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17th Leipzig Research Festival for Life Sciences 2024

ABSTRACT BOOK

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Prof. Dr. Tobias Piegeler
Prof. Dr. Steffi G. Riedel-Heller
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(Hrsg.)

17th

Leipzig Research Festival for Life Sciences

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Faculty of Medicine, Leipzig University
Faculty of Life Sciences, Leipzig University

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Prof. Dr. Tobias Piegeler
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Preface

Dear colleagues and guests,

We are very pleased to announce that the Research Festival for Life Sciences will take place again on January 18, 2024 in the Clinical Trial Center of the Faculty of Medicine. The event had to take a break for three years due to the pandemic. After this long time, we are motivated and excited to bring the scientific community together again and to promote dialogue on current research topics.

The Research Festival for Life Sciences at the University of Leipzig was launched in 2002 and has since then provided a platform for the presentation of numerous talented young scientists from Leipzig's university landscape. The festival focuses on relevant and innovative scientific topics in the fields of medicine and life sciences. At the same time, it serves to promote interdisciplinary networking between different departments and disciplines.

We welcome poster submissions from all fields of medicine and life sciences, including psychology, pharmacy, chemistry, biology, physics, computer science, and others. We would also like to receive applications from the Max Planck-, Fraunhofer- and Helmholtz Institutes, the Helmholtz Centre for Environmental Research UFZ, the German Center for Integrative Biodiversity Research iDiv as well as the groups of the Centre for Biotechnology and Biomedicine BBZ, and from regional biotechnology companies. Contributions from the field of applied clinical research are also warmly welcome.

As in previous editions of the festival, prizes for the best poster presentations will be awarded in recognition of young scientists. In addition to the young scientist prizes, which will be determined by a panel of experts, we will also award an audience prize. All winners will be asked to present their work briefly in 2-minute lightning talks during the award ceremony to give visitors an insight into the winning research.

Our aim is to promote the exchange of knowledge, showcase innovative research, and encourage young scientists to present their work to a wider audience. It is also an opportunity for all participants to network, explore potential collaborations, and learn from the experiences of other researchers.

We look forward to shaping the 17th Research Festival for Life Sciences with you!

Dr. Dr. John T. Heiker | Prof. Dr. Tobias Piegeler | Prof. Dr. Steffi G. Riedel-Heller |
Prof. Dr. Michael Schaefer | Prof. Dr. Michaela Schulz-Siegmund | Prof. Dr. Andreas Thum

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Abstracts Research Festival 2024

Poster 1

Development of Lipoplex-loaded surface-coatings for contact triggered transfection

Krabbes M., Kampik V., Krieghoff J., Schulz-Siegmund M., Wölk C.

Leipzig University, Medical Faculty, Institute of Pharmacy, Pharmaceutical Technology, Leipzig, DE

The release of bioactive substances from smart biomaterials after implantation holds great potential in regenerative medicine due to its ability of precisely targeted, local application. Layer-by-Layer (LbL) surface coatings provide the possibility to create accurately constructed thin-film coatings. Therefore, it is widely used in the field of surface functionalization. The development of LbL coatings loaded with biological active substances is of special interest to control cell behaviour. An upcoming field is the functionalization with nucleic acids to achieve an in situ transfection for controlled protein expression.

In this study the LbL technique is applied to generate lipoplex embedding surface coatings that are composed of hyaluronic acid and chitosan. By using LbL it is intended to create a surface coating which results in high transfection efficiency and good biocompatibilty. Our aim is to get insights in the properties and the control of the transfection activities.

Basal polyelectrolyte multilayers (PEM) are prepared by alternating incubation of the surface with the oppositely charged polyelectrolytes. After a stable basal PEM is fabricated, the lipoplexes are adsorbed and cover layers are added, which have a protecting effect on the lipoplexes.

The gene expression analysis is performed by encapsulating a GFP-encoding pDNA in the lipoplexes, allowing a quantification of transfection events by fluorescence microscopy and flow cytometry.

By adjusting the assembly conditions it was possible to create more reliable, densely loaded films and therefore improve the system.

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Poster 2

Generation of microparticles from three-armed, biodegradable macromers by oil-in-water emulsion and subsequent photo-cross-linking

Krieghoff J.[1], Kubat J.[1], Hacker M. C.[1,2], Schulz-Siegmund M.[1]

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Introduction

As macromolecules with at least one polymeric block and two or more reactive groups, macromers are a suitable basis for adaptable biomaterials. Previously, we developed the TriLGA macromers with lactic (LA) and glycolic acid (GA) in the biodegradable oligoesters [1] [2]. Films from these macromers were used as models for subsequent surface modifications [3].

Objective

Fabrication of injectable microparticles from the TriLGA macromers was investigated as a potential avenue to translate the concept of the surface-modifiable material into a medical device.

Materials and methods

The macromer, a photo-initiator and an optional anchor molecule were dissolved in an organic solvent. This organic phase was added to a stirred poly(vinyl alcohol) (PVA) solution (aqueous phase). The formed droplets were cross-linked to microparticles by UV light, which were subsequently characterized (visual, laser diffraction, SEM).

Results

With the oil-in-water emulsion technique and subsequent UV cross-linking, microparticles were successfully generated from the TriLGA macromers. The reference formulation reproducibly gave spherical, porous particles with sizes between 60 and 160 µm. Multiple TriLGA variants could be used for particle fabrication. Choice of organic solvent as well as PVA concentration significantly affected microparticle size and morphology. As optional anchors for surface modification, glycidyl methacrylate as well as methacrylic acid could be co-polymerized. In the next step, the TriLGA microparticles will be investigated for subsequent surface modification.

References:

[1] Loth R. et al, (2015), Highly adjustable biomaterial networks from three-armed biodegradable macromers, Acta Biomaterialia, 82–96, 26

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[3] Müller B. et al, (2017), Surface modification of copolymerized films from three-armed biodegradable macromers - An analytical platform for modified tissue engineering scaffolds, Acta Biomaterialia, 148–160, 51

Poster 3

siRNA Delivery & Microtissue Assembly Via Gelatine Microparticles For Bone Tissue Regeneration

Mitrach F.[1], Liebezeit A.[1], Springwald A. H.[1], Schmid M.[1,2], Hinkelmann S.[1], Wölk C.[1], Hacker M. C.[1,2], Schulz-Siegmund M.[1]

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[2]Institute of Pharmaceutics and Biopharmaceutics, Heinrich-Heine-University Düsseldorf, Düsseldorf, DE

Objective:
Bone tissue engineering using mesenchymal stem cells (MSCs) is a promising strategy for to support bone regeneration. The cultivation and differentiation of cells in a three-dimensional environment preserves cell-cell interactions and enhances biological functions. However, the densely packed cell layers lead to necrotic core formation [1]. In this study, we used cross-linked gelatin microparticles (cGM) that can serve for two purposes:
1) as a cell-adhesive biomaterial to reduce necrotic core formation and
2) as a siRNA delivery system to decrease expression of anti-osteogenic targets.

Methods:
MSCs were aggregated with various amounts of cGM and stimulated with osteogenic supplements. siRNA-loading of cGM was done via oligomer-stabilized calcium phosphate nanoparticles (CaP-NP). These showed to be a highly effective siRNA transfection reagent with a high siRNA loading efficiency in a previous project [2]. We determined siRNA silencing efficiency using the BMP-2 antagonist Chordin as a molecular target and evaluated effects on the osteogenic differentiation.

Results:
Analysis showed that cultivation of MSCs with cGM increased cell viability and osteogenic differentiation. The loading of cGM with siRNA-carrying CaP-NP resulted in an increased silencing efficiency of Chordin in MSCs that led to a remarkable increased mineralization.

