efflux is impaired after ischemia. Our aim was to analyse bile GSH in NMP livers and hypothesize that it correlates with transplant factors.

Methods: Fifty-eight NMP livers were included in the study. After one and four hours, as well as at the end of the perfusion bile was analysed biochemically. An ELISA assay was performed to detect changes in GSH content during perfusion and correlated with clinical and perfusate parameters.

Results: Overall, 41 of 58 perfused livers were transplanted after NMP. Total preservation time was 22.5±6.7 hours, NMP duration was 16.1±6.3 hours and mean cold ischemia time 6.3±2.6 hours. The GSH content in bile showed a significant increase over time (hour 1: 24.1±30.3µM vs. end: 96.6±85.0µM, $p<0.001$). GSH after one hour of NMP correlated negatively with bile lactate ($p=0.018$). GSH at four hours correlated significantly with bile pH ($p=0.0391$), bile bicarbonate ($p=0.047$) and negatively with the difference of perfusate lactate and bile lactate ($p=0.019$).

Conclusions: Our data indicates that there is an active increase of GSH in bile during liver NMP and GSH content correlates with bile composition. The negative correlation between lactate levels and GSH content might indicate the important role of GSH in protecting cells from oxidative damage and maintaining redox homeostasis.

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# Intensity of cholestasis and cytolysis in rat liver under the influence of different doses and duration of chlorpromazine administration

Chlorpromazine is one of the most commonly used drugs in psychiatric practice. As reported chlorpromazine has a distinct hepatotoxic effect.

Materials and methods. Chlorpromazine was administered to adult rats intragastrically once daily for 30 and 60 days at doses of 3.5 mg/kg, 7 mg/kg, 14 mg/kg and 21 mg/kg. At the end of the experiment, blood samples were taken from all rats for further biochemical studies.

The cytolysis syndrome was assessed by the level of alanine aminotransferase and aspartate aminotransferase. Cholestatic syndrome was assessed by the level of alkaline phosphatase, gammaglutamyltransferase, total and direct bilirubin, total cholesterol. The study of blood serum was performed on a biochemical analyzer "Vital Microlab 300" (USA), reagents from Pointe Scientific Inc (USA) were used.

Results and conclusions. A direct and reliable correlation between biochemical parameters and dose of Chlorpromazine was established, indicating the severity of liver damage. Cytolysis and cholestasis syndromes have a clear dependence on the dose of the drug administered: the higher the dose of chlorpromazine, the more pronounced the biochemical markers characterizing these syndromes. A longer period of drug administration contributed to the development of a more pronounced cytolysis syndrome in rats, which is explained by an increase in indirect liver damage by chlorpromazine with prolonged administration. Instead, cholestatic changes tended to weaken over time, which can probably be explained by the modification of the cytochrome P-450 system in the biometabolism of Chlorpromazine, and the gradual inclusion of compensatory and adaptive mechanisms for the administration of xenobiotic.

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# Development of a multiplex immunofluorescence staining for immune-profiling of formalin-fixed and paraffin-embedded human skin tumor tissue

Tumor infiltrating immune cells can strongly affect the development and progression of cancer as well as the responsiveness to therapy. We investigated the microenvironment of different skin cancer types such as actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and melanoma. The presence and localization of dendritic cells (Langerin, CD1a+) and T cells (CD3+) within the tumor microenvironment were analyzed using immunofluorescence. For this purpose, we retrieved formalin-fixated paraffin-embedded samples of different skin tumor types from the archives of the Department of Dermatology, Medical University Innsbruck, and performed immunofluorescence stainings with antibodies against CD1a and CD3. We found CD1a+ dendritic cells and CD3+ T cells in different locations. Higher levels of CD1a+ dendritic cells than CD3+ T cells were detected within the tumor and the epidermis. Most CD3+ T cells were in close vicinity to the tumor margin.

Our next aim is to gain data from the established multiplex staining of dendritic cells, T-cells and NK cells in the tumor microenvironment. In addition, we want to compare these data with data from flow cytometry analysis obtained from the same tumor.

The architecture of normal and diseased tissues strongly influences the development and progression of disease as well as responsiveness and resistance to therapy.

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# Development of automated synthesis and quality control for [177Lu]Lu zadavotide guraxetan

Aim: PSMA-targeted therapy with the radioisotope 177Lu has become an important cornerstone in the treatment of metastatic castration-resistant prostate cancer. The two most prominent radiopharmaceuticals employed are [177Lu]Lu vipivotide tetraxetan and [177Lu]Lu zadavotide guraxetan. In-house production of these drugs is common, although to date official quality standards have not been published in the European Pharmacopeia.

We present data for a new automated synthesis method and quality control of [177Lu]Lu zadavotide guraxetan.

Methods: Radiolabelling of zadavotide guraxetan with [177Lu]LuCl3 was performed on a Modular-Lab PharmTracer® synthesis module with custom-made disposable cassettes.

For quality control instant thin-layer chromatography (ITLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) among other tests were performed immediately after production and at certain time points post-production to assess stability.

Results: Radiochemical purity (RCP) determined with HPLC was 96.9±0.5 % and 96.3±0.9 % for each method, respectively. After 48 h RCP was still above 95 %, if samples were diluted with ascorbic acid solution and stored at -20 °C. ITLC on silica gel with sodium citrate 0.1 M as mobile phase showed only traces of unbound 177Lu (< 0.1 %).

Conclusion: Results from quality control confirm the non-inferiority of our synthesis method without solid-phase extraction purification.

Concerning quality control, HPLC with phosphate buffer/acetonitrile on both columns demonstrates robust results and peaks without tailing as seen with commonly used TFA solvents. If combined with ITLC with sodium citrate solution, radiochemical purity can be assessed reliably.

Stability of the product formulation over 48 h could be confirmed if stored at -20 °C.

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# Consequences of α2δ subunit mutations linked to brain disorders on neuronal calcium channel trafficking and synapse composition

Recent literature suggests an important role of α2δ proteins in synapse formation beside their role as auxiliary subunit of voltage-gated calcium channels. Therefore, it is not surprising that α2δ proteins have been linked to various neurological and neuropsychiatric disorders.

Here we aimed to investigate human mutations in α2δ proteins by addressing their synaptic functions, besides their role as channel subunit, to shed light on the underlying pathophysiological mechanisms.

We characterized two mutations, the autism-associated mutation p.Arg351Thr in α2δ-1 and the epilepsy-related mutation p.Arg596Pro in α2δ-2, by employing primary cultured hippocampal neurons and tsA201 cells as homologous and heterologous expression system, respectively. First, we quantified plasma membrane trafficking and analyzed potential consequences on synapse composition. Second, to determine potential effects on the biophysical channel properties, we performed electrophysiological recordings of either CaV2.1 or CaV1.3 expressed together with wildtype or mutated α2δ and auxiliary β subunits in tsA201 cells. In addition, we studied the consequences on presynaptic Ca2+ transients in response to action potentials.

Live-cell labelling of cultured hippocampal neurons transfected with α2δ subunits revealed a significant reduction in membrane expression as well as in synaptic targeting of both mutants. Surprisingly, the mutations did not affect the current properties of the presynaptic CaV2.1 channel but modulated currents of the L-type channel CaV1.3. Finally, the two mutations differentially modulated trans-synaptic signaling of α2δ splice variants lacking exon 23.

Taken together, our data show that disease-associated α2δ mutations can alter channel-dependent as well as synaptic functions, both of which may contribute to pathophysiological mechanisms.

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# Effects of HIF-1α stabilisation with Roxadustat on cardiac repair after myocardial infarction in mice

Background: The homing of CXCR4+ cells through CXCL12 activation is known to facilitates myocardial repair. Physiological upregulation of CXCL12 lasts only for 48-72 hours, which limits its reparative potential. After this time period, pathological remodeling processes lead to ischemic cardiomyopathy and heart failure. CXCL12 and CXCR4 are target genes of the Hypoxia-induce factor 1 alpha (HIF-1α), which in turn is stabilized under hypoxic conditions. Our intention is to stabilize HIF-1 α by administration of the prolyl hydroxylase inhibitor (PHI) Roxadustat over 28 days after myocardial infarction to prolong CXCL12 and CXCR4 upregulation and amplify myocardial repair.

Methods: CXCL12 and CXCR4 expression were analysed in vitro with human umbilical vascular endothelial cells (HUVEC) after administration of FG-4592 (20µM to 1000µM). Western Blots were performed and effects on HIF-1α -signaling pathway were analysed and quantificated by densitometry. To estimate the effects of the HIF-1α upregulation after induction of myocardial infarction in vivo, optimal doses (50mg/kg i.p.; 4 cycles every 72h) Roxadusat were administered to Bl6/J mice. A group of 10 mice were treated with Roxadustat and compared to a placebo-treated control group by measuring the cardiac function 28 days after myocardial infarction by echocardiography.

Results: In our preliminary analysis there was a significant preserved ejection fraction (EF) in Roxadustat treated mice after MI (49,5%±4,3 vs. 30,4%±11,1; n=10/12; p=0,004) compared to the placebo-treated group.

Conclusion: Roxadustat appears to have a favourable effect on the course of healing after myocardial infarction. However, it remains to be ensured that all hearts were actually infarcted.

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# Gender-related illness perceptions in COPD patients

Background

COPD is a progressive and mainly irreversible pulmonary disease characterized by respiratory symptoms. Although clinical symptoms may be similar, evidence suggests that men and women may differ in their perception of illness and its impact on their daily life.

The aim of this pre-analysis was to evaluate gender-related illness perception in COPD patients.

Methods

This study represents a pre-analysis of The Brief Illness Perception Questionnaire (BIPQ) as part of the CRITIC-Study, a prospective observational study. The BIPQ is a 9-item questionnaire designed to rapidly assess cognitive and emotional illness perception. The following cut-off levels for the total BIPQ-score were considered: < 42 (low experienced impairment/threat due to the disease), 42-49 (moderate impairment/threat) and ≥ 50 (high experienced impairment/threat).

Results

In total 38 questionnaires (28 (73.7%) of men and 10 (26.3%) of women) were evaluated. The mean age was 65.9 (SD 9.1) years (men 66.4 (SD 9.4), women 64.9 (SD 8.6)). Two-thirds (66%) of patients were experiencing substantial symptom burden (COPD group B or D), comprising 80% of women and 61% of men (p=0.27). According to BIPQ, half of the patients reported being only mildly affected by COPD (54% of men, 40% of women, p=0.46), whereas about one-fifth claimed to be severely impacted by the disease, including 14% of men and 40% of women (p = 0.09).

Conclusion

Although not significant, we could show a trend for women to present with a higher symptom burden and to be more cognitively and emotionally impaired by COPD compared to men.

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# Evaluation of the clinical impact of the Ceruloplasmin variant p.Thr551Ile in liver cirrhosis

Background: Aceruloplasminemia is a recessive disease, caused by pathogenic variants in ceruloplasmin (CP) and is characterized by iron accumulation in liver, pancreas and brain. Heterozygous CP variants have been identified to predispose to hyperferritinemia, hepatic siderosis and fibrosis progression in patients with non-alcoholic fatty liver.

Methods: A cohort of patients referred to the Hepatology Laboratory (Medical University Innsbruck) for PNPLA3 genotyping was retrospectively assessed. Patients diagnosed with cirrhosis and available serum CP concentrations were included (n=568). Demographic, biochemical and clinical parameters were collected. Genotyping for the CP variant p.Thr551Ile was performed by allelic discrimination PCR.

Results: Genotyping results revealed 2 homozygous and 27 heterozygous patients for p.Thr551Ile corresponding to an allele frequency of 2.73% in the liver cirrhosis cohort, which is not different from the general population (according to gnomAD: 2.76%). Biochemical surrogates of liver disease severity and serum iron parameters did not show significant differences when patients were stratified according CP genotype. Reduced CP concentrations were detected in 17.2% of patients carrying the variant and in 11.7% in the normal group. Median time of transplant-free survival was significantly reduced in the group with decreased CP concentration, but not when patients were stratified according CP genotype. Cox-regression analysis showed that CP serum concentration but not p.Thr551Ile genotype was an independent predictor of transplant-free survival.

Conclusions: Reduced serum ceruloplasmin is common in patients with liver cirrhosis and independently associated with reduced transplant-free survival in unselected patients with cirrhosis. The variant p.Thr551Ile shows no association with serum iron parameters or transplant-free survival.

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# Sex differences in Diabetic Kidney Disease: An analysis based on the Provalid Study

Data on how sex affects clinical presentation (proteinuric vs. non-proteinuric phenotype) and progression of diabetic kidney disease (DKD) is inconsistent. Our aim was to investigate sex differences in DKD based on data of the Provalid study, a prospective, observational cohort study in patients with type 2 diabetes (T2DM) in Europe.

Progressive kidney function decline was defined as a sustained drop of eGFR >40%. Progression of albuminuria was defined as a sustained increase in albuminuria of at least 30% including a transition in albuminuria class.

In total, 3131 patients were included. 43% of study participants were female. Median follow up time was 4.4 years [1st, 3rd quartile; 3.1, 5.7], median duration of T2DM at inclusion was 9.0 years [4.0, 14.0] in female vs. 8.0 years [4.0, 13.0] in male participants (p=0.002). Prevalence of DKD was significantly lower in female participants (66.4% vs. 70.5%, p=0.004). At baseline, 82.9% female vs. 76.6% male participants have normal to mildly increased albuminuria (p<0.001). Treatment with RAS-Inhibitors was similar for both groups (67.0% females vs. 67.5% males, p=0.757). The Odds Ratio (OR) for progressive kidney function decline was 1.95 (95% CI 1.2 – 3.1, p=0.006) for female compared to male participants. The OR for a progression of albuminuria was 0.74 (95% CI 0.56 - 0.98, p=0.034) for female compared to male participants.

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# Albuminuria Variability in Diabetic Kidney Disease

Diabetic kidney disease (DKD) is a heterogenous and complex entity. To allow for concise classification, assessment of eGFR and albumin excretion is recommended. Correct assessment of albuminuria is impeded through its high biological variability. Additionally, the ideal classification confirmation strategy is somewhat controversial.

We calculated the coefficients of variation (CV) of the albumin-creatinine-ratio (ACR) in a cohort (n= 776) of European patients with Type 2 Diabetes mellitus. Additionally, we compared the diagnostic quality of different confirmation strategies with the gold standard, the two-out-of-three method.

The median CV of the ACR for the overall cohort is 41%. The median CV of urinary creatinine is much lower than the corresponding CV of urinary albumin (25.9% vs. 45.4%). Baseline characteristics that are associated with a higher CV of the ACR are, among others, higher age, duration of DM, eGFR and amount of albuminuria influencing drugs. The agreement of other classification strategies with the two-out-of-three method is partly suboptimal, in general, the less samples are used, the worse the agreement. There were no clear associations of "misclassification" compared to the two-out-of-three-method with demographic variables.

In conclusion, a solid understanding of the characteristics, variability and limitations of a classification variable are needed to allow for a clinically meaningful and evidence-based usage in patient care. Our data will provide some information regarding albuminuria in DKD staging.

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# Molecular mechanisms of hypophosphatemia after intravenous iron therapy

Background: Administration of the intravenous (IV) iron formulation ferric carboxymaltose (FCM) results in hypophosphatemia due to high FGF23 serum concentrations in the majority of treated patients with normal kidney function. We aim to identify the molecular mechanism causing hypophosphatemia after IV iron therapy.

Methods: FCM, ferric derisomaltose (FDI) and iron dextran (ID) were investigated in charge and phosphate-binding properties by isoelectric focusing, ion exchange chromatography and size exclusion chromatography (SEC). The impact of different IV iron formulations on DMP1 binding to its cell surface receptor αVβ3 integrin was assessed by ELISA and radio-ligand binding studies. DMP1-αVβ3 integrin signaling was investigated in osteoblastic precursor MC3T3-E1 cells with LC-MS/MS and western blotting.

Results: At physiological pH, FCM was positively charged, while FDI and ID were negatively charged. When co-incubated with phosphate buffer only FCM changed its charge properties. High-affinity phosphate binding by FCM was confirmed by SEC followed by phosphate quantification. Phosphoproteomics analysis of MC3T3-E1 cell extracts revealed that treatment of cells with DMP1, a negative regulator of FGF23, causes activation of the integrin signaling pathway via the MAP kinases. FCM but neither FDI nor ID, strongly reduced DMP1 binding to αVβ3 integrin, as well as ERK phosphorylation as an indicator of activation of the MAP kinase activity.

Conclusion: We demonstrate that FCM inhibits the DMP1 binding to αVβ3 integrin and thus the activation of the MAPK pathway in osteoblast precursor MC3T3-E1 cell line. This together with the high affinity of FCM to phosphate could be a possible explanation for FCM-induced hypophosphatemia.

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# Organ manifestations of patients with systemic sclerosis: a gender-specific update from the COLIPRIS-registry

Introduction: Systemic sclerosis is a systemic connective tissue disease characterized by progressive fibrosis primarily affecting the skin with various organ involvement. Women are more commonly affected than men; however, severe organ manifestations seem to be more common in men.

Methods: This prospective observational study of systemic sclerosis patients aims to gather clinical examination, laboratory testing, lung function parameters, and radiologic findings at baseline, six and twelve months to characterize the study cohort and identify the prevalence of organ manifestations and gender-specific aspects. Long-term follow-up analyses are planned for three, five, and ten years after study entry.

Results: Currently, 64 patients with systemic sclerosis were included in the analysis, 57 (89%) females and 7 (11%) males with a mean age of 48 (2%) and 54 (3%) years at study entry. The mean disease duration at study entry was 130 (18%) months in females and 88 (%22) months in males. At baseline, computed tomography showed signs of interstitial lung disease in 19 (44%) of women and 5 (71%) of males. Follow-up CT scans at twelve months displayed similar results, with 16 (55%) females and 2 (67%) males affected. 34 (59%) females and 2 (33%) males showed esophageal motility disorders, while cardiac involvement was rarely observed.

Discussion: Males develop interstitial lung disease early in the disease course, while females initially often present with esophageal involvement. A gender-specific approach in treating systemic sclerosis patients and large prospective trials focusing on the impact of gender on disease course are warranted.

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# Determining factors of adherence to daily monitoring by patients in a pediatric oncology setting

Background: Telemedical assessment of patients' symptoms during therapy can enable early symptom detection and thereby better care. However, to maximize clinical usability of the data, patients need to regularly complete assessments. At the Pediatric Oncology unit in Innsbruck, childrens' symptoms are assessed daily during anticancer therapy. This exploratory analysis investigates how completion rates change over time and which clinical factors influence regular assessment completion.

Methods: Patient data from the Pediatric Oncology unit at the LKI Innsbruck between 11.5.2020 and 15.11.2021 were analyzed. Patients completed daily symptom assessments using a web-based patient portal. We analyzed patient assessment completion during their first year of telemonitoring using a linear mixed model. Factors included in the model were patient age, time, admission status, and patient and clinician-reported symptom severity.

Results: 35 patients (65.7% male) were included in the analysis. Patients were 10.3 (SD = 3.9) years old on average. Median time in monitoring was 148 days and median daily assessment completion rate 58.1%. Significant predictive factors for a decrease in completion rate were time in telemonitoring and admission to the intensive care unit. Hospitalization to the normal unit was a significant predictor of increased completion.

Discussion: Our findings show high completion rates for daily monitoring in children can be achieved even over longer time periods. The most relevant factor for noncompletion was time, indicating that long-term solutions to keep participation high are needed. Future research should focus on investigating and testing different methods to improve completion rates.

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# Gender disparities in living donor liver transplantation: a single centre experience

Rationale

Organ donation brings together complex medical, social and ethical aspects. In the last decades, living liver donation became increasingly important. Generally, parents or close relatives serve as donors. In this study gender aspects of organ transplantation, more specifically gender differences in parental donors are addressed. Therefore, information on all patients who underwent liver transplantation (LT) for biliary atresia at the University Hospital Innsbruck during the last 23 years (1997-2020) and their donors were collected and analyzed.

Results

49 patients were included with LT at median age of 8 months (IQR=4 months). The sex ratio was 3:2,5 female to male. In 91,8% living donor liver transplantation was performed, in 3 cases close relatives donated. Overall fathers donated less often than mothers (36,7% vs. 49%). However, if we look at the gender ratio in different periods, between 1997-2009, twice as many mothers donated as fathers; whereas between 2010-2020, the ratio was balanced at 1:1.

Conclusion

In a retrospective single-centre analysis mothers were more likely than fathers to donate a part of their liver to their child. Overall, however, the gender difference in parents as living donors has narrowed over the past twenty years.

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# Gender differences in oncological outcome in KRAS- and EGFR-mutated NSCLC

Lung cancer remains the leading cause of cancer related deaths worldwide. As gender differences are becoming a rising interest, its influence on oncologic outcome for patients with KRAS- and EGFR-mutated NSCLC is unknown, also if Wnt signaling pathway amplifies this.

Immunohistochemical staining of proteins in the KRAS- and Wnt-pathways on tumor specimens of early stage lung cancer are correlated with oncologic outcomes retrospectively.

In total, 39 patients' samples were analyzed including 24 female patients and 15 male patients. The median age of the total cohort was 67 years (range 47 – 82). Women had a significantly higher incidence of mutations (KRAS and EGFR) compared to male patients ($p = 0.036$). The majority of the wildtype subgroup were male patients ($n = 8$; 62%). LRP-6 and cMyc expression was higher in female patients but not statistically significant.

One, 3- and 5-year survival of all patients was 100%, 96% and 89%. In the KRAS-mutated subgroup, the 1- and 3-years survival was 91% and 64%. Gender did not influence survival in both analyses.

One and 3-year disease-free survival (DFS) was 89% and 68%. In the KRAS-mutated group, the 1- and 3 years DFS was 91% and 64%. The 1- and 3-years DFS in the EGFR-mutated group was 75% and 54%. In both groups, there was no difference in gender.

Women did show a higher rate of genetic-driven NSCLC. However, in this study of early stage lung cancer, we did not find any disparities in terms of oncologic outcome with respect to gender.

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# Investigating the role of Fumarylacetoacetate hydrolase domain containing protein 1 (FAHD1) in the mouse heart

The eukaryotic protein fumarylacetoacetate hydrolase domain-containing protein 1 (FAHD1) has recently been described as mitochondrial oxaloacetate decarboxylase (ODx), which contributes to the regulation of oxaloacetate levels in the mitochondria. In our model, depletion of FAHD1 increases the levels of mitochondrial oxaloacetate. It is commonly known that oxaloacetate is a competitive inhibitor of succinate dehydrogenase (SDH, COMPLEX II of OXPHOS machinery), and decreased COMPLEX II activity would directly result from lower FAHD1 activity. This has recently been demonstrated for breast cancer cells, like BT-20, where FAHD1 appears to be essential for cell survival.

Here we present preliminary data on the role of FAHD1 in the mouse heart. In our model, Fahd1-KO promotes a delay in the development of the heart from fetal to post-natal, as suggested by assessment of several heart development markers. Both RNA seq. and qPCR data have confirmed high expression of typical embryonic genes, like myosin heavy chain 7 (Myh7), in 2 month-old Fahd1-KO hearts. We also measured reduced sarcomeric distance, another developmental marker in Fahd1-KO isolated cardiomyocytes (CM). Reduced sarcomeric distance could contribute to the systolic dysfunction observed in Fahd1-KO mice, which may well lead to premature cardiomyopathy in older mice. In parallel, Fahd1-KO CM showed reduced mitochondrial fitness, and COMPLEX II-activity, similarly to the defect we previously observed in breast cancer cells.

Our data suggest that FAHD1 may play a decisive role in heart development, especially in the fetal to postnatal transition, and heart physiology, demonstrating how critical it is to understand the underlying molecular mechanisms.

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# The Role of p14 in Iron Acquisition

Macrophages play a crucial role in iron homeostasis by recycling iron from erythrocytes, which is then further use to nurse new forming ones.

p14, part of the LAMTOR complex, has been associated with endocytic traffic as well as with mTOR and MAPK signaling. In this research, we used mice with a macrophage-specific p14 deletion (p14 KO mice) to study the role of p14 in iron metabolism. For that, we isolated bone marrow-derived macrophages (BMDMs), treated them with iron and performed q-PCRs, atomic absorption spectrometry, western blot and, flow cytometry analysis, among others.

We show that p14 KO mice experience an iron-deficiency anemic phenotype, characterized by low amount of red blood cells, but hold an excess of iron in the spleen and serum. In vitro experiments show that p14 KO BMDMs contain significantly less iron than WT, which in-turn leads to an impair expression of iron-related proteins and RNA. Flow cytometry analysis demonstrates that p14 KO BMDMs seem to express more Transferrin receptor 1 (TfR1) on the membrane than WT but lower protein level of TfR1 in general. Finally, p14 KO BMDMs appear to experience an impaired endocytosis of beads and less transferrin uptake than WT.

In conclusion, p14 loss in macrophages seems to impair TfR1 endocytosis or recycling, leading to a dysfunctional iron uptake in p14 KO BMDMs that leads to iron-deficiency anemia in mice. However, more data is needed to fully understand the mechanism behind p14 and TfR1 endocytic traffic and the implications for that in iron metabolism.

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# YOUhealTH – a randomized controlled interventional study to improve cardiovascular health in adolescents and adults

Background: Recent studies have shown that cardiovascular disease (CVD) already starts in early live. The majority of CVDs is preventable by timely elimination of responsible risk-factors. However, effective primary prevention strategies are scarce and lack evidence, especially in adolescents. Participative, group-based intervention-studies have shown promising results. The YOUhealTH-study aims to test a lifestyle-intervention focusing on diet and physical activity in students and at least one legal guardian to improve cardiovascular risk factor profiles.

Methods: The YOUhealTH-study is a single-center prospective cluster-randomized controlled parallel 2-arm intervention study with a planned participation of 150 – 200 adolescents (14 to 17 years) and at least one legal guardian each from 6 different Tyrolean schools. Participants will be assessed in a baseline examination. This will include questionnaires, measurements of anthropometrics, blood pressure, pulse-wave-velocity, bioimpedance, transient-liver-elastography and sonography of the carotid artery, the aorta and the abdominal fat tissue. Additionally fasting blood sampling and analysis of the mother-child booklet will be performed. Participants of the intervention-group will undergo a health-promotion developed by citizen scientist (students of the respective school) for the duration of 1 year. The efficacy of the health-promotion, measured by means of change for the components of diet and physical activity, will be evaluated and compared to the control-group in a follow-up-examination identical to the baseline-examination.

Discussion: The aim of the YOUhealTH-study is to test the efficacy of a health promotion intervention over the course of 1 year to improve cardiovascular health of adolescents (14-17 years) and at least one legal guardian.

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# Ex vivo organ-perfusion/conservation of metastatic livers – a novel cancer model

BACKGROUND: Cancer research is constrained by insufficient pre-clinical models, such as artificial in vitro systems or animal studies, lacking the complexity of the human tumor microenvironment (TME). In the present study, we aim to establish a novel ex vivo tumor model of colorectal liver metastases using normothermic machine perfusion (NMP) and assess its potential use for cancer research.

METHODS: Surgically resected livers/liver sections harboring colorectal metastasis were subjected to NMP (OrganOx Metra) for up to 8 days. Biopsies of the cancer lesion and the adjacent liver tissue were collected during ex vivo machine-perfusion. Comprehensive mapping of the tumor compartment as well as the adjacent liver tissue was performed using single cell RNA sequencing, multidimensional flow cytometry (mFCM) and confocal microscopy.

RESULTS: We developed a single-cell atlas containing 74,899 cells comprising 20 samples (10 tumor samples and 10 adjacent liver tissue samples) from 5 patients. 13 different cell populations were detected, including a colorectal cancer (CRC) cell cluster with distinct gene expression pattern. We observed persistent immune cell infiltration into the tumor tissue. Remarkably, the composition of the TME as well as the cancer cell cluster could be preserved during ex vivo NMP. These findings were also validated by mFCM and confocal microscopy.

CONCLUSION: We aim to demonstrate that NMP of cancer organs is an outstanding, highly innovative experimental technique to study cancer biology ex vivo under nearly physiological conditions.

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# Establishment of a mixed tumor model to study the therapeutic potential of an immune-modulatory cargo (IMC)-expressing oncolytic virus

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 to stimulate the immune responses by eliminating 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 infectivity of tumors to VSV-GP infection in vivo. This in turn not only reduce oncolytic capacity but also limits the production of IMCs encoded by the virus making difficult to evaluate potential therapeutic efficacy. To overcome this limitation and enable therapeutic potential we established a tumor model by mixing transformed murine lung epithelial cells (TC-1) expressing HPV-derived oncoproteins and the VSV-GP permissive IFNalpha-receptor knock-out TC-1 (TC-1-IFNaRko) cells. Using this mixed model, we investigated the efficacy as well as the CD8+ T cell responses after treatment with VSV-GP and VSV-GP-IMC. Whereas virotherapy of the original TC-1 did not result in improved tumor control the mixed tumor model virus treatment showed a prolonged survival. Thus, we found an enhanced therapeutic efficacy using VSV-GP-IMC compared to VSV-GP treatment. This together with the phenotypic differences of activated CD8 T cells between VSV-GP and VSV-GP-IMC treatments supports an improved effect of IMC in mixed tumor model.

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# Individualized flow-controlled ventilation versus pressure-controlled ventilation in a porcine model of intra-abdominal hypertension – a prospective, randomized porcine study

Goal of the study

Flow controlled ventilation (FCV) guarantees a continuous and stable flow during the whole ventilation cycle. Coupled with direct intratracheal pressure measurement ventilator settings can be adjusted to achieve the highest dynamic compliance as a personalized approach. Aim of this study was to investigate individualized FCV compared to pressure-controlled ventilation (PCV) in a porcine model of intra-abdominal hypertension (IAH) with high ventilation effort.

Methods

To induce IAH a gas-tight tracheostomy tube was placed in the peritoneal cavity and air was insufflated in the abdomen until a constant pressure of 15 mmHg (IAH grade I) was reached via continuous positive airway pressure system. After one hour IAH grade II (20 mmHg) and subsequently IAH grade III (25 mmHg) were induced and kept constant for one hour each. Ventilation was either performed with individualized FCV or PCV.

Results and discussion

Oxygenation was similar in both groups at baseline and every subsequent IAH grade. However, respiratory minute volume was significantly reduced in FCV (7.6 vs 14.4, MD -6.8 (-8.6 to -5.1) l/min; $p<0.001$) to achieve normocapnia. Additionally, in FCV at each IAH step the resulting applied tidal volume was reduced and the respiratory rate increased as an expression of reduced available lung tissue at an increasing abdominal pressure. The calculated mechanical power (MP) was significantly reduced in FCV compared to PCV, where the mechanical impact of artificial ventilation was increasing at each IAH grade and most pronounced at IAH III (24.2 vs 57.6, MD -33.4 (-41.4 to -25.4) J/min; $p<0.001$).

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# A comprehensive study of wt and Omicron Mpro mutations

Nirmatrelvir is the first protease inhibitor developed against the SARS-CoV-2 main protease (Mpro) licensed for clinical use. Our aim is to safely identify and characterize mutations that confer resistance to this protease inhibitor (PI).

We engineered a chimeric vesicular stomatitis virus (VSV) that is fully dependent on Mpro activity. Replication of this chimeric VSV is thereby inhibited by nirmatrelvir. To safely generate Mpro mutations that confer resistance to the PI, we apply nirmatrelvir to chimeric VSV, select resistant mutants and sequence the Mpro. Characterization of the mutants includes resistance analysis with cellular-based assays and molecular modelling.

In a previously published study, we selected 39 different Mpro mutations, single or in combination. To deepen our understanding of the currently circulating strains, we generated a chimeric VSV bearing the Omicron Mpro sequence and again applied selection pressure with nirmatrelvir. We also applied further selection pressure to a previously generated resistant strain, Mpro-L167F. We obtained a total of 47 distinct mutations in the Omicron strain and 26 second-generation mutations in the L167F strain. We quantified the resistance phenotype of the most interesting mutations, which were chosen according to the proximity to the inhibitor binding site and coverage on sequence databases (GISAID). Moreover, we provide their putative mechanism of resistance by in-silico molecular modelling.

In this work, we were able to safely generate and characterize a panel of Mpro mutations, single or in combination, which will help to understand protease-inhibitor-resistant SARS-CoV-2 strains and inform treatment decisions.

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# The impact of curative cancer treatment on sexual health – Secondary analysis of the EORTC QLQ-SH22 phase IV study

Background: Cancer disease and treatment can severely impair patients' sexual health (SH). Hence, the EORTC recently developed a sexual health questionnaire for cancer patients. In this study, we present results from a secondary analysis of the EORTC QLQ-SH22 phase 4 validation study.

Objective: To investigate the impact of cancer treatment on SH in patients with curative treatment intend.

Methods: We extracted data of a subsample of cancer patients (n=335) with curative intent from the original study, complemented with additional data of breast cancer patients (n=59). We analyzed differences in SH of patients undergoing cancer treatment compared to survivors, and changes across the treatment trajectory.

Results: Compared to survivors, patients undergoing cancer treatment experienced less sexual satisfaction (p=.021, Cohen's d =.36) and libido (p<.001, d=.60) and had higher levels of fatigue (p<.001, d=.50). Importance of sexual activity, masculinity and femininity did not differ between patients on and off treatment. Treatment effects on sexual activity decrease with treatment discontinuation (p<.001, d=.50). In addition, patients receiving intensified treatments (including chemotherapy, radiation and/or endocrine treatment) report more treatment effects compared to patients undergoing surgery only.