References:
[1] Schmitz et al.: Front. Bioeng. Biotechnol. 2021, 9: 611837
[2] Mitrach et al.: Pharmaceutics 2022, 14(2): 326

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Poster 4

Review of mechanisms for cellular uptake and cytosolic payload release of nanoparticle RNA formulations

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The advent of RNA therapeutics in recent years has increased the demand for carrier systems such as lipid or polymer nanoparticles that can transport RNA into target cells. Lipid nanoparticles composed of ionisable lipid composites demonstrated the power of such delivery systems for mRNA delivery as part of the vaccine formulations of BioNTech/Pfizer and Moderna. For effective or even rational design of these carrier systems it is crucial to understand the mechanisms behind internalization and release of the payload into the cytosol. We aim to give a detailed overview over cellular uptake mechanisms of nanoparticles, what overall role they play in transmembrane transport and how the release of cargo within target cells is facilitated. For cell uptake we specifically look at clathrin-, caveolae-mediated and independent-endocytosis, macropinocytosis, phagocytosis and membrane-fusion.[1] [2] For payload release, we will focus on different pathways that pathogens such as the Influenza A virus can hijack to escape lysosomal degradation and facilitate payload release.[3] Furthermore, we analyse various attempts to decorate the surface of nanoparticles to increase transfection efficiency. Overall, literature shows that nanoparticles are mostly internalized by classical endocytotic pathways and therefore automatically accumulate in endosomes.[1] This indicates that lysosomal escape without degradation of the cargo is an important step for delivery into the cell. However, the detailed mechanisms for this are not yet well studied and further research is needed to conclusively understand them.

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Poster 5

Impact of rubella virus infection on syncytialization of human trophoblast cell line BeWo

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Background:
The human trophoblast choriocarcinoma cell line BeWo mimics syncytilization of the placental villous trophoblasts. Incubation with cyclic adenosine monophosphate (cAMP) or forskolin as its inducer, results in fusion and syncytia formation. Here we tested the impact of syncytialization on infection with rubella virus (RuV), a representative pathogen known to cross the placental barrier.

Methods:
Incubation of BeWo with forskolin for 72 hours was used to induce fusion. Syncytialized BeWo were subjected to RuV infection followed by further incubation for 72 hours. To address the impact of RuV infection on syncytialization, infected cells were plated followed by induction of syncytialization. Infection and syncytia formation were monitored through staining of the viral capsid protein and of E-cadherin, respectively.

Results:
Induction of fusion through incubation of BeWo with forskolin interfered with RuV infection. Viral capsid antigen was detected in cells surrounding syncytia, but not within syncytilized BeWo. RuV infection reduced cell viability. Thus, plating of RuV-infected BeWo cells in comparison to uninfected cells followed by fusion induction with forskolin required normalization to the number of attached cells. For normalization, the crystal violet assay was validated.

Conclusion:
We have established BeWo as an in vitro model for syncytialization of human cytotrophoblasts in the context of RuV infection. Further studies will contribute to our understanding of transplacental transmission in pregnancies.

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Poster 6

Lipophilic DNA origami nanostructures for sensing membrane domains

Mamaghaniyeh R., Franquelim H.

Leipzig University, Interfaculty Center for Bioactive Matter (b-ACTmatter), Leipzig, DE

Several crucial biological processes, such as signaling, membrane trafficking, and viral infection are mediated by lipid bilayers. Due to the heterogeneity of lipids found in biological membranes, these can segregate into nanoscopic/microscopic domains that exhibit, for example, different liquid-ordered and liquid-disordered phases properties. It is therefore advantageous to controllably target such lipid domains in model and biological membranes for better mimicking and studying cell functions. In this work we propose to develop fluorescently-DNA origami nanostructures modified displaying different types and numbers of lipophilic moieties with selective affinity for specific lipid phases. Using confocal laser scanning microscopy, membrane binding of the various DNA origami nanostructures was detected on giant unilamellar vesicles (GUVs), which are excellent models for biological membranes. Here, we demonstrate that modified DNA nanostructures bind to liquid-ordered and liquid-disordered domains of GUVs depending not only on the type of lipophilic anchor present on the DNA origami, but also on the stiffness of the liquid-ordered membrane phases. To conclude, these results will help us to understand the physicochemical rules that govern lipid phase selectivity, and to design useful toolkits for detecting membrane properties for a variety of applications within cell membrane studies.

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Poster 7

Adsorption of laminin and cellular response of neurons and glial cells on ion implanted titania nanotube scaffolds

Frenzel J.[1], Kupferer A.[1,2], Mayr S.[1,2]

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Brain-machine interfaces have found extensive application in neuroscience, ranging from real-time monitoring of neural activities to addressing neurodegenerative disorders. Current cultivation platforms, such as cellulose filters, often face issues such as loss of sustained adhesion, rejection reactions, and glial scarring. Also, they may not facilitate electrical contact because of their insulating features. Our research proposes the use of ion implanted titania nanotube scaffolds (TNS) as a promising solution to these challenges, due to their superior biocompatibility and considerable electrical conductivity. In our study, we elucidate how the alterations in surface characteristics, brought about by ion implantation, impact laminin absorption and the viability and adhesion of neurons and glial cells. We correlate the increase in zeta potential and decrease in tube diameter with the impeded laminin adsorption caused by implantation. A possible interface material is suggested by the high and persistent neuron survival on all TNS but the inhibited glial cell development of implanted TNS. We sincerely thank SMWK (100331694) for funding.

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Poster 8

Characterization of lipophilic anchors with distinct hydrophobicities for binding of DNA origami to lipid membranes

Singh S., Franquelim H.

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In recent years, DNA origami has proven to be an exceptional engineering tool when combined with lipid membranes. Most reported membrane-active DNA nanostructures, however, rely on the use of commercially available cholesterol modifications to achieve efficient binding to cell and model lipid membranes. In this work, we aim to investigate new alternative strategies to bind DNA origami to lipid membranes, especially via weakly lipophilic anchors containing aromatic and short aliphatic moieties of different hydrophobicity (logP) values. As model DNA origami, we used a linear Atto488-labeled 20-helix bundle functionalized with lipophilic moieties at the bottom facet. The binding affinity of DNA origami displaying different numbers and types of strongly to weakly lipophilic anchors were quantitatively studied on various model lipid systems using confocal laser scanning and other biophysical detection tools. Furthermore, the interference of weak anchors on the membrane phase localization property of origami were analyzed by determining the cooperativity effect in conjunction with cholesterol as a strong anchor. In the end, we were able to distinguish the minimal hydrophobicity required for binding DNA nanostructures to homogeneous membranes, depending on the class of lipophilic anchor, along with the influence of combined anchors on the membrane phase partitioning properties. Overall, our strategy opens new avenues for mapping membrane vesicle properties, demonstrating the unique advantages of DNA nanotechnology for future membrane trafficking and biosensing applications.

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Poster 9

Exploring the impact of stretching: endoplasmic reticulum dynamics in alveolar type II cells from rat lungs

Mätzschker A., Kuhn H., Frille A., Wirtz H.

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Introduction:
The abnormal accumulation of unfolded or misfolded proteins within the endoplasmic reticulum (ER) is implicated in the pathogenesis of various diseases. To maintain cell homeostasis, cells engage an intracellular dynamic signaling pathway known as the unfolded protein response (UPR). This study aims to elucidate the expression of UPR signaling pathway proteins following physiological (p-stretching) and non-physiological stretching (np-stretching) of alveolar type II (ATII) cells.

Material and methods:
ATII cells were isolated and cultured from Sprague-Dawley rat lungs, they were stretched for 2, 4 and 6-hours, as well as for 24-hours according to a physiological and non-physiological pattern. Subsequently, cell lysates underwent analysis for UPR proteins, including BIP, ATF6α, IRE1α, XBP-1, p-eIF2α, ATF4, and CHOP, utilizing commercial ELISA kits and Western blotting.

Results:
Our investigations revealed the activation of the IRE1α signaling pathway during np-stretching, evidenced by the increased levels of XBP-1. The reduction in BIP indicates activation of IRE1α and PERK in response to np-stretching. Enhanced activity of the PERK signaling pathway is further demonstrated by elevated levels of ATF4 and p-eIF2α. Np-stretch also transiently activates the ATF-6 signaling pathway. However, CHOP remained undetected in the examined cell lysates.

Conclusion:
Our results suggest a correlation between the stretching pattern and UPR activity, with heightened activity observed across all three signaling pathways during np-stretching.