Conclusion: As expected oncological treatment severely impacts on SH. According to our results, SH impairments increase with treatment intensity. SH domains such as sexual satisfaction and libido improve after treatment completion; however, for other aspects of SH (e.g. masculinity and femininity) no changes were reported. In clinical care, SH should be monitored during cancer treatment and beyond to meet patients' sexual health care demands and reduce symptom burden.

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# Iron Deficiency and Treatment Effects on Bone Muscle Axis

Background: Iron is critical for oxygen delivery in the skeletal muscle and essential for the respiratory chain and mitochondrial iron-sulphur cluster proteins. Consequences of iron deficiency on skeletal muscle and energy metabolism are poorly understood. Expression of genes, metabolomics analysis and muscle fiber typing were investigated in animal models of iron deficiency (ID) and iron deficiency anemia (IDA).

Methods: ID and IDA was induced in C57Bl/6 mice by iron deficient diet and phlebotomy. Mice fed a normal diet were used as controls. Hemoglobin (Hb) concentration was measured in all mice. Gastrocnemius, plantaris, soleus and the myocardium were used for further analysis. Gene expression was quantified by RT-qPCR. Metabolic changes were analyzed by LC-MS/MS. The fiber types, which have various energy sources, were visualized by immunofluorescence staining.

Results: Mean Hb concentrations were lower in ID and IDA mice. Differential gene expression patterns and specific changes in response to ID and IDA were observed in skeletal muscle and myocardium. Metabolomics data indicate a shift towards glycolytic and anaerobic metabolites in ID/IDA. Muscle fiber type staining showed a decrease of the slow-oxidative muscle fiber type in the plantaris of IDA mice when compared with controls.

Conclusion: Iron deficiency and iron deficiency anemia in the mouse model could be validated by Hb concentrations. RNA expression in myocardium is more affected than in skeletal muscle. Data of the metabolomic analysis and fiber-typing indicates a metabolomic and structural shift in skeletal muscle. The specific changes observed suggest a strong effect of IDA on muscular energy metabolism.

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# The impact of resilience training versus body-oriented yoga on stress perception and depressive symptoms in patients with major depressive disorder: a randomized control trial protocol

Major depressive disorder (MDD) is often stress-associated and represents a serious, ever-growing problem for health care. Although pharmacotherapy, cognitive behavioral therapy (CBT), mindfulness interventions, and physiotherapy are effective in reducing symptoms and stress perception, new affordable alternative approaches are required. This study aims to evaluate the efficiency of the RASMUS resilience training for coping with stress compared to an active control condition of body-oriented yoga in outpatients with MDD. This is a randomized controlled trial with two parallel groups assigned to 10 x 60-minute training per week each. We plan to recruit 154 participants, 77 per intervention arm in three waves in two years and to complete the trial in the beginning of 2026. Eligible participants will be randomized in a 1:1 ratio to one of two instructor-led intervention groups and will learn the skills for coping with stress either with the RASMUS or the body-oriented approach. The primary outcomes are depression severity and stress perception measured by the Beck Depression Inventory and the Stress-Process Questionnaire, respectively. Secondary outcomes include the level of anxiety, resilience, quality of life, mindfulness and self-compassion. Assessments will be done at baseline, after the interventions, and in a 6-months follow-up. We hypothesize that RASMUS resilience training will lead to a greater reduction of depressive symptoms and stress perception compared to yoga in the short- and in the long-time perspective. The same effect is expected in regards of mindfulness, self-compassion, and quality of life.

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# The impact of the Spindle Assembly Checkpoint on sex specific B-cell development and function

One mechanism leading to genomic instability is the emergence of aneuploid cells as a consequence of errors during mitosis. Chromosomal instability (CIN) that precedes aneuploidy is prevented by the mitotic spindle assembly checkpoint (SAC) that blocks cell cycle progression in the presence of unattached kinetochores by inhibiting the anaphase promoting complex (APC). Deficiencies within the SAC frequently leads to cell death in healthy cells. Therefore it plays a vital role in B-cell development and B cell differentiation. The specific B-cell immune response in humans as well as in mice differs between male and female sex. Generally, females show higher levels of B-cell produced immunoglobulins. Furthermore, as a consequence of distinct sex hormone distribution between male and female sex, B-cell genes important for proper immune response are higher expressed in females than males. This leads to improved immune response for females when challenged by pathogens, but also a higher incidence of autoimmune disease like lupus erythromatodes.

To investigate the impact of CIN on sex differences in B-cell development, a mouse model was created where monopolar spindle kinase 1 (MPS1), a key-components of the SAC, was conditionally mutated, using floxed alleles in combination with Mb1-Cre.

We hypothesized, that due to differential gene expression between B-cells of the different sexes, B-cell numbers, antibody production and B-cell proliferation measured by Eμ-Myc driven lymphomagenesis is higher in female mice.

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# Sex differences in the therapeutic management of COVID-19 sepsis

Introduction

Although, systemic reviews showed more men were admitted to intensive care units (ICU) due to a SARS-CoV-2 infection (1), sex differences in treatments and outcome were rarely analyzed. Investigations in non COVID-19 cohorts showed sex specific differences (2).

Methods

Patients, who were admitted to a critically ill COVID-19 patients treating ICU in Tyrol in the period from 1st February 2020 until 18th July 2022, were included.

We determined interventions like invasive mechanical ventilation, renal replacement therapy (RRT) and the use of vasopressors as well as outcomes including acute kidney injury (AKI) and hospital mortality rate.

Results

1042 patients were included. Over all four waves, approximately two-thirds of the ICU admissions were men.

Female patients were significantly older, however no difference in disease severity, evaluated by SOFA score and SAPS III score at the time of ICU admission, could be observed.

Comparing the rates of therapeutic procedures, IMV and the requirement of vasopressors was nearly the same. The occurrence of AKI and performing RRT shows a trend to be more often performed in men, but not statistically significant.

It was shown that there were no statistically significant differences regarding sex differences in the hospital mortality.

Discussion

Age as a known risk factor for bad outcome was higher in female patients. However no significant difference in disease progression was observed between the sexes with respect to respiratory, hemodynamic, or renal deterioration and resulting therapies, which is also reflected in the similar mortality rates of the sexes.

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# Sex –related differences of inflammatory parameters after micro incision cataract surgery

Purpose

Microincision phacoemulsification surgery (MICS) is the standard of care for the treatment of the cataractous lens. Anterior chamber instability and/or posterior capsule protrusion remain the most important risk factors for complications like posterior capsular rupture and postoperative cystoid macular edema. This study aims to compare inflammatory parameters between two different surgical devices. In addition, the objective of this sub-study is to compare postoperative inflammatory ocular parameters between women and men.

Methods

Prospective, randomized, controlled, observational study involving 120 patients with mature cataract scheduled for MICS. Patients are operated by three experienced phacoemulsification surgeons using an identical technique, and are randomly included in one of two study arms using the DORC Eva phacoemulsifier or the Centurion-AS at comparable fluidics settings. All patients receive the same postoperative treatment.

Inflammatory ocular parameters are assessed pre- and postoperatively on day 1 and 7.

Results

Patient recruitment for this study is ongoing and 24 cases have been recruited by now, showing a significant trend towards the thickness increase of macular volume in women 7 days after surgery. The study is still ongoing and we expect to have half of the patients enrolled and evaluated by the publication date in April.

These results necessitate further analysis on the influence of sex-related differences on the final outcome.

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# Primary patient-derived microtissues (PMTs) for functional drug testing and prediction of immunotherapy efficacy

Introduction and aims:

Checkpoint immunotherapies (IO) have shown remarkable success in a subgroup of non-small cell lung cancer (NSCLC) patients. However, a significant patient population still don't benefit from IO.

Thus, we established a functional in vitro drug testing model to evaluate IO efficacy and to increase IO prediction.

Material and Methods:

Fresh tumor tissues were collected immediately after surgery from NSCLC patients. These samples were then stepwise dissected and enzymatic digested in order to obtain a single cell suspension. Next, primary derived microtissues (PMTs) were generated from this single cell suspension in ultra-low attachment plates. Conditions were optimized to maintain the original cell composition including the different immune cell populations. After tissue maturation (six days) they were treated with various chemotherapies, targeted agents, drug combinations and IO over 14-days. Treatment response was defined by growth inhibition or volume reduction of PMTs.

Results:

We established a standardized protocol for the production and maintenance of PMTs by testing different conditions to optimize growth and reproducibility of the model. PMTs were maintained in culture up to 14 days and initial cell compartments (cancer cells, stromal cells and immune cells) were preserved. Drug incubation led to a dose depending decrease in tumor cell volume. Furthermore, IO alone or in combination with chemotherapy showed a significant decrease in volume of PMTs.

Conclusion:

The present PMT model combined with a clinically relevant drug response analysis is one of the first to show checkpoint inhibitor-mediated cancer cell killing in a scalable long term in vitro assay.

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# A content analysis of common patient-reported outcome measures in cancer patients using the International Classification of Functioning, Disability and Health and other health frameworks

Background

Patient-reported outcomes (PROs) are frequently used for assessing endpoints in cancer clinical trials and play an increasingly important part in the context of drug approvals. The most commonly used PRO questionnaires in oncology differ regarding the content of the functional health and symptom domains that are measured which may compromise converting results from one questionnaire metric to another (crosswalks). Therefore, the objective of this study was a detailed analysis of questionnaire content of the following frequently used PRO measures: EORTC CAT Core, EORTC QLQ-C30, SF-36, FACT-G, and PROMIS measures.

Methods

Relying on the framework of the International Classification of Functioning, Disability and Health (ICF) and other health frameworks, we categorized the item content of all questionnaires and compared content of similar domains.

Results

For the key domain "physical function" 118 items from the comparator PRO measures were investigated. All physical function items of the EORTC measures but one were assigned to the first-level ICF categories 'Mobility' and 'Self-care', all within the component 'Activities and Participation'. The SF-36 additionally included item content related to 'Community, social and civic life' and the PROMIS Short Form for Physical Function 20a also included content related to 'domestic life'. Results on further questionnaires and PRO domains will be presented at the conference.

Discussion

Our results complement quantitative information on psychometric characteristics of these measures, provide a better understanding of the possibilities of establishing crosswalks for score conversion, and allow for a more in-depth understanding of what is measured by these questionnaires.

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# Average length of stay after a cardiology day clinic stay compared to a normal ward: A propensity score matched analysis

Background:

The aim of this study was to evaluate the performance, efficacy and safety of the newly opened day hospital, especially in comparison to the normal cardiology ward.

Methods:

During a retrospective study were recorded data from 1,085 patients of the normal ward and 924 day-care patients who were admitted for diagnostic coronary angiography (CAG) in the period from the opening of the CDC in October 2020 up to the end of January 2022. The primary efficacy outcome was the number of overnight stays per patient comparing the two study cohorts. Therefore, propensity score matching (PSM) was performed to directly test the association of the primary effectiveness endpoint between patients with similar characteristics.

Results:

Considering the predefined covariates, there was no more significant difference after PSM.

When a CAG was performed, the length of stay in nights after PSM was significantly shorter in the newly established CDC than in the normal cardiology ward (0.60 nights 95%-CI: 0.45-0.76 vs. 1.82 95%-CI: 0.45-0.76, $p < 0.001$).

Conclusion:

The study conducted was able to confirm the a priori elaborated hypothesis of the superiority of outpatient CAG compared to the current "standard of care" inpatient admission.

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# Patients' and health-care professionals' understanding of the graphical presentation of patient-reported outcome data

Background:

A high level of patient involvement is a key objective of modern healthcare. Patient-reported outcomes (PROs) allow the standardized assessment of the patient's perspective on a disease and its treatment. Electronic routine PRO monitoring (ePROM) comprises collection, analysis and documentation of patient-reported symptoms in daily clinical practice. This allows treatment monitoring, quality assurance and clinical research.

Objective:

The aim was to investigate patients' and health care professionals' (HCPs) understanding of longitudinal graphs showing PRO data from psychosomatic patients. In addition, we investigated the possible variation of understanding across patients' educational level.

Method:

In semi-structured interviews 40 patients and 13 HCPs were shown longitudinal PRO graphs. The participants were asked to answer questions about changes over time and about specific time points for four assessed constructs. Additionally, participants were asked to provide feedback on any aspect of the graphs they considered important.

Results:

In our sample, 80% of patients answered at least 9 out of 12 questions on the information shown in the graphs correctly, while 84.7% of HCPs answered at least 15 out of 16 questions correctly. Patient's educational level showed a statistically significant association (p=0.026) with the number of correct responses.

Conclusion:

The graphical understanding of PRO results was high in psychosomatic patients and HCPs. The observed impact of patients' educational level on understanding suggests the need for further improvements of the graphical format. Easy access and interpretation of PRO data is vital for the wider use of such data in daily clinical practice.

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# Histopathological Diagnosis in Type 2 Diabetes Patients with Chronic Kidney Disease – A Personalized Approach

Introduction

A positive family history for diabetes (FHD) is a risk factor for type 2 diabetes (T2D). This retrospective study investigated the effect of FHD and sex on insulin use and comorbidities.

Methods

Data from 3231 women and 4635 men with T2D from the Tyrolean Diabetes Registry presenting from 2012 until 2020 were analysed. Insulin therapy, micro- and macrovascular comorbidities in patients with any diabetes in the family were compared to controls. Additional propensity score matching was performed to adjust for differences in age, sex, body mass index (BMI) and haemoglobin A1c (HbA1c).

Results

1989 men and 1646 women with FHD and 2646 male and 1585 female controls were included. FHD patients were younger, had lower insulin initiation age and higher BMIs.

Fewer macrovascular events occurred in those with a FHD (17.03 vs 19.97%, $p<0.01$) mainly due to lower rates in strokes (4.24 vs 5.58%, $p=0.01$) and peripheral artery disease (3.82 vs 5.11%, $p=0.01$).

Patients with FHD showed higher rates of neuropathy (8.42 vs 6.64%, $p<0.01$) and retinopathy (2.94 vs 1.77%, $p<0.01$), but lower rates of diabetic kidney disease (11.42 vs 14.18%, $p<0.01$).

Stroke rate remained lower in people with FHD after matching for age, sex, BMI and HbA1c. Furthermore, matching revealed higher neuropathy rates in FHD men and higher rates of retinopathy in FHD women.

Conclusions

FHD was associated with insulin therapy at younger age and microvascular disease, but with less macrovascular disease, especially stroke. Male sex may contribute to neuropathy risk and female sex to retinopathy risk.

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# ASTI of 4min in electroconvulsive therapy, living on the edge

Introduction:

Major depression affects millions of people and up to twenty percent of patients do not respond to combined psychotherapy and antidepressive therapy. For these patients the electroconvulsive therapy (ECT) is considered to be most effective and safe treatment option. State of the art ECT treatment is performed under general anaesthesia. The time interval between the administration of the anaesthetic to the electrical stimulation (ASTI) should be two to four minutes and is associated with better seizure quality. The depth of anaesthesia is a relevant factor that influences the quality of the seizure: light anaesthesia is associated with better ictal characteristics. EEG monitors, like the Narcotrend (NCT) can be used to optimize the depth of anaesthesia.

Aim of this study was to evaluate a new NCT-guided ECT management compared to a ECT management based on an ASTI of four minutes.

Materials and Methods:

Patients scheduled for ECT with a score >18 on the Montgomery-Asberg Depression Rating Scale (MADRS) were included in the study. Every patient was randomly assigned either into the NCT group, or into the ASTI group. In the NCT group the seizure was induced, when the patient reached a NCT value between 41 and 64. In the ASTI group the seizure was induced four minutes after the start of administration of Thiopental. Primary study endpoints were the three levels of seizure quality.

Results:

31 patients were included in the study. Ultimately, 228 interventions were analyzed for primary and secondary endpoints. Further results will be presented on the poster.

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# Cell behavior of human-fetal-osteoblasts (hfOB) and Staphylococcus aureus on Nanostructured Titanium Alloy Ti-13Nb-13Zr (NanoTNZ)

Currently, 1–2% of all prosthetic joint surgeries are followed by an infection. These infections cause approximately 4% of deaths in the first year after surgery, while the 5-year mortality rate is up to 21%. Prosthetic joint infections are mainly caused by Staphylococcus aureus or Staphylococcus epidermidis strains. Both species share the capability of biofilm formation and methicillin resistance. The formation of biofilm helps bacterial cells to withstand critical environmental conditions. Due to their tolerance against antibacterial substances, biofilms are a significant problem in modern medicine. Nanostructured titanium alloys have shown promising results on controlling bacterial adhesion and growth in comparison to adhesion of osteoblasts. To accomplish the requirements for lighter, more elastic materials with a nanostructured surface, Ti-13Nb-13Zr shows promising properties.

In this study we investigated cell adhesion and proliferation of human-fetal-osteoblasts (hFOB 1.19) and Staphylococcus aureus ATCC 29213 on nanostructured Ti-13Nb-13Zr, a second-generation biomedical β-rich (α+β)-titanium alloy. We performed all experiments on Titanium CP 4 and Ti6Al4V ELI as controls. To evaluate the adhesion and proliferation of hFOB 1.19 we performed cell counting with a hemacytometer, following RNA extraction and RT-PCR. We performed scanning electron microscopy to gain insights into cell morphology of hFOB 1.19 on nanostructured Ti-13Nb-13Zr. Bacterial adhesion and proliferation of Staphylococcus aureus ATCC 29213 was studied by colony forming unit counting. We examined the gene expression of biofilm-associated genes and regulators. We performed RT-qPCR after RNA extraction of Staphylococcus aureus ATCC 29213. Scanning electron microscopy was performed to investigate biofilm morphology on the studied implant material.

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# Anterolateral tenodesis protects anterior cruciate ligament reconstructions - A biomechanical in vitro study

Introduction

Anterior cruciate ligament reconstructions (ACLR) can be supported by a lateral extra articular tenodesis (LET). The LET is intended to restore knee kinematics and prevent ACLR ruptures. The aim of this study was the direct measurement of forces in the ACLR and LET in various knee flexion angles under external joint loading.

Methods

Six human knee specimens were used for testing in a knee joint test bench at 0°, 30°, 60°, and 90° of flexion. Specimens were loaded with an anterior tibial translation force (ATF), internal tibial torque (IT) and combinations of both (IT+ATF), while graft forces of the ACLR and LET as well as knee kinematics were measured. The following conditions were investigated: (1)native, (2)resected ACL, (3)additional anterolateral instability, (4)isolated ACLR and (5)combined ACLR+LET.

Results

During IT and IT+ATF, ACLR graft forces were reduced up to 61% by the LET. Forces in the LET of up to 112 N were measured. For an isolated ATF the effect of the LET was negligible. Only the combination of ACLR+LET was able to restore the native internal rotation under internal tibial torque loading. For 30° of flexion, ACLR+LET caused a non-physiological reduction of internal tibial rotation.

Conclusion

ACLR graft forces under internal tibial torque loading can be reduced with an additional LET and residual rotational laxity after isolated ACLR can be decreased. This might reduce the risk of ACLR ruptures, in particular for pivoting motions. Care should be taken not to overconstrain the internal tibial knee joint motion with an LET.

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# Sex Differences in the Association of Peripheral inflammation and Panss Scores in Schizophrenia

BACKGROUND

There is a growing literature regarding a relationship between neuroinflammation and schizophrenia, however, little is currently known about a potential sex-specific relationship between psychopathology and peripheral inflammation when starting antipsychotic monotherapy and its change over one month.

METHODS

The sample consisted of 116 (52.6% male) patients with schizophrenia who were started on monotherapy with a second-generation antipsychotic. Sociodemographic and clinical data were collected at baseline. Psychopathology was rated at baseline and after 2 and 4 weeks of treatment using the Positive and Negative Syndrome Scale (PANSS). Blood samples (full blood count, CRP) were taken at the same points in time. Besides CRP, the integrative immune inflammation markers neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and systemic immune-inflammation index were considered. Data of 116 cases were available for baseline analysis and of 57 cases at weeks 2 and 4.

RESULTS

PANSS (sub)scores decreased significantly from baseline to consecutive follow-ups, and the two sexes did not differ in this regard. Similarly, no significant sex differences were found in CRP levels, NLR, MLR, and SSI. The results of Spearman rank correlation revealed no statistically significant association between PANSS (sub)scores, neuroinflammation markers, and sex at any time of investigation.

CONCLUSION

In the light of these findings, the two sexes do not differ in regards of changes in psychopathology and inflammatory biomarkers.

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# Non-alcoholic fatty liver disease (NAFLD): Risk factors and sex-specific aspects in Tyrolean adolescents – Preliminary data from the EVA4YOU study

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children, with a prevalence of 7.6 %. Patients with NAFLD are at markedly increased risk of adverse outcomes, including overall mortality. Therefore, early detection of the disease is crucial. The aim of this study is to identify risk factors for elevated liver fat content in our cohort and to illustrate sex-specific differences.

Methods: From January 2021 to June 2022, 1098 Tyrolean adolescents aged between 14 and 19 years were screened for cardiovascular and metabolic risk factors. Liver fat was measured using transient elastography (FibroScan®, Echosens, Paris, France) – a non-invasive method that determines the degree of fatty liver disease via the Controlled Attenuation Parameter (CAP). Risk factors for elevated CAP-values were analyzed using linear regression adjusting for sex and age. Male sex as an independent risk factor for NAFLD was analyzed using independent samples t-test and linear regression adjusting for age, BMI z-score, triglycerides, and insulin.

Results: 681 (62%) of the participants were female and 417 (38%) were male with a mean age of 17.2±1.3 years. Mean CAP-values were significantly higher in male adolescents (mean difference = 18.8 dB/m [14.2-23.4 dB/m], $p<0.001$). This difference remained significant in a linear regression model after adjusting for other determined risk factors for elevated CAP-values (age, BMI z-score, triglycerides, and insulin), $p<0.001$.

Discussion: In our cohort male sex was a significant independent risk factor for elevated CAP-values.

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# Temporal changes in cardiac autonomic function in patients with acute ST-elevation myocardial infarction undergoing percutaneous coronary intervention

Background/Aim: Periodic Repolarization Dynamics (PRD) and Deceleration Capacity (DC) are novel ECG-derived parameters quanifying cardiac autonomic function: PRD as a measure of sympathetic activity and DC as a measure of vagal activity. The aim of this study was to investigate temporal changes of these parameters in the acute setting of patients undergoing percutaneous coronary intervention (PCI) for ST-elevation myocardial infarction (STEMI).

Methods: Patients presenting with acute STEMI were included in the study and monitored from hospital admission to 24h after PCI with high resolution ECG. Each recording was divided into 1 pre PCI segment and 24 post PCI segments of 1-hour length each. PRD and DC were calculated for each segment according to previously pubished methods.

Results: In total 23 patients (age 63 [56-70], 17 (74%) male) were included in the study. PRD decreased from 7.8 (IQR 6.4-10.5) $deg^2$ at hospital admission to 4.1 (IQR 2.8-6.2) $deg^2$ at 6 h ($p = 0.004$) and 4.6 (IQR 2.3-7.4) $deg^2$ at 24 h post PCI ($p = 0.131$). DC on the contrary increased from 4.7 (IQR 2.8-5.7) ms before PCI to 6.5 (IQR 5.38.1) ms at 6 h ($p = 0.018$) and 5.5 (IQR 3.7-6.8) at 24 h after PCI ($p = 0.557$).

Conclusion: Our study reveals that patients presenting with acute STEMI suffer from severe autonomic dysfunction by means of sympathetic hyperactivation and concomitant vagal suppression at hospital admission. Revascularization of the affected vessel by acute PCI significantly improves cardiac autonomic dysfunction within the first hours after PCI.

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# Gender Differences in Patients with Cardiac Amyloidosis

Introduction

Cardiac amyloidosis (CA) is an infiltrative cardiomyopathy resulting from extracellular deposition of amyloid fibrils in the myocardium. Gender modulates the prevalence and morpho - functional manifestations of CA. This study aimed to examine gender differences in echocardiographic parameters in CA.

Methods

We retrospectively identified 120 patients with CA diagnosed at our university hospital between 2016-2022. Echocardiographic data were collected from time of diagnosis. Cohorts were divided based on sex for gender analysis.

Results

24 patients (20%) were female. Interventricular septum (IVS) was less thick (15.2mm vs. 16.4mm, p=0.07) and left ventricular (LV) ejection fraction was higher (58.9% vs. 55.0%, p=0.18) in women. LV mass was significantly lower in women than in men (145.5g/m2 vs. 164.5g/m2, p=0.04). Left (LA) and right atrial (RA) diameter were smaller in women (LA: 41.3mm vs. 44.3mm, p=0.11; RA: 39.0mm vs. 46.5mm, p=0.001). Additional gender variations included significant differences in LV end-diastolic- (LVEDV) and end-systolic volume (LVESV), as well as in LV end-diastolic- (LVEDD) and LV end-systolic diameter (LVESD) (LVEDV: 66.0ml vs. 98.0ml, p<0.001; LVESV: 25.0ml vs. 39.8ml, p<0.001; LVEDD: 39.8mm vs. 45.7mm, p=0.01; LVESD: 27.4mm vs. 32.3mm, p=0.03). There was no relevant gender difference in posterior wall thickness (p=0.49) or strain analysis (p=0.54).

Conclusion

Women with CA presented with lower IVS thickness, higher LV ejection fraction, and smaller LV size compared to men. Hence, women are less likely to meet the diagnostic thresholds for CA screening. These findings contribute to the underdiagnosis of CA in women. Physicians need to be familiar with gender differences in CA.

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# Gender-specific differences in the correlation between suicide risk and moral-religious beliefs: interim analysis

In 2019, 1113 people committed suicide in Austria (13 per 100.000 inhabitants); the number of suicide attempts is difficult to ascertain and commonly estimated to be 10-30x higher. Men account for more than ¾ of completed suicides in Austria; women commit more suicide attempts. Suicide risk is very high after discharge from a psychiatric hospital (135 per 100.000 discharges in the first 3 months). Therefore, factors predicting a likely relapse of suicidal ideation are of great interest to clinicians.

In this ongoing single-center observational study, 50 patients admitted to involuntary confinement because of suicidal ideation are recruited immediately before or after discharge/transfer and given three questionnaires (Brief Reasons for Living, Spiritual and Religious Attitudes in Dealing with Illness, Subjective Determinants of Treatment Effect in Suicidal Ideation). Additionally, history of suicidal ideation/attempts and sociodemographic data are collected. We hypothesize that relevant reasons for living, strong moral-religious beliefs and the experience of receiving subjectively helpful treatment are protective factors regarding relapse. One year after recruitment, outcomes (relapse of suicidal ideation, re-admission to involuntary confinement and/or completed suicides) will be considered and correlated to values. A descriptive interim analysis with a focus on gender specific differences is presented.

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# Edema-like symptoms are common in ultra-distance cyclists and driven by overdrinking, use of analgesics and female sex – a study of 919 athletes

Background: Ultra-endurance cyclists regularly report various extents of bodily decline during long-distance bicycle rides, including potential kidney function-related symptoms such as swelling of body parts and urine changes. This study aimed to assess the prevalence of these symptoms in a representative cohort of ultra-endurance cyclists and shed light on potential predictors related to the ride, the rider and the rider's behavior.

Methods: Between November 26 and December 14, 2020, 1350 people participated in an online survey investigating potential kidney-related symptoms of ultra-distance cycling. Frequency and severity of edema-like ("swelling") symptoms and perceived changes in urine output, concentration and quality were associated with ride-related factors, demographic parameters and rider behavior-related variables.

Results: A total of 919 participants met the predefned inclusion criteria. The majority (N=603, 65.6%) stated that they sufered from at least one potential kidney function-related symptom, out of which 498 (54.2%) stated one or more edema-like ("swelling") symptoms. In correlational and multiple regression analyses, female sex, intake of analgesics and drinking strategies correlated with swelling symptoms. Further analyses indicated that drinking due to thirst and/or drinking adapted to ambient sweating and temperature negatively correlated with swelling symptoms, whereas "drinking as much as possible" enhanced these. Intake of analgesics was moderately positively correlated with swelling symptoms.

Conclusions: According to our survey, edema-like symptoms occur in the majority of ultra-distance cyclists and female sex, drinking strategy and intake of analgesic drugs are major predictors thereof. Studies are needed to investigate the underlying pathophysiological processes of such symptoms

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# Augmenting microscopic navigated surgery

The aim of this work is to identify 3D anatomical ear-nose-throat (ENT) structures in live video streams provided by a high-precision stereo-microscopic navigation system during ENT surgeries. The surgical microscope is tracked by optical navigation is utilized to create an augmented reality setup. The goal of 3D anatomical detection is to demonstrate ENT structures in order to create automatic segmentation of anatomical structures and to augment microscopic navigated surgery. In our research, the custom modified Leica M500N stereo microscope will be used, which is equipped with two high resolution IDS cameras. As the first step, the cameras are calibrated for each zoom level in order to assign the camera position. For this purpose, a symmetric object with specific size and dimensions is prepared and captured at the same time in different directions. We also use Blender Environment to create grund truth of the undistorted images of the same object to use deep learning for camera calibration.

The can bus communication existed in our in-house software provides the feasibility to connect the microscope to the camera. The parameters for zoom and focus tuning will be sent to the microscope and proper calibration values will be read out. The obtained parameters from calibration technique will be used to rectify images captured from the cameras in order to create the block matching.

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# Sex specific differences in body composition and varying classification for overweight and obesity comparing BMI and FMI in adolescents: Data from the prospective EVA4You cohort study

Background: Overweight and obesity are one of the main risk factors for chronic non-communicable diseases (NCDs.) However, it is not increased body weight that is crucial for the development of such diseases, but excess body fat. Body mass index (BMI) is widely used to assess adiposity but fails to distinguish between fat mass (FM) and fat-free-mass (FFM), which vary according to age, sex, and ethnicity.

Objective: The aim of this study was to assess the different categorization of the risk factors overweight and obesity for a pediatric population based on measured height and weight versus using the proportion of fat and muscle mass.

Design: Sex-specific FMI reference percentiles were applied to 919 adolescents (347 boys, 572 girls; 16-19 years) of the EVA4You study population and categorized into normal weight, overweight, and obese using the WHO international BMI guidelines. The different FMI and BMI categorizations were then compared.

Results: 77.5% of all participants were BMI normal weight, 18.0% overweight, and 4.5% obese. 92.1% were FMI normal weight, 5.7% overweight, 2.2% obese. Boys showed a significant higher average BMI ($p=0.011$), but a significant lower average FMI than girls ($p<0.001$).

When comparing the two indexes, 66.0% of BMI overweight/obese showed a normal FMI with a significant higher muscle-mass-index (MMI) ($p<0.001$).

Conclusion: Using BMI for classification of adiposity in adolescents can lead to misclassification because the differentiation between fat mass and muscle mass is not considered and therefore classification by FMI would be an almost equally simple and more precise tool to assign adiposity.

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# Effect of ex-vivo application of von Willebrand factor concentrate on complement system and contact pathway in critically ill patients under extracorporeal support devices with and without sepsis

Human von Willebrand Factor (vWF) plays a major role in haemostasis. It is synthetized by megacaryocytes, subendothelial and endothelial cells in form of ultra-large vWF-multimeres (ULVWM). In case of vascular trauma it will readily bind to collagen, facilitating platelet adhesion and aggregation by binding to platelet receptors and protecting factor VIII from degradation. vWF will acts as an inductor of inflammatory response by the induction of thrombin and bradykinin via the coagulation cascade, as well as activation of contact and complement pathways.

Enzymatic breakdown of vWF occurs under calcium binding or mechanical shear stress via the metalloproteinase ADAMTS13 at a partially buried cleavage site, leading to smaller, less efficient vWF-molecules.

Under mechanical circulatory support (MCS) like extracorporeal membrane oxygenation (ECMO) or Impella pumps patients' blood is exposed to higher levels of shear stress due to turbine effects within the machine. In consequence, more breakdown of vWF via ADAMTS13 will occur, which may lead to an acquired coagulopathy due to loss of ULVWM as a form of acquired vWF-syndrome (AVWS).

In cases of AVWS application of recombinant vWF concentrate (rvWFC) such as vonicog alfa (Veyvondi®) may be appropriate, but there is little data on its effects in the setting of MCS, especially in regards to complement and contact pathway activation.

The goal of our project is to evaluate the coagulatory effects of ex-vivo application of rvWFC, as well as complement and contact pathway activation in order to provide more evidence on its effects in MCS settings with and without sepsis.

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# Extracorporeal life support in acute myocardial infarction induced cardiogenic shock: gender differences

Objectives:

Extracorporeal circulation support is a valuable option in patients facing acute myocardial infarction (AMI). So far, little data, investigating a potential profit of extracorporeal membrane oxygenation (ECMO) support in female patients with AMI induced cardiogenic shock (CS), is available.

Methods:

A total of 536 consecutive patients with AMI induced CS are investigated. Of which 22.8% (n=122) were of female gender. Primary analysis outcome was mid-term survival. Multivariable logistic regression analysis was performed in order to identify independent predictors for survival.

Results:

Out of 536 patients, 174 patients (32.5%) received ECMO support. Women significantly less often received ECMO support than men (26.2% versus 34.3%, p=0.016). Mid-term survival significantly differed between female and male patients receiving ECMO support (14.3% versus 50.4%, p<0.001).In multivariate Cox-regression analysis, female gender was an independent predictor for impaired survival ( HR: 1.75, 95% CI 1.04-2.93; p= 0.035 ). Complete revascularisation is achieved more frequently in females (68.8% versus 50.5%, p=0.034).