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Poster 10

Influence of local anesthetics on the mechanical properties of circulating ovarian cancer cells

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Background:
Ovarian cancer is typically treated with surgical resection and chemotherapy. However, the risk of seeding new micro-metastasis by shedding circulating tumor cells (CTCs) is increased during the peri-operative period. The use of local anesthetics (LA) is not only a well-established part of the perioperative anesthesia regimen, but might also be an option to counteract the metastatic cascade as these substances might affect membrane proteins of CTCs. Here, we used the optical stretcher (OS), to measure the mechanical behavior of ovarian cancer cells, treated with different concentrations of LA.

Methods:
With the OS active and passive mechanical resistance of ovarian cancer cells SKOV3 was measured at 875mW laser power, while mimicking the conditions of the blood stream. Further, the mechanical properties were determined 30 min after treatment of the cells with the LA ropivacaine at clinically relevant concentrations ranging from 1nM to 100µM. Negative controls (NC) using saline solution only were included.

Results:
SKOV3 cells showed a decreased elliptic deformation (0.01) when treated with ropivacaine within the range of 1nM to 100nM compared to the NC (0.015). Using 1µM to 100µM, however, the elliptic deformation was increased (0.025) indicating that the cells appear to become softer at the µM range. The median elasticity coefficient, a parameter for the ratio of stress to strain, increased from 32.16 at the NC to 34.79 under treatment with 1nM ropivacaine.

Conclusion:
Physical parameters appear to indicate a well-defined reaction to the LA treatment.

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Poster 11

Mechanical Properties of the Premature Lung

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Premature infants with respiratory distress syndrome are likely to be dependent on mechanical ventilation. Nevertheless, this essential treatment and related mechanical stress can lead to serious respiratory complications induced by the ventilation process. In a novel approach, we mimicked the positive pressure in the lung during mechanical ventilation with uniaxial compression experiments, as opposed to tidal breathing associated with negative intrathoracic pressure with uniaxial tension experiments. Using fetal and adult rat lung tissue, we determined mechanical properties via rheology experiments, applying distinct deformation velocities to investigate how mechanical stress affects lung tissue. Hyperelastic features were observed in fetal tissue under both types of deformation, whereas adult lungs exhibited this response solely under compressive loads. Young‘s moduli of adult controls were consistently lower than their fetal counterparts, indicating dissimilar tissue mechanics. A clear dependence of the Young‘s modulus of fetal lung tissue on the different compression rates indicated its viscoelastic nature, suggesting an increased susceptibility to mechanical ventilation-induced inflation as opposed to normal breathing. Primary fetal distal lung cells were also studied electrophysiologically in an Ussing chamber under varying hydrostatic pressure gradients. Increasing pressure affected the function of the epithelial sodium channel and the sodium-potassium pump, disrupting vectorial sodium transport, which is crucial for alveolar fluid clearance.

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Poster 12

Improved diagnosis of syndesmotic injuries via CT imaging utilizing the influence of non-neutral foot positions

Heilemann M.[1], Souleiman F.[2], Hennings R.[2], Hepp P.[2], Gueorguiev B.[3], Gehweiler D.[3], Richards G.[3], Osterhoff G.[2]

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Issue

Diagnosis of an isolated unstable syndesmotic injury is challenging. CT imaging, which is usually performed in a neutral position of the foot, is poorly responsive.

Aim

The aim of this study was to analyze whether detection of an unstable syndesmotic injury via CT imaging is possible based on different non-neutral foot positions.

Methods

Fourteen paired human cadaveric distal tibiofibular joints (DTFJ) were positioned in a particularly designed frame and pneumatically loaded (700 N) to simulate body weight. CT scans were performed in several foot positions: neutral position (N), internal/external rotation (IR/ER), and dorsal/plantar flexion (DF/PF) before and after cutting all syndesmotic ligaments.

For each position three 3D-parameters were analyzed: Clear space difference, translation angle, and vertical offset of the fibula. All three parameters describe the change in configuration of the DTFJ caused by the syndesmotic injury.

Results

The syndesmotic injury resulted in a significant posterior translation of the fibula in ER (4.34°; SD 1.63°) and DF (1.32°; SD 1.16°) as well as a significant anterior translation in IR (-2.08°; SD 1.65°). The clear space between the tibia and fibula was significantly enlarged only in ER (0.46 mm; SD 0.46 mm) due to the injury to the syndesmosis.

Conclusion

For foot rotation, especially ER, the injury of the syndesmosis led to the most significant differences in configuration of the DTFJ. This effect may be utilized in the future to detect unstable syndesmosis injuries using CT imaging.

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Poster 13

Influence of local anesthetics on inflammatory states of human keratinocytes in vitro

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Introduction:
Local anesthetics (LA) are frequently used in clinical practice, e.g. in the context of tissue transplantation. Rejection of allogenic tissue transplants is a common phenomenon. LA showed anti-inflammatory effects in experimental models, so there might be beneficial effects in tissue transplantation. Other studies showed cytotoxic effects of Ropivacain (RC) and a negative influence on wound healing. However, the influence of LA on the inflammatory response of the skin in vitro has not been examined yet.

Methods:
The expression/secretion of inflammatory markers was induced in immortalized human keratinocytes (HaCat) by application of TNF-α/IFN-γ. Lidocain (LC) and RC were added in clinically relevant concentrations to analyze their effects. We characterized HaCat cells using ELISA, Western Blot (WB) and resazurin-based viability assay (RVA).

Results:
Here we show that TNF/IFN induce an inflammatory state in HaCat that might be modulated by LA. In RVA the combination of TNF/IFN and LA significantly reduced cell metabolism or cell number. LA do not influence the secretion of IL-1b, IL-8 or IL-6. LC reduced the expression of EGF by approx. 40%, while LC and RC do not seem to affect the expression levels of other proteins like (p)NFkB and (p)p38.

Conclusion:
LA might be able to alter inflammatory states of ceratocytes on the intracellular level but not their paracrine function. The effect of LA on cell viability in the context of inflammation requires further research. We anticipate our project to be a starting point for more complex research on LA and inflammation.

Poster 14

Crosslinking-mass spectrometry as a tool to study the different polymerisation steps during the CCA-addition of a canonical tRNA

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Crosslinking mass spectrometry (XL-MS) has emerged as a powerful tool for studying protein-protein interactions. In this study, we employed XL-MS to investigate the individual polymerization steps during CCA addition, using the human CCA-adding enzyme and a canonical tRNA from yeast. By irreversibly crosslinking enzyme and substrate with the chemical crosslinker DSBU at various stages of CCA-addition, we aim to elucidate the structural changes occurring during this crucial tRNA maturation step. The crosslinked product is subjected to mass spectrometric analysis. Fragmentation of the peptides in the mass spectrometer results in the cleavage of the crosslinker at specific sites, giving rise to two distinct peaks. By identifying these peaks, the crosslinked amino acids can be determined. This information allows inferring the structural movement of the CCA-adding enzyme. Importantly, this approach offers an advantage over traditional techniques, as it enables the investigation of enzymes that cannot be easily crystallized. Furthermore, the utilization of small quantities of enzyme and substrate in the XL-MS workflow significantly reduces experimental time and equipment requirements, facilitating rapid data acquisition within a week. In conclusion, XL-MS provides a valuable method for studying the intricate polymerization steps occurring during CCA-addition. The ability to decipher enzyme-substrate interactions in a time-efficient manner makes XL-MS an indispensable tool for unraveling the molecular mechanisms underlying critical biological processes.

Poster 15

Unveiling the GPS Cleavage Mechanism in ADGRL1 with QM/MM

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Adhesion G-Protein Coupled Receptors (aGPCR) are a class of 33 proteins with a defining conserved GPCR autoproteolysis inducing (GAIN) domain with a GPCR-proteolysis site (GPS). The autoproteolytic mechanism has been previously proposed, but remains to be validated since. [1][2]

Using a multiscale QM/MM approach - combining computational quantum mechanics with classical molecular dynamics - to study the GPS cleavage mechanism in silico on ADGRL1 reveals the likely sequence of events at the electronic level, suggesting relative energies for the individual states during the reaction and provides insight into the structural determinants for a successful GPS cleavage exceeding the catalytically active GPS triad.