Conclusion:

Female patients with AMI induced CS significantly less often received ECMO treatment in comparison to men. Despite more completeness of revascularization in female patients undergoing ECMO treatment, early and mid-term survival was significantly impaired compared to survival rates in male patients.

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# Acute Kidney Injury in 1000 critically ill COVID-19 patients in Tyrol, Austria

Introduction:

Acute kidney injury (AKI) is common in critically ill COVID-19 patients and associated with increased mortality. We aimed to investigate the incidence, outcome and risk factors of AKI in critically ill COVID-19 patients in Tyrol, Austria.

Methods:

This prospective register study included all patients with a SARS-CoV-2 infection, who were treated in one of the 12 ICUs during 1st February 2020 and 1st May 2022. AKI was classified according to the Kidney Disease: Improving Global Outcomes guidelines.

Results:

In total, 1042 patients were included. The median age of the overall cohort was 66 years.

Of the included patients, 244(23.2%) developed AKI during the ICU stay. According to the KDIGO classification 7.8% had AKI stage 1, 5.0% AKI stage 2 and 11% AKI stage 3.

Patients with AKI were older, had more often the pre-existing diagnosis of hypertension, cardiovascular disease, or a known renal comorbidity. In total 126(12.3%) required renal replacement therapy (RRT). In patients with AKI the IMV rate was significantly higher (88% [n=227]vs.41%[n=312],p<0.001). The most important risk factors for AKI were IMV (OR=4.5 p<0.001), age (OR=1.01, p=0.013), vasopressor use (OR=3.13, p<0.001) and renal comorbidities (OR=2.17, p<0.001) in a multivariable logistic regression analysis. Overall hospital mortality in our cohort was significantly higher in the AKI group (52.1% [n=138] vs. 17.2% [n=131], p<0.001).

Conclusion:

As in non-COVID-19 patients AKI seems to be associated with an increased mortality in critically ill COVID-19 patients. Among known risk factors IMV has been an independent predictor of AKI in our analysis.

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# Gender-related characteristics of NSCLC tumor microenvironment

Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide. Compared to men, women with NSCLC are more likely to be of younger age, non-smokers, have adenocarcinoma histology and a driver-gene mutation. Gender differences in immune and tumor cell composition have not yet been evaluated in-detail.

Aim: To evaluate differences in the cellular composition and transcriptomic profile of men versus women with NSCLC.

Method: We used our recently published NSCLC single-cell atlas that comprises data of 18 published plus our own datasets, covering 556 tissue samples 322 patients (tumor and control patients) with in total 1.283.972 cells. In this sub-investigation, we analyzed data of NSCLC patients with gender annotation, focussing on differences in cell type composition and gene expression in both tumor and normal lung tissue.

Results: 222 patients have a gender annotation, including 100 women. Overall, male samples accounted for 551.026 cells, female samples for 438.400. In tumor samples, male samples showed a higher relative amount of tumor cells compared to female (16.0% vs 10.4%), whereas female samples comprised higher rates of macrophages (m: 8.8% vs. f: 12.2%), CD4 T cells (m: 15.3% vs f: 17.6%) and neutrophils (m: 1,3% vs f: 2.97%). Differentially expressed genes in these cell types will be evaluated.

Conclusion: First analyses show higher proportions of tumor cells and lower proportions of certain immune cell subtypes in male compared to female samples, suggesting a gender-specific composition of the NSCLC tumor microenvironment.

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# Less than half of trials use patient-reported outcome measures to evaluate supportive interventions for children with cancer. A systematic review

Background

Children with cancer suffer from numerous symptoms and side-effects, making supportive interventions indispensable to improve their health-related quality of life (HRQOL). The gold standard for evaluating HRQOL are patient-reported outcome measures (PROMs). This systematic review investigated the current practice of clinical outcome assessment (COA) in clinical trials on supportive interventions.

Methods

ClinicalTrials.gov and EudraCT were searched for clinical trials (registered 2007-2020) on supportive interventions in paediatric oncology (up to 21 years). The use of different COAs (PROMs, performance-based, clinician-, observer-reported outcomes) was analysed, focusing on PROMs. Associations with trial characteristics were investigated using univariate and multivariable analyses.

Results

Of 4789 identified clinical trials, 229 were included. PROMs were used in 44.1% of the trials. The proportion of trials using PROMs did not significantly differ over time. In the multivariable analysis, intervention type (behavioural > medical interventions) and cancer type (mixed and solid tumours > haematological) were significant predictors of PROM use. Most trials using PROMs (59.6%) measured more than one health domain, with physical health being the most frequently assessed domain (92.6%).

Conclusion

Less than half of registered trials investigating supportive interventions for children with cancer used PROMs. While this proportion is higher than in clinical trials evaluating anti-cancer treatments for paediatrics, it is inferior to that in adult oncology research. The result is striking since supportive care explicitly aims to improve patients' HRQOL, which is best assessed using PROMs. Our systematic review underlines the need to identify and overcome barriers for PROM implementation in childhood cancer clinical trials.

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# Temporal Dynamics of Somatic Mutations in the Context of Systemic Inflammation

Introduction: Clonal hematopoiesis of indeterminate potential (CHIP) refers to a population of related myeloid cells with an acquired mutation of a leukemia-associated gene (with a variant allele fracture [VAF] >2%) in patients with normal peripheral blood counts and no clinical or pathological evidence for a WHO defined hematologic malignancy. The majority of these mutations has been accounted to only a handful of genes, namely DNMT3A (50-60%), TET2 (10-15%) or ASXL1 (8-10%). As could be expected, the risk of developing leukemia is approximately 10-times higher in individuals with CHIP as compared to those not harboring CHIP. Interestingly it was found, that the mutations are associated with inflammatory imprinting of circulating immune cells that invade atherosclerotic plaques and secrete cytokines that maintain local inflammation. Therefore, individuals with CHIP have also a markedly increased risk for coronary heart disease, myocardial infarction and ischemic stroke.

However, it is not known how inflammatory-driven cardiovascular events (i.e. stroke) influence expansion of CHIP-clones and how this impacts long-term clinical outcomes.

Methods: Targeted NGS will be performed at the time of event (stroke) and at 12-month post-event to identify the mutational profile and expansion of CHIP-clones. This will be correlated to measures of systemic inflammation and clinical outcome data.

Expected Results: The project will help to understand the post-stroke inflammatory response and could help to identify stroke patients that may benefit from individualized anti-inflammatory or anti-clonal treatment strategies.

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# Optimized Cell Culture Experiments to Elucidate the Role of Coagulation Factors in Bone Metabolism

In vitro experiments of the effects of coagulation factor VIII on osteoblasts are necessary for the understanding of low bone mineral density (BMD) in haemophilic patients, as haemophilia A does not only go along with spontaneous bleeding into joints or soft tissues, but also leads to osteopenia in 43% and osteoporosis in 27% of the patients - independent from common risk factors. So far, assessments of such effects have not been performed in vitro because of low sensitivity of Alizarin Red S assays.

To optimize Alizarin Red S assays, supplementation of calcium chloride (1-10 mM) was tested to improve mineralization of human (SaOs-2) and murine (MC3T3-E1) osteoblasts when cultured for three or four weeks, depending on the size of the wells. Addition of 2.5 to 5 mM calcium chloride to the osteogenic cell culture medium led to increased bone matrix mineralization with higher sensitivity of Alizarin Red S staining by 36.3 to 61.0-fold and 1.9 to 3.4-fold in human and murine osteoblasts, respectively. Surface area and incubation times showed only minor effects. Calcitonin without addition of calcium chloride did not show any effect on the osteoblast cell lines.

These optimized Alizarin Red S assays are now used to elucidate interactions between the coagulation system and osteoblasts. Understanding of involved signaling pathways will provide additional guidance for the development of effective and safe treatment strategies in order to prevent low BMD in haemophilia A patients.

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# Are there gender differences in myocardial infarction size depending on the applied cardiac magnetic resonance sequence? A comparison between the standard "bright blood" and an innovative "dark blood" late enhancement technique

Background: With the standard "bright-blood" late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) technique the discrimination of ischemic scar and adjacent blood-pool may be limited. The recently introduced "Flow-Independent Dark-Blood Delayed Enhancement" (FIDDLE) sequence addresses this specific limitation and may have an advantage in the assessment of smaller infarct scars.

Purpose: To prospectively compare the infarct size between women and men in LGE and FIDDLE sequences.

Methods: The conventional "bright blood" LGE and FIDDLE sequences were acquired in 109 patients (60.4 ± 9.7, 19% women) within 7 days (n=8 women), 4 months (n=7 women) and 12 months (n=6 women) after acute ST-elevation myocardial infarction (STEMI), respectively. By applying the "Full Width at Half Maximum" method using the semi-automated postprocessing software (CVI42 Circle Cardiovascular Imaging) the infarct sizes (infarct volume (ml) were assessed on both, the standard short-axis LGE and on FIDDLE images.

Results: In the woman cohort the median infarct volume as assessed by standard LGE was 9.6 ml (interquartile range (IQR) 5.7-15.1) and the median infarct volume for FIDDLE was 9.5 (IQR 6.8-13.4, p=0.601). In men the median infarct volume for LGE was 14.3 ml (IQR 8.3-20.9) and for FIDDLE 14.8 ml (IQR 9.3-21.6), p=0.862. T-test revealed no statistically significant difference in infarct size between women and men for both conventional LGE and FIDDLE sequences (p=NS).

Conclusion: Our prospective CMR study revealed no gender-specific difference in infarct size after STEMI, neither as assessed by the standard "bright blood" LGE nor by the recently introduced "dark blood" FIDDLE CMR technique.

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# Overcoming the Gender Bias with Artificial Intelligence in Cardiac Imaging

Objective: The objective of our study was to analyse whether model bias occurres in the evaluation of coronary artery calcium score (CACS) in thoracic computed tomography.

Methods: This was a secondary analysis of a prospective, multicenter observational cohort study conducted from April 29 to August 12, 2020, to assess pulmonary abnormalities at chest CT of Covid-19 patients. We evaluated CACS semiautimatically with the Syngo Via Software and the full automatic artificial intelligence based software AI Rad Companion.

The results were compared with a Lin's concordance correlation coefficient test.

Results: A total of 80 patients were included. The mean calcium volume evaluated with syngo via was 126mm$^3$. The mean volume evaluated with the artificial intelligence software was 139mm$^3$.

There was an excellent correlation with a Lin's coefficient of concordance of 0.99 (95% CI: 0.98).

In a separate evaluation of men and women, women also showed an excellent correlation with a Lin's coefficient of concordance of 1.0 (95% CI: 0.99).

Conclusions: With the help of artificial intelligence-based diagnostic software, gender bias in the assessment of CACS can be avoided.

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

Introduction. Dual Energy CT (DECT) determines the liver iodine content on enhanced CT images and the creation of virtual non-enhanced images (VNC). If validated, this could offer the opportunity to reduce radiation dose by omitting true non-enhanced images (TNC) and improving efficiency of CT scans.

Aim. The purpose of this retrospective clinical study was to compare the average liver density on VNC derived from arterial (aVNC) and delayed phase (dVNC) to TNC and to evaluate the potential differences between genders.

Methods. Preliminary data of 122 patients (83 male, 39 female) was pre-analysed. Within each gender group and for each patient the average liver density (HU) on aVNC, dVNC and TNC were obtained. Subsequently the differences aVNC-TNC and dVNC-TNC were calculated, and their means compared. In addition, the cut-off value of 10 HU commonly used in literature was applied for analysis of density differences. Within each gender group and separately for aVNC and dVNC, two groups with ≤ 10 HU and > 10 HU difference in average density were formed and their means are compared.

Results. The mean difference for liver density was 58 HU (male)/ 56 HU (female) on aVNC, 57 HU/ 56 HU on dVNC and 49 HU/ 52 HU on TNC. 66,3% (male)/ 89,7% (female) had less than 10 HU difference in average liver density between aVNC/ TNC, respectively 65,1%/35,9% between dVNC/ TNC. P-values will be provided.

Conclusion. There are slight differences regarding average liver density between genders. aVNC from female patients showed the least differences to TNC.

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# Establishing thresholds for clinical importance for EORTC questionnaire modules for specific cancer patient populations

Aims

The aim of this study is to establish thresholds for clinical importance (TCIs) for a set of European Organisation for Research and Treatment of Cancer (EORTC) questionnaire modules for specific cancer populations. These modules, covering the majority of cancer cases in Europe.

Methods

In line with the target populations of the above EORTC questionnaires, we will recruit 225 patients in seven European countries for each of the following eight patient groups: elderly patients (>=70years), patients with breast, prostate, colorectal, endometrium, lung, head and neck, and ovarian cancer. Patients will complete the relevant EORTC questionnaire module and an anchor item questionnaire assessing limitations of everyday life, worries, and need for help/treatment for each individual domain in the questionnaire modules. The anchor items will be used to create binary criteria for clinical importance to inform the development of TCIs for each domain. The statistical analyses will comprise receiver operating characteristic analysis and multivariable logistic regression analysis to determine TCIs and to assess their variability across patient groups.

Results

Recruitment for this project is currently ongoing. First results from an interim analysis will be presented at the conference.

Conclusion

The TCIs established in this project will aid in the interpretation of absolute PRO scores obtained from an individual patient at a single point in time. Such thresholds complement e.g., minimal important differences that guide interpretation of score change or group differences. TCIs will facilitate the use of the above EORTC questionnaire modules for symptom screening in daily oncological practice.

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# What do we know about visualisation of group-level PRO data from cancer clinical trials for patients? Results from a systematic literature review

Aims: As part of the SISAQOL-IMI project, currently developing standards for the use, analysis and interpretation of patient-reported outcome (PRO) data in cancer clinical trials, work package 4 (WP4) performed systematic literature searches to gather existing knowledge on how to visually present PRO trial data to different stakeholder groups. This abstract reports findings for patients. Methods: Web of Science, EBSCOhost and the Cochrane Library were searched to identify visualisation advice for PRO data. Two independent reviewers screened each reference and one researcher performed data extraction (subjected to quality control). According to the aim of WP4, only references investigating group-level PRO data visualisations were included in the qualitative synthesis. Results: The selection process resulted in 19 references providing information on the visualisation of group-level PRO data. No clear preference of any type of visualisation was found. Line graphs, pie charts and bar charts are considered reasonable options for patients. Longitudinal data presentation is favoured. Statistical details are rather found confusing, though numerical reporting of values and highlighting of statistically significant results is appreciated. Labels indicating the direction of better and worse scores and textual explanations aid interpretation. Literature generally suggests simple visualisations for patients, but educational differences can affect preferences and understanding. Conclusion: Although literature provides some specific recommendations on the graphical presentation of PRO trial data to patients, further research is needed to address their particular needs, which might vary within this stakeholder group. Disclaimer: The content of this abstract MUST NOT be taken as recommendations of the SISAQOL-IMI consortium.

F. Gross 1, L. M. Wintner 1, R. Peacock 2, F. Boele 2, J. I. Arraras 3, G. Velikova 2, J. Chang 4, B. Holzner 1, submitted on behalf of the SISAQOL-IMI Work Package 4

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# Gender influences on symptoms after stereotactic radiofrequency ablation (SRFA) of liver tumors

Purpose

To assess gender influences on symptoms after stereotactic radiofrequency ablation (SRFA) of liver tumors.

Methods

This is a sub-analysis of a recently conducted health-related quality of life (HRQoL) questionnaire study after SRFA of liver tumors. Among all patients receiving SRFA treatment for liver tumors at our institution between 2011 and 2017, 303 patients (90 women; mean age 64 [range 9-87 years]) completed and returned 363 HRQoL questionnaires and were included in this analysis. Data on the queried symptoms (i.e, pain, fever, poor wound healing, digestive disturbances, reflux, night sweats, nausea/vomiting, lack of appetite, and weakness) were compared between gender and correlated with possible other influence factors (i.e., tumor size, tumor location, and major complications) in multivariate analysis.

Results

Fifty (16.5%) patients returned > 1 questionnaire due to multiple SRFA sessions. Women reported significantly more pain (61/96 [63.5%] vs. 132/264 [50%]; p=.023) and weakness (63/95 [66.3%] vs. 126/263 [47.9%]; p<.01) than men after SRFA of liver tumors. Scores on a pain scale of 1-10 were also significantly higher in women than in men (5.11 vs. 4.20; p<.001). In multivariable analysis, gender proved to be the only and independent predictor of pain and weakness after SRFA of liver tumors.

Conclusions

Gender significantly and independently affects pain or weakness after SRFA of liver tumors. Women report more frequent and more severe pain after SRFA compared to men. No gender differences were observed in other symptoms after SRFA (fever, poor wound healing, digestive disturbances, reflux, night sweats, nausea/vomiting, lack of appetite).

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* These authors contributed equally.