A stable π-edge interaction with a conserved phenylalanine provides an interaction which is crucial to generate the initial reaction step, where the His-1 abstracts a proton from the Thr+1 hydroxyl moiety for nucleophilic attack on the peptide carbon, forming a hydroxy-oxazolidine intermediate.[3]

By investigating the pathway from different angles, we further find surprising involvement of an acid/base catalysis mediated via water molecules by a glutamate residue.

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Poster 16

Integrative Analysis of Allosteric Networks in G Protein-Coupled Receptors

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This scientific abstract outlines a project amalgamating expertise in protein modeling, MD simulations, and evolutionary analysis. It aims to employ allosteric network analysis to understand GPCR molecular mechanisms, focusing on Neuropeptide Y receptors, crucial in physiological processes like appetite, circadian rhythm and anxiety.[1][2][3] Additionally, the project aims to merge expertise in protein modeling, molecular dynamics simulation (MD) with data generated by wetlab experiments. The integration of experimental and computational methods, disulfide crosslinking data of NPY2R's N-terminal guides protein modeling, is used as an example for a successful integration of both disciplines. Through Rosetta and AlphaFold, various N-TER conformations are generated and explored via all-atom MD simulations. The analysis sheds light on the complex NPY2R N-TER, deepening our understanding of its structure.

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Poster 17

Elucidating the mechanism of ligand control of PAR1 receptor activation: A molecular dynamics simulation study

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The protease-activated receptor 1 (PAR1) is a key regulator in homeostasis. Current drug research targets PAR1 to prevent thrombotic events, with an already clinically used antagonist vorapaxar (VPX). However, the distinct signalling outcomes of PAR1 in response to different proteases challenge the application. VPX administration not only blocks proinflammatory thrombotic effects but also interferes with the cytoprotective signalling induced by activated protein C, potentially leading to bleeding complications. The rational design of a biased PAR1 modulator, which would maintain cytoprotective signalling while inhibiting proinflammatory signalling, is hampered by the lack of knowledge on the structural basics of biased PAR1 signalling. Here, the effects of different ligands on the conformational free energy landscape of PAR1 activation were investigated using molecular dynamics (MD) simulations. A molecular model was generated that reveals the binding mode for the thrombin-generated tethered ligand (tTL) on PAR1 and is in good agreement to experimental studies. Subsequent MD simulations with enhanced sampling uncovered the free energy landscapes of the ligand-free, tTL-bound and VPX-bound PAR1 receptor complexes. Comparison of these landscapes revealed an activation increasing effect for tTL and a hampering effect for VPX consistent with experimental data. These findings shed light on the structural basics of PAR1 signalling and motivate further investigations of more ligands to characterize their differential effects and uncover distinct conformational receptor states.

Poster 18

Detecting common ligand binding sites in Ion channels using structural and sequence alignment.

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In this study, we created a workflow for analyzing common binding sites in ion channel families using structural alignment, sequence alignment, and fraction of common contacts clustering.

Identifying common ligand binding sites in ion channels can provide valuable information to researchers, such as finding suitable starting points for docking experiments or estimating cross-reactions of potential drugs. Research is usually limited to one (sub)family. Here we present a workflow for identifying common ligand binding sites that can be applied to any ion channel family and easily adapted to other transmembrane proteins.

To achieve this, multiple structural alignments were performed on all known ion channel structures available in the PDB. Ion channel structures were grouped into families according to the notation in the TCDB and OPM. For each ligand, contacts to the ion channel were identified and compared with ligands in other structures. Ligands with at least 50% common contacts were grouped into a ligand cluster.

Further analysis of the ligand binding sites was performed, such as calculating conservation and comparing sequence identity between subfamilies. We were able to identify promiscuous binding sites in different ion channel subfamilies, identify highly conserved ligand binding sites and residues, and compare binding sites between subfamilies of the same family.

We aim to make the workflow, including a graphical user interface, available to all researchers via a Streamlit app.

Poster 19

Establishment of a computational vaccine development pipeline for emerging viral diseases – a case study on Lassa virus

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The SARS-CoV-2 pandemic has highlighted that zoonotic viruses are a major threat to global health. It is therefore of special interest to establish novel vaccine development strategies for the fast and effective production of vaccine candidates. Computational protein design might be a major facilitator for the rapid stabilization of viral glycoproteins for formulation in mRNA vaccines. As part of a global coalition for vaccine development, these technologies will be refined using multiple test cases and held in preparedness for the worst case.

One virus family with great zoonotic pandemic potential are Arenaviruses. Here, the Lassa virus (LASV) is of special interest as it can cause Lassa fever, which can have a high mortality rate. Therefore, our goal is to establish a novel vaccine development pipeline using LASV as example case, which can then be employed for other viruses.

The LASV glycoprotein complex (GPC) is composed of glycoprotein 1 (GP1), GP2 and the stable signal peptide (SSP) and located on the virus surface. It is the dominant immunogen of LASV making it a prime target for vaccine-induced immunity. The GPC is a class I fusion protein that undergoes dramatic structural changes during cell entry. It will therefore be stabilized in its prefusion conformation by the strategic introduction of disulfide bridges and single-point mutations using biophysically driven protein design and artificial intelligence-based methods The resulting GPCs will be screened and characterized based on their binding properties.

Poster 20

Insights into M2R-Arrestin Interactions in the Live Cell by Genetically Encoded Crosslinkers

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The muscarinic acetylcholine receptor M2 (M2R) is, as most G-protein coupled receptors (GPCR), regulated by β-arrestins. However, it lacks the phosphorylated C-tail typical of other GPCRs that is often necessary for arrestin binding. Instead, it binds arrestin through its exceptionally long third intracellular loop (ICL3). Although a cryo-EM structure of the full length M2R-arrestin 2-complex exists, only the key phosphorylation cluster in ICL3 is resolved.

We incorporate photo-activatable as well as proximity-enabled crosslinkers into the two β-arrestins to map their interaction with the M2R. Occurrence of crosslinking is analyzed by western blot and ELISA. Using photocrosslinking, we compiled a complete footprint of the M2R on the N-domain and central crest of arrestin 3 and could identify arrestin residues responsible for interactions with the M2R.

We then used arrestin 2 for proximity-enabled crosslinking. That allowed us to not only map interactions similar to the published structure, but also to identify additional interactions between receptor and arrestin that are not resolved in any structure. This crosslinking data suggests a semicircular path of the M2R ICL3 around the arrestin N-domain. In this way, we want to unravel the whole path the receptor ICL3 takes on the surface of arrestin 2.

Our aim is now to identify more interactions between the M2R and arrestin 2, to use this information to build an accurate model of the M2R-arrestin 2-complex and to determine the functional role of the interactions for arrestin recruitment.

Poster 21

Intracellular Loop 3 Critically Regulates GPCR Phosphorylation

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G protein-coupled receptor (GPCR) activation and desensitization depends on precise temporal control of receptor signaling by two small regulatory protein families: GPCR-kinases (GRKs), and beta-arrestins (β-arr). Here, we examine the critical role of the GPCR intracellular loop 3 (ICL3) in the process of GRK and β-arr recruitment. Using a cutting-edge biochemical toolkit, we study GPCR-GRK and GPCR-beta-arr interactions at single-residue resolution directly in living cells. We outline a presently unknown regulatory mechanism that controls kinase recruitment to GPCRs and therefore guides receptor desensitization.

Poster 22

Performance of multiple protein design protocols in germline-targeting immunogen development

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Germline-targeting vaccines utilize modified priming immunogens with improved affinity for precursors of broadly neutralizing antibodies to selectively activate target B cells. Most germline-targeting immunogens developed to date have relied heavily on iterative cycles of time-consuming in vitro optimization by directed evolution and computational protein design using RosettaDesign. Recently, ProteinMPNN, a machine-learning based approach that relies on a deep neural network instead of physics-based calculations, showed improved performance for fixed-backbone design. Here, we compare ProteinMPNN, RosettaDesign fixBB, a combined method of ProteinMPNN and RosettaDesign, and directed evolution by yeast surface display to develop germline-targeting immunogens for the Hepatitis C virus (HCV). We design interface residues on HCV envelope glycoprotein E2 core to increase the affinity for unmutated precursors of broadly neutralizing antibodies. In vitro high-throughput screening experiments of immunogens designed with ProteinMPNN, and the combined method expressed significantly better than proteins from yeast display. On average, Rosetta-designed proteins showed significantly better binding to the target antibody compared to ProteinMPNN-designed immunogens. However, the overall best-binding immunogen resulted from ProteinMPNN. Taken together, these results highlight strengths and weaknesses of different immunogen design methods and yield HCV immunogen candidates with improved properties.

Poster 23

Studying TCR-pMHC interactions: Binding of computationally stabilized single-chain TCRs to a cancer epitope

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In the advent of targeted personal immunotherapy interest in the T cell receptor (TCR) interaction with its natural ligand, the peptide loaded major histocompatibility complex (pMHC), has grown. With the heterodimeric nature of TCRs impeding handling as drugs and in research there is a need for single-chain TCRs (scTv) that combine the assets of their antibody counterparts, single-chain variable fragments (scFv), with the specific TCR binding properties. To address the low stability of scTvs, computational protein design was performed to increase stability and expression yield. Finally, a binding assay was established to better study the interaction between the pMHC and its ligands.

Single-chain TCRs were designed for improved stability with ProteinMPNN and Rosetta, starting from the known TCR 1G4 (PDB ID 2F53). The variable fragment was extracted, designed with ProteinMPNN and the most promising point mutations evaluated in Rosetta. Protein expression was performed in a human embryonic kidney cell line and E. coli. Binding was measured using biolayer interferometry (BLI).

Computational protein design of the scTv led to improved expression rates for all chosen constructs compared to wild type. In addition, predicted improvement in stability correlated well with the final expression yields. A BLI essay was established and used to measure binding affinities between pMHC and scTvs. Measured affinities agreed with KD values from literature. In conclusion, we introduced a pipeline for the computational stabilization, expression and in vitro verification of scTvs as pMHC binders.

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Poster 24

Substrate Affinity Versus Catalytic Efficiency: Ancestral Sequence Reconstruction of tRNA Nucleotidyltransferases Solves an Enzyme Puzzle

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CCA addition, executed by CCA-adding enzymes, is an highly accurate step during tRNA maturation and is essential for aminoacylation and consequently for translation. CCA-adding enzymes of class I, containing bacterial and eukaryotic nucleotidyltransferases, are highly efficient in it's catalysis, but exhibit a surprisingly low substrate affinity to tRNAs. To get insights into this obvious contradiction, the evolution of CCA-adding enzymes needs to be investigated. In this project, we investigate an 2-billion-years old candidate for an ancestral CCA-adding enzyme from Gammaproteobacteria and compare it with it's modern equivalent from Escherichia coli. We figured out, that the ancestral candidate catalyzes CCA-addition error-free. Interestingly, the ancestral CCA-adding enzyme exhibits a high binding affinity and the E.coli enzyme shows a low affinity to tRNA substrates. The consequence of the enhanced substrate affinity of the ancestral enzyme is a fast catalysis of the reverse reaction, which removes the CCA-end from the tRNA. Furthermore, the ancestral candidate polymerizes in an processive way by adding the CCA-end at one step. These features lead to a lower efficient CCA-synthesis of the 2-billion-years old enzyme. In contrast, the E.coli enzyme lowered its tRNA affinity to counteract the reverse reaction and catalyzes CCA-addition step by step with an distributive polymerization mode. This leads to an highly efficient modern CCA-adding enzyme with interesting, at the first glance contradictory features approved as beneficial during evolutionary development.

Poster 25

Vaspin Interactions with LDL Superfamily Receptors and Influence of DNA on Protease Inhibition

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Vaspin is a serpin expressed primarily in liver, kidney, skin, brain and adipose tissue. It counteracts obesity-related inflammation and metabolic dysfunction mainly by inhibiting target proteases such as kallikrein 7 (KLK7) or by interacting with cell surface receptors. The cell surface localized interaction partners of vaspin in adipocytes and potential intracellular downstream effects remain unknown. To investigate the cellular fate of vaspin in primarily adipocytes, we fluorescently labeled recombinant vaspin and variants, performed binding affinity ELISA, fluorescence microscopy, Western blot analyses, electrophoretic mobility shift and kinetic assays.

We identified several LDL superfamily endocytosis receptors as binding partners, including LRP1 and LDLR. In addition, we have extensively characterized LRP1 as a major endocytosis receptor in adipocytes.

LDL receptors are primarily described as clearance receptors to remove the serpin protease complex via endocytosis and lysosomal degradation. To further characterize potential catalysts of serpin inhibition that enhance vaspin complex formation, we identified DNA as another activator in addition to previously described glycosaminoglycans.

Our results reveal distinct LDL receptors for vaspin in different tissues and a defined receptor-mediated uptake in adipocytes. In addition, we demonstrated enhanced inhibition of KLK7 by vaspin in the presence of DNA. Taken together, these results contribute to a better understanding of the cellular fate of vaspin as well as potential therapeutic targets for the treatment of obesity.

Poster 26

Incorporating Nanopore Sequencing into a Diverse Diagnostic Toolkit for Incontinentia Pigmenti

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Incontinentia pigmenti (IP) is a rare, hereditary multisystemic disorder affecting 1.2 in 100,000 live births, predominantly females. Conventional genetic analysis through short-read sequencing is complicated due to the presence of a highly homologous pseudogene. Long-range PCR is employed in order to overcome this challenge, however, detection of skewed X-Inactivation (XCI) can also aid in correctly assigning a variant to IKBKG. We employed a comprehensive multi-method approach, incorporating whole-exome sequencing (WES), long-range PCR, RT-PCR, XCI analysis, and nanopore sequencing, to identify and phase a small heterozygous deletion to the IKBKG gene in a family affected with IP. After detection of the variant through WES, XCI analysis and IKBKG-specific long-range PCR both indicated the variant to be located in IKBKG in both the proband and her mother. On RNA level, the variant was undetectable, suggesting nonsense-mediated mRNA decay of the transcript containing the variant. We further utilized nanopore sequencing not only to pinpoint and map the variant to the IKBKG gene but also to analyze methylation status of both alleles. This allowed us to confirm the skewed XCI, with the variant-carrying allele found to be predominantly inactivated. Therefore, nanopore sequencing serves as a valuable tool in genetic diagnosis, enabling the precise localization of the variant in either the gene or the pseudogene. This method facilitates the determination of whether the variant is predominantly located on the activated or inactivated X-chromosome.

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Poster 27

ICAM-1 as a potential predictive marker for immune checkpoint inhibitor response in bladder cancer

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Background:
ICI treatment has been approved for MIBC, but its effectiveness can be limited to patients with specific biomarkers, such as PD-L1 expression. However, a substantial number of patients do not respond to ICI even if they are PD-L1 positive. The study aimed to explore ICAM-1 as a potential predictor of ICI response.

Methods:
The study analyzed tissue samples from 385 MIBC patients using immunohistochemistry and digital quantitative image analysis. They evaluated the expression of PD-L1 and ICAM-1, as well as the density of CD8+ cytotoxic T cells (CTL) in the tumors. Optimal cut-off values for predicting overall survival were determined using statistical methods. The same analysis was repeated with a second cohort of 22 MIBC patients who had received ICI treatment.

Results:
Patients were categorized into three groups based on PD-L1 and ICAM-1 expression: PD-L1high/ICAM-1^{high} (10.6%), PD-L1^{high}/ICAM-1^{low} (45.9%), and PD-L1^{low}/ICAM-1^{low} (43.2%). The double expression of PD-L1 and ICAM-1 was associated with better overall survival and increased CTL density. In MIBC patients treated with ICI, those with high ICAM-1 expression and high CTL density showed significantly improved overall survival after therapy.