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# NLRP3 Inflammasome and Orthodontic Tooth Movement

Our study aims to investigate the role of the NLRP3 inflammasome in orthodontic tooth movement (OTM). Further, effects of tooth movement on inflammatory pathways, with special emphasis on the expression of pro-inflammatory cytokines should be clarified.

MATERIALS AND METHOD: NLRP3 depleted mice (NLRP3-/-) and wild-type littermates (WT) were used in a model of experimental OTM induced by nitinol coil-springs that were bonded between the left first maxillary molar and the upper incisors. Teeth in the right maxillae were served as contralateral controls. After 7, 14, and 21 days, mice were sacrificed, the jaws were removed, and analyzed by micro-computed tomography (distance for mesial movement of the first molar) and decalcified histology (TRAP staining). Cytokine levels of IL-1β were measured in blood plasma by enzyme-linked immunosorbent assay (ELISA). Additionally, general health parameters (blood, weight) were analyzed.

RESULTS: The amount of tooth movement was significantly lower in NLRP3-/- mice than in WT mice. Further, the number of TRAP-positive cells were suppressed in NLRP3-/- mice on the compression side.

ELISA showed higher serum levels of IL-1β in WT mice due to inflammasome activation. Weight and blood analyzation gave a survey of a good general health and a sufficient nutritional status of the mice.

CONCLUSIONS:

This study reveals the possibility that NLRP3 plays a mechanosensory role in OTM.

Inflammation during OTM needs to be well controlled, as dysregulated inflammatory reaction leads to tissue destruction. Understanding these mechanisms has crucial clinical implications, as it will allow OTM to become more efficient and safer in humans.

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# Inflammatory processes in Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (CML) is a myeloproliferative disease with need for better prognostic markers. In a prospective study on CML patients undergoing Nilotinib treatment we have previously established and published sCD62L levels at baseline as a very consistent biomarker for the prediction of molecular remission. Preliminary data from this and other studies suggest HLA-DR expression on classical monocytes as well as plasma tryptase as additional predictive markers.

We have investigated a prospective cohort of 183 patients treated with Dasatinib at baseline to confirm these biomarkers. 37% achieved an MR4 at 15 months. When dichotomized by sCD62L levels (using ROC analysis), 11% in the sCD62L high cohort achieved MR4 at 15 months compared to 55% in the sCD62L low cohort (HR 7.09, $p<0.001$). Similarly, when dichotomized by HLA-DR expression on classical monocytes (using ROC analysis), 55% in the HLA-DR high compartment achieved MR4 at 15 months compared to only 22% (HR 0.33, $p=0.002$). Likewise, patients with high plasma tryptase levels (dichotomized using ROC analysis) achieved an MR4 in 28% compared to 65% in the tryptase low group (HR 3.56, $p<0.001$).

When the characteristics where combined, patients in the favorable cohort achieved an MR4 in 91% compared to only 5% in the unfavorable group(HR: 45.23, $p<0.001$).

To sum up, we were able to confirm these predictive biomarkers for achievement of molecular response in CML patients at baseline and combine them to an impressive risk score. This method of risk stratification discriminates patients much better than any of the risk scores available.

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# Temporal trends in infarct severity outcomes in ST-elevation myocardial infarction: A cardiac magnetic resonance imaging study

Background and Aims: Severity of myocardial tissue injury is a main determinant of morbidity and mortality related to ST-elevation myocardial infarction (STEMI). Temporal trends of infarct characteristics at myocardial tissue level have not been described. This study sought to assess temporal trends in infarct characteristics through a comprehensive assessment by cardiac magnetic resonance imaging (MRI) at a standardized time-point early postSTEMI.

Methods: We analyzed STEMI patients treated with percutaneous coronary intervention (PCI) at the University Hospital of Innsbruck who underwent a cardiac MRI between 2005–2021. The study period was divided into terciles. Myocardial damage characteristics were assessed using a multiparametric cardiac MRIprotocol within the first week after STEMI and compared between groups.

Results: A total of 843 STEMI patients (17% female) with a median age of 57 (IQR 51-66) years were analyzed. While age, sex and the clinical risk profile expressed as TIMI risk score were comparable across the study period, there were differences in guideline-recommended therapies. At the same time, there was no significant change in infarct size (p=0.25), microvascular obstruction (p=0.50) and intramyocardial hemorrhage (p=0.34). Left ventricular remodeling indices and left ventricular ejection fraction remained virtually unchanged (all p>0.05). Major adverse cardiovascular events at 4 (IQR 4-5) months were similar between groups (p=0.36).

Conclusions: In this MRI study, investigating STEMI patients treated with primary PCI over the last 15 years, nochange in infarct severity at myocardial level has been observed. Clinical research on novel therapeutic approaches to reduce myocardial tissue injury should be a priority.

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# Missing data handling and sensitivity analyses for patient-reported outcome endpoints in breast cancer randomized controlled trials: a systematic scoping review on current reporting practice

Aims: This review addresses the common problem of missing patient-reported outcome (PRO) data in clinical trials by assessing the current practice of reporting sensitivity analyses and missing PRO data in breast cancer randomized controlled trials (RCTs). If methodological rigor in study design and study administration fails to avoid missing data, adequate and transparently reported handling can minimize the potential of drawing erroneous conclusions from clinical research.

Methods: We conducted a systematic literature search in PubMed to identify breast cancer RCTs with at least one PRO endpoint published between January 2019 and February 2022. Only trials that randomized at least 50 patients evaluating biomedical treatments were eligible. Two trained reviewers independently assessed the eligibility of the publications and extracted pre-specified information on reporting of missing PRO data & related sensitivity analyses.

Results: The systematic literature search identified 1.598 publications listed in PubMed, of which 140 met the inclusion criteria. Apart from the report of the extent of missing PRO data (74.6%), none of the other aspects of missing PRO data reporting (i.e. reasons for missingness) exceeds 40%, the reporting of the mechanism of missingness, statistical approaches to deal with missing data and sensitivity analyses remains below 20%.

Conclusion: The reporting of missing PRO data and related aspects is rare in breast cancer RCTs. As overcoming underreporting would contribute to a more reliable evaluation of the clinical benefit of new oncological treatments, future efforts should further endorse transparent and detailed reporting on missing PRO data and procedures to deal with it.

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# COVID-19 and its continuing burden after 12 months: a longitudinal observational prospective multicenter trial

Background: Recovery trajectories from COVID-19 call for longitudinal investigation. We aimed to characterize the kinetics and status of clinical, cardiopulmonary and mental health recovery up to 1-year following COVID-19.

Methods: Clinical evaluation, lung function testing (LFT), chest computed tomography (CT) and transthoracic echocardiography (TTE) were conducted at 2-, 3-, 6- and 12 months after disease onset. Submaximal exercise capacity, mental health status and quality of life (QoL) were assessed at 12 months. Recovery kinetics and patterns were investigated by mixed-effect logistic modeling, correlation and clustering analyses. Risk of persistent symptoms and cardiopulmonary abnormalities at the 1-year follow-up were modeled by logistic regression.

Findings: Out of 145 CovILD study participants, 108 (74.5%) completed the 1-year follow-up (median age: 56.5 years, 59.3% male; 24% ICU patients). Comorbidities were present in 75% (n=81). Key outcome measures plateaued after 180 days. At 12 months, persistent symptoms were found in 65%, 33% suffered from LFT impairment, 51% showed CT abnormalities, and 63% had low-grade diastolic dysfunction. Number of persistent symptoms, predominately self-reported fatigue and dyspnea, but not cardiopulmonary findings correlated with diminished QoL and poor mental health. Inflammatory biomarkers (interleukin 6, C-reactive protein, D-dimer) and anti-SARS-CoV-2-IgG antibody levels at early follow-up were identified as risk factors for persistent cardiopulmonary findings.

Conclusion: One year after COVID-19, three recovery trajectories are emerging, separating almost complete recovery from patients with a post-acute inflammatory profile and cardiopulmonary residuals from a female-dominated post-COVID syndrome with reduced mental health status. These observations set the stage for further mechanistic and therapeutic considerations

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# Regulation of innate immune system orchestrates inflammation in calcific aortic valve disease

Calcific aortic valve disease (CAVD) is the most common type of valvular diseases with rising prevalence. Valve interstitial cells (VICs) are the predominant cell type within the aortic valve and responsible for stability of valve function. During disease development, VICs differentiate into osteoblast-like cells, actively producing bone, thereby impairing leaflet function. The trigger for this phenotypic switch is still unknown. The lack of mechanistic insight stymies the development of effective intervention strategies and replacement of the aortic valve via invasive surgery is the only treatment option. Non-invasive pharmaceutical approaches for CAVD are urgently needed.

In recent work we discovered, that Tlr3-/- and Bgn-/- mice are protected from CAVD and observed, that Toll-Like receptor 3 (TLR3) activation promoted the pathological phenotype. We uncovered the proteoglycan biglycan (BGN) as an endogenous TLR3 ligand and provided evidence that xylosyltransferase 1 (XYLT1) - dependent maturation of BGN was crucial for TLR3 activation. A genome wide association meta-analysis of two large-scale cohorts with >300.000 individuals revealed that genetic variations at loci relevant to the XYLT1-BGN-TLR3 pathway were significantly associated with clinically relevant aortic valve calcification.

To pursue our goal of developing a pharmacological therapy option we aim to characterize the interaction of TLR3 and BGN, find genomic variants indicative for CAVD development and finally develop a pharmacological inhibition of the XYLT1-BGN-TLR3 axis to prevent from or halt the development of CAVD. Thereby we could not only improve the quality of life for affected patients, but also reduce the socio-economic health burden of the disease.

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# Assessment tools in ex-situ heart perfusion – an evaluation in a pig model

BACKGROUND: Ex-situ heart perfusion (ESHP) enables the resuscitation and assessment of donor hearts. Therefore, a comprehensive analysis of myocardial function and metabolism is crucial. Currently, the only used assessment tool for quality control is sequential lactate measurement from the perfusate. The aim of this study was to investigate new assessment tools in ESHP.

METHODS: 12 German domestic pigs were used as heart and blood donors. "Donation after brain death" (DBD) (n=6) and "donation after circulatory death" (DCD) (n=6) were simulated and 6 hours of normothermic ESHP was performed. Different assessment tools were used to analyse functional status (left ventricular pressure balloon [LVPB], visual cardiac score [VCS]) and myocardial damage and metabolic in the perfusate (lactate, myoglobin, high-sensitive [hs] troponin t).

RESULTS: Parameters of myocardial damage increased in both groups and were significantly higher in the DCD group: myoglobin (T1: DCD 1154±444.7 vs. DBD 385.2±113.8µg/l, p=0.011; T3: DCD 2002±571.4 vs. DBD 652±117.2 µg/l, p=0.003), hs troponin T (T1: DCD 44796±46581 vs. DBD 12229±6447ng/l, p=0.159; T3: DCD 446742±315830 vs. DBD 102496±33448 ng/l, p=0.044). Functional assessment revealed a decline during ESHP in both groups. LVPB did not differ between the groups (T1: DCD 160±97 vs. DBD 204±83mmHg, p=0.354; T3: DCD 66±38 vs. DBD 104±40mmHg, p=0.250), whereas VAS was significantly lower in DCD group (T1: DCD 7.3±0.8 vs. DBD 7.8±1.2, p=0.490; T3: DCD 2.7±1.0 vs. DBD 5.8±1.5, p=0.002).

CONCLUSIONS: Additional assessment tools in ESHP are urgently necessary. Biomarker and microdialysis are both feasible and able to detect metabolic and functional.

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# Clinical PhD Talks

## Wednesday

| Presenting Author | | Abstract title |
|---|---|---|
| Nikolaus | Kögl | Lumbar disc herniation - surgical treatment and its timing |
| Vera | Filippi | MR-Spectroscopy: Investigating neurochemical changes in brain metabolism in migraineurs before and after CGRP-Antibody treatment – a randomized, controlled, open-label trial. |
| Florian | Hofer | Characterising autonomic dysfunction through ECG-derived biomarkers in patients with Takotsubo cardiomyopathy |
| Anel | Karisik | Psychological Consequences of Post-Stroke Dysphagia and its Association with Depression |
| Simon | Staggl | Effects of HIF-1α stabilisation with Roxadustat on cardiac repair after myocardial infarction in mice |
| Nicolas | De Cleene | Olfactory training in COVID-19 associated loss of smell (SMELL) |
| Katharina | Müller | YOUhealTH – a randomized-controlled interventional study to improve cardiovascular health in adolescents and adults |
| Astrid | Knell | Analyse und Charakterisierung des Immunresponse nach Influenza-, SARS-CoV-2- und Pneumokokken-Impfungen (IMMUNE-Study) |
| Fritz | Oberhollenzer | Optimized risk stratification using cardiac magnetic resonance imaging in patients with ST-segment elevation myocardial infarction |
| Clemens | Plattner | Histopathological Diagnosis in Type 2 Diabetes Patients with Chronic Kidney Disease – A Personalized Approach |
| Anna | Lindner | Infectious complications in patients with intracerebral hemorrhage – sex-specific aspects |
| Philipp | Spitaler | Schrittmacher-basiertes Schlafapnoe Langzeit-Monitoring |
| Nicole | Campese | Pain in people with multiple system atrophy: a single-center, cross-sectional, observational, web-based survey |
| Raphael | Gmeiner | Biomechanical effect of a novel „tether pedicle screw“ in long-segment spinal instrumentation |
| Frederik | Eisendle | Safeback Trial |
| Franziska | Schmidt | The Use of Augmented Reality as an Educational Tool in Minimally Invasive Transforaminal Lumbar Interbody Fusion |

# Clinical PhD Talks

## Wednesday

| Presenting Author | | Abstract title |
|---|---|---|
| Philipp | Lichtenberger | Effect of ex-vivo application of von Willebrand factor concentrate on complement system and contact pathway in critically ill patients under extracorporeal support devices with and without sepsis |
| Julian | Wagner | Effects of head-up versus supine CPR on cerebral oxygenation and metabolism during advanced life support in a porcine model |
| Clara | Dosser | Temporal Dynamics of Somatic Mutations in the Context of Systemic Inflammation |
| Vera | Kleinveld | Neurological manifestations in wild-type transthyretin amyloidosis |
| Cristina | Alomar Dominguez | In vitro determination of the optimal Fibrinogen to FXIII ratio in a diluted coagulopathy model |
| Anna-Katharina | Gerstner | Evaluation of Dual Energy Computed Tomography (CT) of diffuse liver disease and HCC |
| Michael | Eller | CGRP and other neuropeptides in acute and subacute stroke |

## Lumbar disc herniation - surgical treatment and its timing

Patients with intervertebral disc herniation undergo surgical removal of herniated disc material in case of persisting symptoms or neurologic deficits. While motor deficits often prompt surgery, little is known about the optimal timing of surgery in these cases.

Methods:

The aim of this prospective registry study was to prospectively evaluate the impact of timing of disc surgery on motor recovery.

Results: In total, 120 patients with sciatica and or sensorimotor deficits due to a lumbar disc herniation were surgically treated at the authors' center within a 3 months period. In 60 patients motor deficits were present at the time of admission. Motor function was assessed using manual muscle testing and subdivided according to the Medical Research Council (MRC) scale. Patient demographics, neurologic deficits, duration of motor deficits, treatment characteristics, and outcome were assessed. At a minimum follow-up of one year functional recovery and complications were collated. Patients were subdivided into groups according to the severity of the paresis (MRC ≤3/5 vs. MRC 4/5). Intra-group differences were compared based on the duration of the neurologic deficits. Patients with moderate and severe paresis (MRC ≤3/5) benefit from treatment within 72 hours as they were shown to have a significantly higher complete recovery rate at 1-year follow-up (75% vs. 0%; $p<0.001$).

Conclusion:

Immediate surgery should be offered to patients with moderate and severe motor deficits to increase the likelihood of neurologic recovery. This prospective data may have an impact on emergency triage in these patients.

N. Kögl

# MR-Spectroscopy: Investigating neurochemical changes in brain metabolism in migraineurs before and after CGRP-Antibody treatment – a randomized, controlled, open-label trial.

Background: Imaging techniques have revealed important aspects of the underlying pathophysiological mechanisms of migraine, suggesting abnormal energy metabolism and increased cerebral hyperexcitability as triggers for a migraine attack. Since only a small percentage of monoclonal CGRP antibodies crosses the blood-brain barrier, their main site of action outside the blood-brain barrier is discussed. It is uncertain whether they lead to central effects through their action outside the blood-brain barrier or exert direct central effects. Objective: To investigate whether neurochemical, structural, and functional changes in the migraine brain are associated with CGRP-antibody treatment. Methods: This prospective, randomised, controlled, open-label study will enrol 38 patients diagnosed with episodic migraine (w/o aura) according to ICHD-3 criteria. All participants will undergo an initially stratified 1H-, 31P- MR-Spectroscopy and resting-state fMRI interictally. Half of the participants (n=19) will receive CGRP mAB treatment (Fremanezumab 225mg monthly) after the first scan for three months according to local standard guidelines for CGRP mAB treatment. MR-spectroscopy and resting-state fMRI will be repeated after the treatment. Controls will be measured in an identical setting at the same time points but without CGRP mAB treatment. Future aspects: Investigating the effects of CGRP mAB on the metabolism and its association to functional connectivity in the migraine brain provides in-depth knowledge about the mechanism of action of the CGRP antibody and permits individualized treatment.

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# Characterising autonomic dysfunction through ECG-derived biomarkers in patients with Takotsubo cardiomyopathy

Takotsubo cardiomyopathy is an acute and transient dysfunction of the left ventricle. Patients with Takotsubo (TTS) present similarly to patients with ACS (acute coronary syndrome). The disease was first described 1990 in Japan, and has received more and more attention in the last years. Takotsubo was initially thought to be a relatively benign disease, but more recent data show that it is associated with complications and mortality to a similar extent as acute coronary syndrome (ACS). Although the understanding of this disease has made some progress in the last years, its exact pathophysiology is not understood up to date. Theories on its pathogenesis include adrenergic hormones/stress (overstimulation of the sympathetic nervous system), decreased estrogen levels, altered microcirculation, endothelial dysfunction etc.

In our study we will investigate the autonomic dysfunction in patients with TTS through two ECG-derived parameters: PRD and DC.

Repolarisation of the heart is modulated in the low frequency spectrum (<0.1 Hz). This modulation is caused by phasic efferent sympathetic activity presumably. This low frequency modulation was first characterised in 2014 under the name ‚Periodic repolarization dynamics' (PRD).

Deceleration capacity was first described in 2006 to distinguish between vagal and sympathetic cardiac activity. It was postulated that a diminished deceleration-related modulation of the heart rate may be a relevant prognostic marker in cardiovascular disease.

Our goal is to characterize autonomic dysfunction in patients with TTS through PRD and DC and in a second step to validate PRD and DC regarding their prognostic relevance in this patient group.

F. Hofer

Cardiovascular medicine (CVM)

# Psychological Consequences of Post-Stroke Dysphagia and its Association with Depression

Background: Post-Stroke Dysphagia has a tremendous impact on quality of life and mortality. The consequences of stroke-related swallowing impairment on psychosocial functioning and its psychological sequelae remains unclear.

Methods: Within the prospective STROKE-CARD Registry study (2020 – 2022) dysphagia was diagnosed during clinical routine by swallowing examination by speech therapists. SINGER Independency Index assessed the presence of swallowing problems at discharge and after 3 months. Affective symptoms were recorded after 3 months based on Hospital Anxiety (HADS-A) and Depression (HADS-D) Scale and Beck Depression Inventory (BDI).

Results: Of 648 patients, 19.3% showed dysphagia at baseline. At hospital discharge and 3-month follow-up, 14.8% and 7.3% reported ongoing swallowing issues, respectively. Depression and anxiety scales at 3 months were higher in patients with longer duration of dysphagia (no dysphagia at presentation, dysphagia at presentation, persistent at discharge, persistent at 3-months follow-up) for the HADS-A (4.4±3.5, 5.4±3.6, 6.0±3.6, 7.0±3.6), HADS-D (4.4±3.7, 7.1±4.2, 7.7±4.4, 9.8±4.3) and BDI (7.9±6.7, 12.5±8.7, 13.5±9.0, 16.5±10.2). In binary logistic regression analysis, initial dysphagia and persistency until discharge and follow-up was significantly associated with more depressive symptoms at 3-month follow-up regardless of age, sex and functional outcome (modified Rankin Scale) when compared to those without dysphagia at baseline as well as those who recovered at any timepoint.

Discussion: Post-stroke dysphagia has an immense impact on psychosocial functioning and is associated with depressive and anxiety symptoms. Moreover, our results highlight dysphagia as an independent co-risk factor in the development of depressive symptoms after ischemic stroke.

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# Effects of HIF-1α stabilisation with Roxadustat on cardiac repair after myocardial infarction in mice

Background: The homing of CXCR4+ cells through CXCL12 activation is known to facilitates myocardial repair. Physiological upregulation of CXCL12 lasts only for 48-72 hours, which limits its reparative potential. After this time period, pathological remodeling processes lead to ischemic cardiomyopathy and heart failure. CXCL12 and CXCR4 are target genes of the Hypoxia-induce factor 1 alpha (HIF-1α), which in turn is stabilized under hypoxic conditions and thus reaches higher levels. Our intention is to stabilize HIF-1 α by administration of the prolyl hydroxylase inhibitor (PHI) Roxadustat over 28 days after myocardial infarction to prolong CXCL12 and CXCR4 upregulation and amplify myocardial repair.

Methods: To estimate the effects of the HIF-1α mediated CXCL12 upregulation after induction of myocardial infarction in vivo, optimal doses (50mg/kg i.p; every 72h) of Roxadusat (FG-4592) will be administered to Bl6/J mice.

1. 10 Mice will be treated with Roxadustat (for 28d) after LAD Ligation. 28 days after MI the cardiac function will be assessed by echocardiography and compared to a placebo-treated control group. The size of the infarcted area will be measured from histological tissue sections.

2. 7 days after MI we will harvest heart and bone marrow tissue of 5 Roxadustat treated and 5 placebo-treated mice to determine neovascularization and apoptosis and characterize infiltrating cells.

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# Olfactory training in COVID-19 associated loss of smell (SMELL)

Olfactory dysfunction (OD) is a common symptom of SARS-CoV-2 infection, responsible for the COVID-19 pandemic, and is increasingly recognized as a persisting post-infectious complication. This could be a result of damage to the sensory olfactory epithelium or by direct neurotrophic effects on the olfactory receptors residing in the neuroepithelium. Potential treatment strategies aim for the neural plasticity of the olfactory system and its potential for recovery.

In this RCT, 100 individuals with COVID-19 related persisting OD (>3 months post-infection) will be included. Data regarding impact on daily life and health, OD-related mood and Quality-of-Life (QoL) will be collected, together with objective OD measurements using the Sniffin' sticks test (identification and discrimination). After randomization, the training cohort will perform olfactory training (OT) twice a day with a 4-odor training set for 12 weeks. The other cohort will not train for the first 12 weeks, making it possible to evaluate the natural history of OD.

Olfactory functioning plays an important role in social relationships and is often impaired during and after COVID-19. However, there are currently no proven therapeutic options for affected individuals. Nevertheless, previous studies have shown olfactory training to be an effective therapeutic option for individuals with post-viral or post-traumatic OD.

By proving the efficacy of OT after COVID-19, we offer a simple treatment option for affected individuals with persistent OD. Even more, we hope to show a correlation between OD and its impact on daily functioning and QoL, thereby stressing the importance for therapeutic options.

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# YOUhealTH – a randomized-controlled interventional study to improve cardiovascular health in adolescents and adults

Background: Recent studies have shown that cardiovascular disease (CVD) already starts in early live and is preventable by timely elimination of responsible risk factors. However, effective primary prevention strategies are scarce, especially in adolescents. The YOUhealTH study aims to test a lifestyle-intervention focusing on diet and physical activity in students and at least one legal guardian to improve cardiovascular risk-factor-profiles.

Methods: The YOUhealTH-study is a single-center prospective cluster-randomized controlled intervention study with a planned participation of 150 – 200 adolescents (14 - 17 years) and at least one legal guardian each from 6 different Tyrolean schools. Each participant will be screened for cardiovascular risk factors in a baseline-examination. Participants of the intervention group will undergo a health-promotion, developed by citizen scientist (students of the respective school), for the duration of 1 year. The efficacy of the health-promotion, measured by means of change for the components of diet and physical activity, will be evaluated and compared to the control-group in a follow-up-examination identical to the baseline-examination.

Discussion: The aim of the YOUhealTH-study is to test the efficacy of a health-promotion-intervention over the course of one year to improve cardiovascular health of adolescents and at least one legal guardian

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# Analyse und Charakterisierung des Immunresponse nach Influenza-, SARS-CoV-2- und Pneumokokken-Impfungen (IMMUNE-Study)

Respiratorische Erreger wie SARS-CoV-2, Influenzaviren und Pneumokokken können schwere Atemwegserkrankungen auslösen und zum Tode führen. Insbesondere vorerkrankte Personen sind einem erhöhten Risiko für einen schweren Erkrankungsverlauf ausgesetzt, was sich in einer höheren Morbidität und Mortalität zeigt. Um diese Personen vor schweren Verläufen zu schützen, wurden Impfstoffe gegen Covid-19, Grippe und schwere Pneumokokkenerkrankungen entwickelt. Die Effektivität der durch den Impfstoff ausgelösten (humoralen und zellulären) Immunreaktionen wird jedoch von mehreren Faktoren beeinflusst und mitunter gemindert.

Es erfolgt eine Rekrutierung von Personen am Tag der Covid-19-, Influenza- oder Pneumokokkenimpfung (Baseline). In einem Follow-up nach 3, 6 und 12 Monaten wird Blut abgenommen, mithilfe dessen sowohl die humorale als auch die zelluläre Immunreaktion auf die Impfung laborchemisch untersucht wird. Erste Analysen zum Covid-19 Impfstoff bei Erkrankten mit chronischer Nierenerkrankung, Vaskulitis der kleinen Gefäße und Nierentransplantat wurden bereits durchgeführt. Zudem wurden 190 Probanden/-innen von den internistischen Ambulanzen für Gastroenterologie, Infektiologie, Rheumatologie und Pulmologie rekrutiert. Serum und PBMCs werden auf spezifische SARS-CoV-2 S1 Antikörper, neutralisierende Antikörpertiter gegen verschiedene SARS-CoV-2 Varianten sowie auf Virus-spezifische T-Zellantwort via IFNgamma ELISpot Assay untersucht.

Der Vortrag stellt das Projekt vor und gibt einen Einblick in erste Ergebnisse, die signifikante Unterschiede zwischen den Erkrankungskohorten erkennen lassen.

A Knell 1

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# Optimized risk stratification using cardiac magnetic resonance imaging in patients with ST-segment elevation myocardial infarction

Acute myocardial infarction (AMI) is considered one of the leading causes of death worldwide. ST-elevation myocardial infarction (STEMI) is the most severe form and is predominantly caused by abrupt occlusion of a coronary artery. The broad implementation of primary percutaneous intervention (PCI) as preferred reperfusion strategy and optimized secondary prevention brought to a significant decrease in mortality associated with STEMI. Nevertheless, AMI continues to be a leading cause of death in the Western world and mortality rates have plateaued during the last years. Moreover, in patients surviving an AMI there is a considerable risk of a recurrent cardiovascular event beyond the acute phase of infarction.

Due to its excellent temporal and spatial resolution, cardiac magnetic resonance imaging (MRI) has emerged as the non-invasive gold standard for morphological and functional tissue characterisation. The thorough assessment of functional and morphologic myocardial tissue alterations, as well as other consequences following STEMI, can only be done using this method.

The aim of this dissertation is to characterize functional and morphologic myocardial tissue changes and assess complications after STEMI by using cardiac MRI. For this purpose, different MRI sequences, electrocardiographic and laboratory biomarkers will be evaluated to enable an optimized risk stratification after STEMI, which in this context could represent an important step towards personalized medicine. An optimized and personalized risk stratification using MRI in patients with STEMI will therefore be the main objective of this research effort.

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# Histopathological Diagnosis in Type 2 Diabetes Patients with Chronic Kidney Disease – A Personalized Approach

Introduction

In common practice early signs of chronic kidney disease in longstanding type 2 diabetes are attributed to diabetes, even though an alternative aetiology may dominate the course of disease.

Methods

In the Innsbruck Diabetic Kidney Disease Cohort (IDKDC), a prospective clinical trial, type 2 diabetes patients aged 18 to 74 years with diabetes duration exceeding 10 years will be studied. Inclusion criteria consist of an estimated glomerular filtration rate (eGFR) between 45 and 60 ml/min/1.73m² irrespective of albuminuria or an eGFR of 60 – 89 ml/min/1.73m² with albuminuria >30 mg/g creatinine. Ultrasound-guided kidney biopsy, subsequent histopathological assessment and biannual follow-up for 5 years will be performed.

Aims

We aim to determine the prevalence of diabetic and non-diabetic kidney disease, the frequency of therapeutic changes following histologic diagnosis, the frequency of biopsy related complications and renal as well as cardiovascular prognosis in this high-risk population. Furthermore, a biobank of kidney tissue, blood and urine samples for identification of prognostic and therapeutic biomarkers will be established.

Conclusions

Current advances in the treatment of diabetic and non-diabetic kidney disease warrant a personalized diagnostic approach.

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# Infectious complications in patients with intracerebral hemorrhage – sex-specific aspects

Objective: Infectious complications(IC) commonly occur in patients with intracerebral hemorrhage (ICH) and are associated with increased length of hospitalization(LOS) and poor long-term outcome. There is little known about sex-related differences in ICH. In this study, we intended to investigate sex-specific differences in the prevalence, severity and morbidity/mortality of IC after ICH.

Methods: We prospectively enrolled 229 patients with non-traumatic ICH admitted to the neurocritical care unit(NICU) of a tertiary care hospital. Patients were screened daily for IC. Multivariable regression models using generalized linear models were used to identify associated factors with the occurrence of IC and to study their impact on functional outcome, which was assessed using the modified Rankin Scale Score(mRS) after 3 months. Unfavorable outcome was defined as mRS≥3.

Results: During NICU stay, a total of 109 (48%) patients developed at least one infection. The most common infections were pneumonia (28%), urinary tract infections(24%), sepsis(4%) and ventriculitis(2%). Overall, we found no sex-related difference in the occurrence of IC (women: 43%, men: 43%). Moreover, there was no difference in LOS between men and women (p=0.618). Having at least one infection during NICU stay was associated with unfavorable 3-month outcome (adjOR=3.0, 95% CI 1.41-6.54, p=0.005). However, in multivariate model, female sex was no risk factor for poor functional outcome.

Conclusion: IC are common in ICH patients and independently associated with unfavorable outcome. Our results could not confirm sex-related difference in the occurrence of IC. Moreover, there was no difference in functional outcome after ICH between men and women.

A. Lindner

Clinical Neuroscience (CNS)

# Schrittmacher-basiertes Schlafapnoe Langzeit-Monitoring

Die ACaSA Studie fokussiert sich auf Patient:innen mit erhaltener Pumpfunktion, die ein konventionelles Herzschrittmachersystem erhalten haben und mittels telemedizinischer Nachsorge in Zukunft überwacht werden sollen. Dadurch können die Nachsorgeintervalle auf mehrere Jahre verlängert werden.

Der Schrittmacher BOREA® der Firma Microport® kann kontinuierlich das Ausmaß von schlafbezogenen Atemstörungen (zum Beispiel Schlafapnoe), die körperliche Aktivität und Arrhythmien messen.

Schwere Schlafapnoe, definiert durch einen durchschnittlichen Respiratory Disturbance Index (RDI) ≥ 20/h, findet sich bei zumindest 30 % aller Patient:innen mit implantiertem Herzschrittmacher. Das Ausmaß der körperlichen Aktivität korreliert mit dem Gesamtüberleben bei Patient:innen mit implantiertem Herzschrittmacher oder ICD.

Störungen der kardialen autonomen Funktion können durch ein Risiko-EKG bestimmt werden: Erhöhte PRD-Werte (periodic repolarisation dynamics) sind mit schweren Arrhythmien bei Patient:innen nach Myokardinfarkt und einer eingeschränkten systolischen Pumpfunktion (LVEF 35 -50%) assoziiert.

Die ACaSA Studie testet die Hypothese, dass ein erhöhter PRD Wert ≥ 5,75 $deg^2$, und Schrittmacher-basiertes Langzeit-Monitoring von Schlafapnoe (gemessen am RDI) und ein bewegungsarmer Lebensstil (gemessen mittels Langzeit-Monitoring der täglichen Stunden mit körperlicher Aktivität) wichtige Informationen bezüglich zukünftiger klinischer Ereignisse liefert. Hier wird ein kombinierter 3P-MACE Endpunkt (Gesamtmortalität, Auftreten eines Myokardinfarkts oder eines Schlaganfalls) kommen. Ebenso wird das Auftreten von Arrhythmien wie Vorhofflimmern und ventrikuläre Tachykardien mit kardialer autonomer Dysfunktion, Schlafapnoe und körperlicher Inaktivität korreliert werden.

Ein weiteres Studienziel ist es, die Prävalenz und Assoziation von Begleiterkrankungen bei Patient:innen mit einem Herzschrittmacher zu charakterisieren und deren Impact auf das Langzeit-Outcome prospektiv zu erfassen.