Conclusion:
The study suggests that ICAM-1 expression is a favorable prognostic factor in PD-L1-positive MIBC. It is associated with an enhanced adaptive anti-tumor immune response. The preliminary findings from a small cohort of patients indicate that ICAM-1 expression and CTL density may serve as predictive factors for the response to ICI therapy in MIBC.

Poster 28

Proteomics approach for identification of membrane proteins in microglia cell membrane microdomains after plant sterol treatment

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Plant sterols (PS) derived exclusively from diet were previously shown to have anti-inflammatory effects. In contrast to cholesterol, PS are able to cross the blood-brain barrier and accumulate in the brain, where they may affect signaling of membrane microdomains. To study whether the changes in cell membrane PS content influence membrane protein distribution, we investigated cell membranes of SIM-A9 mice microglia cells enriched with PS.

For that, we established a detergent-free OptiPrepTM-based isolation of lipid rafts from membranes by gradient ultracentrifugation. The microdomain proteome was characterized by analyzing individual fractions by LC-MS/MS searched against the UniProt Mus musculus database using the MaxQuant software.

In three independent membrane microdomain isolation experiments, we compared the first six pooled microdomain fractions to the latter ten enriched with the endoplasmic reticulum and mitochondrial membranes. Over all 2046 proteins were detected, among which 53 were upregulated in the microdomain and 157 in the mitochondrial and endosomal fractions (FC>1.5; FDR=0.05). The enrichment of known microdomain resident proteins (CD48, CD14, CD147, Protein S100-A10, AHNAK nucleoprotein, and NHERF1) was observed exclusively in the first six fractions.

To summarize, we have characterized the membrane microdomain proteome from three independent isolation experiments. This approach opens new possibilities to study the changes of microdomain proteome upon modifications in its sterol composition.

Poster 29

The top 101 articles in pediatric surgery from an altmetric perspective

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Background

In the era of scientific digitalization, online media platforms gain increasing popularity to accomplish research output awareness. Altmetric analyses assess these online mentions systematically. We aimed to characterize the top 100 articles with the highest Altmetric Attention Score (AAS) in pediatric surgery.

Methods

Publications from core journals (J Pediatr Surg, J Pediatr Surg Case Rep, Eur J Pediatr Surg, European J Pediatr Surg Rep, Pediatr Surg Int, Semin Pediatr Surg) were retrieved in January 2023 from www.altmetric.com and ranked by their AAS. The top 100 articleswere analyzed for their bibliometric measures, study design and quality as well as online media mentions.

Results

The top 101 AAS articles were published between 1974 and 2022, preferentially from the United States (64%) and mainly in J Pediatr Surg (73%), followed by J Pediatr Surg Case Rep, Pediatr Surg Int, Semin Pediatr Surg, and Eur J Pediatr Surg. Their AAS ranged between 21 and 389 (median 33) with Twitter/X being mostly responsible for online mentions (n=2,189; 75%). The number of citations in peer reviewed journals ranged between 0 and 358 (median 16) and did not correlate to AAS. Retrospective study design (33%) with low evidence level IV (43%) dominated.

Conclusions

The altmetric popularity of the top articles is predominantly achieved by their propagation via Twitter/X, irrespective of the study quality and recognition in the scientific community. Thus, active “twitterism” may play the key role to reach high AAS scores.

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Poster 30

Computed Tomography Embolus Texture Analysis as a Prognostic Marker of Acute Pulmonary Embolism

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Texture analysis is a quantitative imaging analysis that provides novel biomarkers beyond conventional image reading. Our aim was to use texture analysis of pulmonary emboli derived from thoracic computed tomography for prediction of mortality and prognosis of acute pulmonary embolism (PE). Overall, 216 patients (116 female, 53.7%) were included in the analysis.

Texture analysis was calculated on axial slices of the contrast enhanced pulmonary angiography of the proximal embolus. Clinical scores, serological parameters, need for intubation, intensive care unit (ICU) admission and mortality was assessed and correlated with the texture features.

In the correlation analysis, there were several associations with mortality in days, the highest for the parameter S(0,5)SumVarnc ($r = 0.43$, $P < 0.001$). Another parameter, S(3,3)AngScMom correlated with sepsis-related organ failure assessment score (SOFA)-score ($r = 0.31$, $P < 0.001$). Several texture features correlated with venous lactate and glucose levels. In discrimination analysis, there were significant differences in regard to texture features between survivors and nonsurvivors and between patients with and without the need for ICU admission ($P = 0.02$, respectively).

These results highlight the potential clinical benefit of texture features in patients with acute PE as novel imaging biomarkers. Further studies are needed to validate these results.

Poster 31

Transrectal in-bore MRI biopsies of the prostate: impact of gland and lesion features on cancer detection rate

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Objectives:
To systematically analyze the diagnostic outcome of transrectal in-bore MRI-guided biopsy as a function of prostate volume, lesion size and lesion location.

Materials and Methods:
This single-center study retrospectively included 224 consecutive patients with transrectal in-bore MRI biopsies and histological analysis after multiparametric (mp) MRI diagnostics of at least one PI-RADS ≥ 3 lesion. Diagnostic and biopsy MRI data were analyzed for specific gland and lesion features: prostate volume, lesion size, lesion location and sagittal lesion depth. Subgroups of these features were then compared for statistically significant differences in the cancer detection rate (CDR) of any as well as clinically significant (cs) prostate cancer (PCa).

Results:
A total of 264 lesions were biopsied detecting any-PCa in 52% and cs-PCa in 21%, respectively. Median prostate volume (range) was 52.5 (13.4-270.9) mL, lesion size 0.69 (0.04-24.09) mL and lesion depth 11 (0-52) mm. The CDR of cs-PCa varied significantly between different ranges of lesion size ($p < 0.001$) but not between groups of prostate volume, segmental index or lesion location ($p = 0.112$-0.305). The CDR of any-PCa differed significantly for prostate volume, lesion size, segmental location (all $p < 0.001$) and sagittal lesion depth ($p < 0.05$).

Conclusion:
Our findings suggest that transrectal in-bore MRI-guided prostate biopsy is a reliable option for challenging biopsy cases in larger glands or with lesions in eccentric positions.

Poster 32

Application of a pseudotargeted high-resolution mass spectrometry approach for metabolomics in a mouse hippocampus sample

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High resolution mass spectrometry (HRMS) is a key technology in the field of untargeted metabolomics. However, identification of low abundant metabolites in complex sample matrices by HRMS is still a challenge. The application of a novel pseudotargeted analytical workflow designed for acquiring fragmentation data of less intense metabolites in a complex sample was the aim of this work.

Hippocampus samples from mice fed standard chow and a high fat diet were prepared by an adapted protocol from Reinicke et al., 2020. Chromatographic separation was achieved on a C-18 Kinetex Core-Shell column (Phenomenex Torrance, CA, USA) and HRMS data acquisition was performed using an Orbitrap Exploris 480 following the novel pseudotargeted AcquireX workflow (Thermo Fisher Scientific).
The number of biomarkers associated with differences in the mouse brain tissue due to the various diets increased six fold with the AcquireX workflow compared to a data-dependent (DDA) workflow. The biomarker set extracted from the AcquireX data includes less intense compounds with lower abundancy compared to the DDA dataset biomarkers. The DDA biomarkers reached annotation certainty levels 3 and 4, while two AcquireX biomarkers were annotated with level 2 (according to Schrimpe-Rutledge et al., 2016).

Significant differences in the brain tissue of mice associated with diet could be established. The data acquisition strategy of untargeted HRMS-based metabolomics influence the quality of component identification. The AcquireX pseudotargeted strategy is suited for identification of low abundant biomarkers.

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Poster 33

PRospective Evaluation of clinical parameters anD initial cerebral CT for the prediction of Malignant Media Infarction (PREDICT MMI)

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Background:
Early identification of patients at risk for space-occupying (malignant) middle cerebral artery infarction (MMI) would allow early initiation of therapies directed against the edema. One approach is to anticipate edema development by measuring the density of infarcted tissue referring to the healthy brain hemisphere in the first native cerebral computed tomography (CT), termed net water uptake (NWU). This retrospective study aimed to investigate whether CT-based parameters, in combination with clinical features, can predict the development of MMI in patients with a large vessel occlusion (LVO).

Methods:
Patients had to present cerebral infarction due to LVO and were treated in the Department of Neurology, University of Leipzig, between 2012 and 2021. Patients were assigned to a MMI or non-MMI group. Using a generalized linear model, we investigated which constellation of 4 developed models provided high predictive accuracy for development of MMI.

Results:
A total of 173 patients (69±12 years; 89 with MMI) were included. With an accuracy of 78.98% (sensitivity 78.57%; specificity 79.45%), the model with native cerebral CT and basic clinical data achieved best prediction of MMI (area under the curve (AUC) 0.86). NWU alone (AUC 0.62) was less predictive than reported in previous studies.

Summary:
In patients with proximal LVO, the combination of the first non-contrast CT and early clinical data achieved the best prediction of MMI. This model is currently being evaluated in a prospective study.

Poster 34

High rate of venous thromboembolism in patients with space-occupying (malignant) cerebral infarction and decompressive surgery

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Introduction:
Venous thromboembolic events (VTE) like deep vein thrombosis or pulmonary embolism are frequent complications in critical ill patients. Anticoagulation for VTE after extensive cerebral infarction is a therapeutic dilemma. The aim of this retrospective study was to investigate clinically apparent VTE in patients with cerebral infarction due to a proximal large vessel occlusion (LVO), its treatment, and the rate of complications.

Methods:
Patients with a first cerebral infarction due to LVO were assigned to one of 4 groups: Space-occupying (malignant) infarction (MMI) in the territory of the middle cerebral artery (MCA) with or without decompressive surgery (DS) and cerebral infarction comprising more than 2/3 or less than 2/3 of the MCA territory. Clinical parameters included risk factors for VTE, type of thromboprophylaxis, treatment of VTE, and treatment associated complications.

Results:
15 (8.7%) cases of VTE occurred in 173 patients. Among MMI patients, VTE occurred only in the DS group (n=11, 17.5%; χ^2 test, $p<0.001$). Patients with a history of cancer had a higher incidence of VTE ($p <0.001$). Only 3 patients had major bleeding events while being anticoagulated (1 cerebral and 2 extracranial bleedings).

Discussion:
Patients with MMI and DS had a high risk of VTE. Despite extensive infarct size and DS, therapeutic anticoagulation required for VTE appeared to be safe regarding cerebral bleeding events. An intensified thromboprophylaxis with pharmacological and non-pharmacological measures seems appropriate, especially in patients with MMI and DS.

Poster 35

Significant improvement of selected cerebrovascular risk factors along with a short interventional program in a neurovascular outpatient clinic

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Introduction:
Best medical treatment (BMT) plays an important role in the (secondary) prevention of IS in patients with carotid artery disease (CAD) and involves, amongst others, optimization of cerebrovascular risk factors (CVRF), lifestyle changes, and health education. However, the implementation of BMT into a real-world setting remains challenging with many patients not reaching specific goals. This prospective study aimed to assess the influence of a short intervention on CVRF in patients of a neurovascular outpatient clinic with CAD.

Methods:
93 patients were examined at three time points (baseline, 6 and 18 months (T1/2)). The short (about 10 minutes) intervention included structured information about BMT, laboratory testing (lipids, HbA1c), and blood pressure (BP) measurement. A report was sent to the general practitioner comprising detailed recommendations to optimize medical treatment.

Results:
Focusing on patients with both follow-ups (FU; n=66 (71%; 69±9 years), we observed a reduction of LDL cholesterol over time (86 to 77 mg/dl at T2). At T2, diastolic but not systolic BP was lower while HbA1c (5.9 to 6.2%) had increased with reference to baseline. At baseline, patients that dropped out thereafter (n=17, 69±11 years) had a similar BP, but higher LDL-cholesterol (111 mg/dl), and a trend to a higher HbA1c (6.3%) than patients attending both FU.

Discussion:
This short and structured intervention to optimize BMT resulted in a significant improvement of selected CVRF. Efforts should be made to identify patients with low adherence to BMT to tailor individual approaches.

Poster 36

Tissue Outcome Prediction in Patients with Ischemic Stroke and Mechanical Thrombectomy: Comparison of univariate and mass-univariate logistic Models

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Background:
Defining ischemic stroke lesion's core and its penumbra (tissue salvageable through reperfusion) offers important aid to selecting patients eligible for mechanical thrombectomy. For patients with acute proximal vessel occlusion in the anterior circulation, mass-univariate generalized logistic models (GLM) have shown to be superior to established single-parameter thresholding. Single-univariate GLM present an alternative with better spatial coverage of the brain.

Method:
We implemented a single-univariate GLM approach handling data imbalance with Random Stratified Sampling. Spatial features were included using static tissue probability maps. Training and validation was based on acute CT perfusion parameter maps (CBF, CBV and Tmax), demographic and clinical data and final lesion maps (ground truth) of 304 patients (172 female, 74±12 years). Subsequently, we compared single- and mass-univariate models regarding volumetric, topographic (area under the curve, AUC) and spatial (Dice-index) accuracy.

Results:
Both models were able to predict lesion's core and penumbra. Furthermore, we were able to predict lesions in case of successful and unsuccessful recanalization. The single-univariate model had better spatial coverage. However, contrary to our hypothesis, it proved to be inferior regarding volumetric, topographic and spatial accuracy compared to the mass-univariate approach.

Conclusion:
In the future, we plan on predicting functional outcomes based on predicted tissue outcomes. In view of the current results, we will therefore rely on the mass-univariate approach.

Poster 37

Optokinetic stimulation training in immersive virtual reality for chronic stroke patients with visuospatial neglect

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Visuospatial Neglect (VSN) is common in right-hemisphere stroke patients and is characterized by failure to visually attend, orient, or respond to the side contralateral to the brain lesion.[1][2][3] Optokinetic stimulation (OKS) is one evidence-based treatment of VSN.[4] It involves directing the patient‘s attention to the contralesional side by fixating on arbitrary moving visual stimuli on a screen.[1][3] However, there is limited control over head and eye movement. Immersive virtual reality (VR) with eye tracking (ET) allows for precise experimental control and automated behavioral measurement, allowing patients to adapt based on online feedback.[1][4] We describe the pilot evaluation of a VR training specifically developed and implemented for VSN patients.

Seven post-stroke VSN patients (2 female, age M=58 ± 15) completed ten VR-OKS training sessions of varying difficulty to redirect their attention to the contralesional side. In each session, participants fixated on moving virtual objects on a conveyor belt while keeping their heads in a fixed, straight position. The VR-OKS system provided immediate feedback on participants‘ gaze direction, head orientation, and the number of objects successfully transported using integrated ET in the VR headset.

First empirical results show no significant increase in cybersickness and the VR-OKS received high ratings in terms of likeability. Further analysis will include evaluating the effect of VR-OKS on VSN symptoms based on behavioral data. The VR-OKS training shows high feasibility and potential to support VSN patients in rehabilitation.

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Poster 38

Profiling PUFAs and PUFA-derived metabolites in polytraumatic patients

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Objectives:
Oxylipids, potent mediators having pro-inflammatory or resolving properties, result from the degradation of arachidonic acid (AA) and related polyunsaturated fatty acids (PUFA). Here, we examined whether circulating PUFA-derived metabolites are potential biomarkers for the post-traumatic time course and outcome of traumatized patients.

Methods:
We profiled PUFAs and their metabolites in 36 patients, showing a wide range of injury severity scores (ISS, 5-75), up to 240 h after injury by targeted liquid chromatography-tandem mass spectrometry and related them to patient‘s clinical data.