P. Spitaler

Cardiovascular medicine (CVM)

## Pain in people with multiple system atrophy: a single-center, cross-sectional, observational, web-based survey

Background: Multiple system atrophy (MSA) is a rare, rapidly progressive neurodegenerative disorder of the adulthood presenting with autonomic and motor dysfunction in various combinations. Pain is frequently reported, nonetheless its prevalence, features and risk factors remain poorly understood in people with MSA.

Objective: Primary aim of this study is to estimate the prevalence of different types of pain in people with MSA. The characterization of pain features, MSA-dependent and independent risk factors for pain, current treatment strategies and impact of pain on patients' quality of life (QoL) represent secondary aims of the study. Additionally, pain-related caregiver burden and impact of patient's pain on caregiver's QoL will be addressed.

Methods: A single-center, cross-sectional, observational, web-based survey to be completed independently by both people with MSA and their informal caregivers will be run between February and May 2023. Two different questionnaires for people living with MSA and their caregivers will be available in English and in German. The link to the survey will be also disseminated through social-media to reach patients not referred to specialized centers.

Results: After completion of the active recruiting phase, data will undergo a data-cleaning process and finally a data analysis. The results of the study will be available upon completion of this process.

Conclusion: Pain is a frequent, yet underrecognized and undertreated feature of MSA, impacting on patients' and caregivers' QoL. A better characterization of prevalence, features and MSA-dependent and independent risk factors will help developing tailored pain assessment and treatment strategies for this rare disorder.

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# Biomechanical effect of a novel „tether pedicle screw" in long-segment spinal instrumentation

Hintergrund

Dieses Forschungsprojekt setzt sich mit der häufigsten Komplikation nach langstreckigen Wirbelsäulenversteifungen auseinander, die sogenannte Anschlussdegeneration (ASD). Allgemein nahmen Wirbelköperversteifungen in den letzten Jahrzenten immer mehr zu und stellen heute bei Wirbelsäulendeformitäten den häufigsten Eingriff dar, weisen jedoch noch immer eine hohe Komplikationsrate, wie Infektion, Schraubenlockerung, Materialbruch etc. auf. Die Anschlussdegeneration wird bei langstreckigen Instrumentierungen und kranialer, kyphotischer Fehlstellung auch als „proximale junktionale Kyhose - PJK" genannt. Diese entsteht in den anschließenden Wirbelkörpersegmenten, da es aufgrund der erhöhten mechanischen Belastung, zu einer verfrühten Degeneration kommt.

In dieser Untersuchung kommt nun eine speziell entwickelte Schraube zum Einsatz. Diese Schraube, eine sogenannte „theter pedicle screw - TPS", enthält ein straffes Band zwischen der Schraubentulpe und dem Gewinde und weist somit eine Restbeweglichkeit auf. Damit soll der kraniale Übergang zwischen stabilisiertem Konstrukt und den beweglichen Segmenten abgefedert und gleichmäßiger verteilt werden.

Material und Methodik:

Wir untersuchen drei verschieden Instrumentierungen an 10 menschlichen Wirbelsäulenpräparaten von BWK7 bis LWK2. Verglichen werden drei Gruppen, die erste Gruppe, standard_group, beeinhaltet eine standard, transpedikuläre

Stabilisierung von BWK10-LWK1 und gilt aus Referenzgruppe. Die zweite Gruppe, TPS_UIV_+1 group, erhält ebenso eine standard, transpedikuläre Stabilisierung von BWK10-LWK1 und eine zusätzliche TPS in BWK9. Die dritte Gruppe, TPS_UIV_+2 group, erhält die standard, transpedikuläre Stabilisierung von BWK10-LWK1 und eine zusätzliche TPS in BWK 9 und BWK 8. Danach erfolgt von der 1. und 3. Gruppe eine Ermüdungsprüfung. Anschließend erfolgt ein Ausrisstest der Pedikelschraube in BWK10 (Gruppe 1), und TPS in BWK7, 8 ,9 + 10 (Gruppe 3).

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# Safeback Trial

Survival of fully buried avalanche victims depends in major part on a triad of hypoxia, hypercapnia and hypothermia and therefore decreases rapidly after burial. 10% of victims die in the first minutes because of major trauma. Additional two thirds of non-survivors die in the next 18 to 35 minutes due to asphyxia. Based on previous research, the Norwegian company Safeback SE (Bergen, Norway) developed a new device using an innovative functional principle. The device, called the Safeback SBX (Safeback SE, Bergen, Norway), should make it possible to prevent asphyxia by delivering fresh air to the air pocket, leading to a prolongation of survival in critically buried subjects up to over 60 minutes. Even if technical tests already provided some promising results, this study is needed to provide the scientific evidence of the effectiveness and influence on physiologic parameters in snow debris buried humans under realistic conditions.

The study will be conducted as an interventional, randomized, controlled and single blinded field trial. We plan to include 26 healthy ASA I participants, men and women.

The main hypothesis of the proposed research is that the use of the Safeback SBX will delay hypoxemia and hypercapnia in subjects critically buried in avalanche debris.

Primary endpoint is a time to Event analysis (reaching 60 minutes of time or a specific SpO2 threshold). Secondary objectives of the proposed study are to evaluate additional effects on physiological parameters and respiration, experienced stress level, neuropsychological stress intensity, gas diffusion in the snow and biological markers.

F. Eisendle

Intensive Care and Emergency Medicine (ICE)

# The Use of Augmented Reality as an Educational Tool in Minimally Invasive Transforaminal Lumbar Interbody Fusion

Purpose: One of the major challenges in training neurosurgical and orthopedic residents the technique for minimally invasive transforaminal lumbar interbody fusion is the lack of visualization of surgical landmarks (pedicle, pars, lamina). Augmented Reality (AR) is an emerging technology, which superimposes digital images onto the real-world environment. The purpose of this study is to assess the utility, accuracy, efficiency, and precision of AR-guided MIS-TLIF and to determine its impact in spine surgery training.

Methods: At two centers, twelve neurosurgical residents performed a one-level MIS-TLIF on a high-fidelity lumbar spine simulation model with and without AR projection into the microscope. For the MIS-TLIF procedures with AR, surgical landmarks were highlighted in different colors on preoperative image data. These landmarks were visualized in the spinal navigation application on the navigation monitor and in the microscope in order to confirm the relevant anatomy. All procedures were recorded for evaluation and time measurements. Post-procedural surveys (NASA task load index) were given to the residents.

Results: 12 residents were included in this prospective, multi-center, randomized-controlled trial. AR-guided procedures had a consistent impact on resident anatomical orientation and workload experience. Procedures performed without AR had a significantly higher mental demand ($p=0.003$) than with AR. Residents reported to a significantly higher rate that it was harder work for them to accomplish their level of performance without AR ($p=0.019$).

Conclusion: AR can bring a meaningful value in MIS teaching and training in order to confirm relevant anatomy in situations where the surgeon will have less direct visual access.

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# Effect of ex-vivo application of von Willebrand factor concentrate on complement system and contact pathway in critically ill patients under extracorporeal support devices with and without sepsis

Human von Willebrand Factor (vWF) plays a major role in haemostasis. It is synthetized by megacaryocytes, subendothelial and endothelial cells in form of ultra-large vWF-multimeres (ULVWM). In case of vascular trauma it will readily bind to collagen, facilitating platelet adhesion and aggregation by binding to platelet receptors and protecting factor VIII from degradation. vWF will acts as an inductor of inflammatory response by the induction of thrombin and bradykinin via the coagulation cascade, as well as activation of contact and complement pathways.

Enzymatic breakdown of vWF occurs under calcium binding or mechanical shear stress via the metalloproteinase ADAMTS13 at a partially buried cleavage site, leading to smaller, less efficient vWF-molecules.

Under mechanical circulatory support (MCS) like extracorporeal membrane oxygenation (ECMO) or Impella pumps patients' blood is exposed to higher levels of shear stress due to turbine effects within the machine. In consequence, more breakdown of vWF via ADAMTS13 will occur, which may lead to an acquired coagulopathy due to loss of ULVWM as a form of acquired vWF-syndrome (AVWS).

In cases of AVWS application of recombinant vWF concentrate (rvWFC) such as vonicog alfa (Veyvondi®) may be appropriate, but there is little data on its effects in the setting of MCS, especially in regards to complement and contact pathway activation.

The goal of our project is to evaluate the coagulatory effects of ex-vivo application of rvWFC, as well as complement and contact pathway activation in order to provide more evidence on its effects in MCS settings with and without sepsis.

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# Effects of head-up versus supine CPR on cerebral oxygenation and metabolism during advanced life support in a porcine model

Background

The aim of the current study was to investigate the effect of head up position (HUP) versus supine position (SUP) on cerebral oxygenation and metabolism during advanced life support (ALS) in a porcine model.

Material and Methods

A total of n=19 pigs were anaesthetized and instrumented (n=10 HUP, n=9 SUP). After 5 minutes of cardiac arrest (CA), the pigs were ALS resuscitated for 15 minutes in either 30° HUP or SUP. Mean arterial pressure (MAP), intracranial pressure (ICP), cerebral perfusion pressure (CePP), cerebral regional oxygen saturation (rSO2), cerebral venous oxygen saturation (ScvO2) and brain tissue oxygen tension (PbtO2) were measured continuously. Cerebral microdialysis and blood samples were collected at baseline, after CA and every 5 min during CPR.

Results and Discussion

ICP was significantly lower in HUP animals ($p<0.021$). Consequently, CePP was significantly higher in the HUP group ($p=0.0107$). However, relative PbtO2 was significantly higher in the SUP group ($p=0.0376$). CePP is not a physiological parameter but is calculated from the difference between MAP and ICP. As such, it is prone to error and may not necessarily reflect the actual prevailing perfusion pressures. Furthermore, blood must be pumped "upwards" during HUP necessitating sufficient forward blood flow.

Conclusion

HUP ALS lowers ICP which is associated with a perceived improvement in CePP. However, CePP alone does not provide sufficient information about cerebral blood and oxygen supply during CPR. Despite higher CePP values, HUP does not improve cerebral metabolism compared to SUP and may even lead to a deterioration of cerebral oxygenation.

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# Temporal Dynamics of Somatic Mutations in the Context of Systemic Inflammation

Introduction: Clonal hematopoiesis of indeterminate potential (CHIP) refers to a population of related myeloid cells with an acquired mutation of a leukemia-associated gene (with a variant allele fracture [VAF] >2%) in patients with normal peripheral blood counts and no clinical or pathological evidence for a WHO defined hematologic malignancy. The majority of these mutations has been accounted to only a handful of genes, namely DNMT3A (50-60%), TET2 (10-15%) or ASXL1 (8-10%). As could be expected, the risk of developing leukemia is approximately 10-times higher in individuals with CHIP as compared to those not harboring CHIP. Interestingly it was found, that the mutations are associated with inflammatory imprinting of circulating immune cells that invade atherosclerotic plaques and secrete cytokines that maintain local inflammation. Therefore, individuals with CHIP have also a markedly increased risk for coronary heart disease, myocardial infarction and ischemic stroke.

However, it is not known how inflammatory-driven cardiovascular events (i.e. stroke) influence expansion of CHIP-clones and how this impacts long-term clinical outcomes.

Methods: Targeted NGS will be performed at the time of event (stroke) and at 12-month post-event to identify the mutational profile and expansion of CHIP-clones. This will be correlated to measures of systemic inflammation and clinical outcome data.

Expected Results: The project will help to understand the post-stroke inflammatory response and could help to identify stroke patients that may benefit from individualized anti-inflammatory or anti-clonal treatment strategies.

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# Neurological manifestations in wild-type transthyretin amyloidosis

Background: Transthyretin amyloidosis (ATTR) is a rare progressive condition characterized by extracellular deposition of transthyretin in organs and tissues, causing a wide spectrum of symptoms, depending on the location of the transthyretin aggregates. ATTR can result from mutations in the TTR gene, causing depositions of abnormal transthyretin (hereditary form, ATTR-v), or by deposition of misfolded non-mutated transthyretin (wild-type transthyretin amyloidosis, ATTR-wt). Neurological manifestations, such as large and small fiber, and autonomic neuropathy are part of the clinical spectrum in ATTR-v. Based on the pathophysiology of ATTR, these neurological complications might also be common in ATTR-wt. Better understanding of neurological manifestations in ATTR-wt may affect clinical management.

Method: 30 ATTR-wt patients with cardial involvement and 30 age and sex-matched controls will be included. Both treatment-naïve and treatment-experienced patients are included. Assessments will include i) nerve conduction studies and clinical neurological examinations to assess poly- and mononeuropathy ii) cardiovascular autonomic function testing, quantitative sudomotor axon reflex testing and Composite Autonomic Symptom Score-31 to measure autonomic nervous function, iii) quantitative sensory testing and skin biopsies in the lower extremity to study small nerve fiber function iv) questionnaires (Norfolk Quality of Life-Diabetic Neuropathy and Chalder Fatigue Scale). These assessments will be repeated during a 1-year follow-up visit to evaluate disease progression.

Discussion: Our aim is to provide a comprehensive clinical and electrophysiological characterization of neurological and autonomic complications in patients with ATTR-wt. The frequency and severity of these complications and their progression over one year are studied.

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# In vitro determination of the optimal Fibrinogen to FXIII ratio in a diluted coagulopathy model

## Introduction

In traumatic injuries, severe bleeding is initially treated with crystalloid and colloid fluids in order to maintain organ perfusion, but also provokes a blood dilution that exacerbates traumatic coagulopathy and worsens the bleeding. Substitution of blood loss then follows with erythrocyte concentrates and fibrinogen. However, fibrinogen needs factor XIII to stabilize the clot. So far, a substitution threashold for factor XIII is not well stablished, and little is known of the effect of both fibrinogen and factor XIII levels on clot stability. In this study we aim to find the ratio of fibrinogen to factor XIII that best restores clot stability in a dilution coagulopathy model.

## Methods

Drawn blood from 20 healthy donors will be diluted 1:3 with a mixture of the colloid Gelofusine® and Ringer's Lactate solution as to model a dilutional coagulopathy. Diluted samples will be spiked with different concentrations of fibrinogen and factor XIII. All samples will undergo the following coagulation tests: viscoelastic tests (ClotPro®:EXtest, INtest, FIBtest, TPAtest), confocal microscopy, blood count and standard global coagulation tests (aPTT, PT, TT), fibrinogen (Clauss' method and immunologic), factor XIII (immunologic) and clot retraction. Spiked samples will be compared to the respective diluted controls. The primary endpoint is defined as the fibrinogen to factor XIII ratio that achieves the optimal ClotPro® FIBtest Maximum Clot Firmness (MCF).

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# Evaluation of Dual Energy Computed Tomography (CT) of diffuse liver disease and HCC

Chronic liver disease (CLD) is a worldwide problem with increasing incidences. The final common stage is liver cirrhosis, the main risk factor for developing hepatocellular carcinoma (HCC). The gold standard for characterizing CLD is a biopsy which however is not suitable for follow up due to the invasiveness of the procedure. Dual Energy CT (DECT) brings with it the advantage of using a material decomposition algorithm providing a wide range of diagnostic possibilities.

The aim of this study is to retrospectively evaluate DECT-based quantification of fat, iron and iodine content of the liver and HCC compared to established methods like MRI, ultrasound-elastography and biopsy. The study was approved by the Ethics Committee of the Medical University Innsbruck. Patients with chronic liver disease receiving DECT of the abdomen acquired from the Siemens Somatom Drive at the University Hospital Innsbruck from March 2021 to March 2023 are included. The data will be collected pseudonymously.

Parametric and non-parametric statistical tests are going to be performed to evaluate the statistical significance of differences between means of the groups.

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# CGRP and other neuropeptides in acute and subacute stroke

Stroke is the second leading cause of death from non-communicable diseases and is the most frequent cause of permanent disability worldwide. As Calcitonin Gene-Related Peptide (CGRP) is known as one of the most potent vasodilative neuropeptides and its role in migraine was found to be substantial, CGRP blockage is emerging in preventive treatment of migraine. As many studies suggest a possible correlation between stroke and CGRP, a rodent model of CGRP blockage showed more severe stroke in female and male mice.

The aim of this study is to collect data of CGRP and other neuropeptide levels in acute and post-acute ischemic stroke patients. Data work-up to establish a bio-databank of CGRP and other neuropeptide levels in stroke patients will be performed.

This study will be set up as a prospective, monocentric cross sectional study enrolling patients (n=80) with ischemic or haemorrhagic stroke. All patients enrolled will undergo three to four consecutive blood collections out of the cubital vein. The first blood sample will be taken in the emergency room immediately after stroke diagnosis. In follow-up, one blood sample will be taken on the following day of stroke and three months after stroke as part of the STROKE-CARD standard of care visit. Patients eligible for thrombectomy will undergo a fourth blood collection out of the affected cerebral artery while thrombectomy. All collection of blood samples will be performed via standard of care blood sample collection in stroke diagnosis and in follow-up.

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# Life Science PhD Meeting

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