Results and Conclusion:
The PUFA levels varied obviously immediately after trauma, independent of the ISS, likely reflecting dietary intake and synthesis, and declined in almost all patients during hospitalization. Consistently, especially in the early post-traumatic phase, the metabolite profiles are also heterogeneous in most patients. Several metabolites distinguish very severely injured patients (ISS>=25, n=14) from the other patient groups (ISS<16, n=11; ISS 16-24, n=11). In this group the levels of various dihydroxyeicosatrienoic acids (DHETs), the stable derivatives of bioactive epoxyeicosatrienoic acids (EETs), suggested to be protective, are lower after injury compared to the other patients. Lowest levels or the most distinct decrease of DHETs are seen in patients with very severe injuries. In conclusion, some PUFA-derived metabolites are potential biomarkers in multi-injured patients.

Poster 39

Dissecting the role of Interleukin-8 attracted neutrophils in T-prolymphocytic leukemia.

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T-prolymphocytic leukemia (T-PLL) is a rare, poor-prognostic, mature T-cell malignancy associated with an aggressive clinical course. Despite exponentially rising WBC, T-PLL patients do not show overt neutropenia, arising the question if neutrophils contribute to T-PLL leukemogenesis. Aiming to dissect the role of neutrophils in T-PLL, we found T-PLL-derived neutrophils being associated with a more activated phenotype and higher migratory activity. Furthermore, T-PLL-derived neutrophils show a better survival in vitro and enhance the activation of T-PLL cells in co-culture experiments. Additionally, activated T-PLL cells can improve the in vitro survival of healthy donor-derived neutrophils. Moreover, we could show that neutrophils are attracted by T-PLL-conditioned medium which is mainly driven by IL8. In line, we found IL8 mRNA being overexpressed in T-PLL cells which also reflects into elevated IL8 levels in T-PLL plasma. Considering TCL1A-promoted TCR- and hyper-activated JAK/STAT-signaling as hallmarks of T-PLL, we found that TCL1A expression increases IL8 secretion upon TCR engagement, and that IL8 induce the activation of JAK1/3 and STAT3/5. Overall, we propose IL8 acting as an autocrine agent promoting hyper-activated JAK/STAT signaling in T-PLL. On the other hand, as a paracrine agent T-PLL cells attract neutrophils with leading to increased neutrophil survival and activation. Simultaneously, IL8-attracted neutrophils enhance the activation of T-PLL cells, which is mainly induced by cooperative TCL1A/TCR-signaling driving the leukemic outgrowth of T-PLL.

Poster 40

In vitro selection of armless tRNAs interacting with mitochondrial EF-Tu

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tRNAs play a central role in protein synthesis, as they function as universal adapter molecules in translation. To be recognized by all components of the translational machinery, tRNAs in all three kingdoms of life exhibit highly conserved cloverleaf-like secondary structures that fold into three-dimensional L-shapes. However, in mitochondria of metazoans, several tRNAs deviate from this consensus structure, as they lack the D or T-arm. In the nematode Romanomermis culicivorax, this miniaturization comes to an extreme, as in nine mitochondrial tRNAs both D- as well as T-arm are replaced by short (single-stranded) connector elements. These bizarre tRNAs can still fold into a boomerang-like tertiary structure and are recognized by different tRNA-interacting proteins. Here, we investigate the function of the connector regions and how they contribute to the interaction with the mitochondrial elongation factor EF-Tu. To this end, we are using an in vitro selection method to isolate mt EF-Tu-binding tRNAs from a starting pool consisting of tRNAs with randomized connectors. This method combines a filter binding approach with high throughput sequencing. Several repetitions can be done to select the best interacting tRNA constructs. With this strategy, we hope to get insights into the mitochondrial translation system of armless tRNAs, its evolution and the adaptation of proteins like EF-Tu to these miniaturized tRNAs.

Poster 41

An in vivo selected CCA-adding enzyme with an altered nucleotide recognition site

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The CCA-adding enzyme represents a ubiquitous and essential activitiy in tRNA maturation. This enzyme adds the indispensable CCA triplet to the 3'-end of tRNAs, generating the site to be charged with the cognate amino acid. The enzyme contains a set of conserved motifs, whose coordinated interplay is crucial for its specificity. As a consequence, alterations within these regions can lead to the manifestation of disease. In an in vivo screening system for the impact of such mutations, we searched for alterations within motif D of the corresponding human enzyme. This motif serves as an amino acid template for proper selection of the incoming nucleotides and is indispensable for the enzymes' specificity. Yet, an unexpected variant E164G emerged in the screening process. In the in vitro characterization of this variant, we could show that the replacement of the acidic glutamate by the inert glycine does not lead to a higher rate of misincorporation, but to a decrease in catalytic activity. The small size of the glycine side chain obviously leads to an increased flexibility of the nucleotide binding pocket, resulting in a promiscuous CTP and ATP binding in the ground state of the variant, while the corresponding state of the wt enzyme is selective for CTP – the first nucleotide to be incorporated into the tRNA substrate.

Our results support the hypothesis that in the wt enzyme, E164 interacts with a flexible loop element that acts as a lever adjusting the binding pocket for switching its specificity from CTP towards ATP during CCA-addition.

Poster 42

CARcoon – Scaling up mRNA CAR-T cell manufacturing onto the Cocoon® platform

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Emily Whitehead, the first chimeric antigen receptor (CAR)-T cell therapy patient, celebrated being cancer-free for eleven years in 2023. The therapy has rapidly advanced ever since with six FDA-approved CAR-T cell therapies at present. Approved CAR-T cell therapies rely on permanent genomic integration of the CAR construct using viral gene delivery methods. This results in long-term side effects, and an expensive production pipeline bearing safety issues. One promising alternative for a faster, cost-improved and safe production of CAR-T cells is the transfer of CAR-mRNA to generate transient CAR-T cells.

This study aims to scale up an established lab-scale protocol to produce mRNA CAR-T cells for the treatment of hematological diseases by transferring it onto the Cocoon® platform (Lonza) – an automated, closed-system bioprocessor. Therewith, we ultimately aim to pave the path to a decentral, safe, and high-quality production of mRNA CAR-T cell therapeutics.

Anti-CD123-mRNA CAR-T cells were applied in this study. Frozen PBMCs or T cells from healthy donors were transfected using mRNA-loaded lipid nanoparticles or electroporation. Viability, T cell purity, T cell activation, and transfection efficiency were analyzed with flow cytometry assays.

Based on the data collected, optimal parameters for large-scale production of transient CAR-T cells were defined. A T cell expansion experiment was successfully carried out on the Cocoon® platform. We will now implement large-scale mRNA-based transfection and continue to optimize the process.

Poster 43

Deciphering Regulatory Biomarkers Associated with Cardiogenic Shock

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The endothelium, a pivotal component of vascular biology, crucially contributes to the orchestration of inflammation within blood vessels. Cardiogenic shock (CS), a severe condition marked by hypoxia and inflammation, entails significant disturbances in metabolic and cytokine environments. Our investigation is focused on characterizing the inflammatory response of Endothelial cells (ECs) to the CLIP biomarker proteins. Initial findings from our investigation unveiled an inversely proportional correlation between IL-6 levels and the proliferative rate of ECs. Conducting a comparative analysis with the control group at distinct temporal intervals (72 hours and 96 hours after IL-6 addition). Our findings reveal a significant reduction by 50% (p=0.001) at the highest IL-6 concentration of 100 ng/ml, and still a 15% reduction at the lowest concentration (10 ng/ml, p=0.003). Later, we did ECs barrier function under identical experimental conditions. Following exposure to IL-6 at different concentrations, cells were subjected to stimuli, including ethanol 1%, histamine 100 uM, and TRAP 50 uM, with continuous minute-by-minute monitoring of changes. Over a 30-minute observation period, cells exposed to IL-6 at 100 ng exhibited a noteworthy 30% reduction in endothelial permeability, while lower IL-6 concentrations (10 and 50 ng/ml) showed a comparatively milder effect. Our data will help to elucidate the effects of CS associated circulating biomarkers on the cardio-vascular endothelium as a mediator of systemic hypoxic and inflammatory side-effects in CS.

Poster 44

Design of synthetic riboswitches to regulate RNA stability

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Structured elements in the viral RNA genome are important for the regulation of many processes in the replication cycle of a virus. An interesting example is the generation of subgenomic RNA to influence pathogenicity and cytopathicity by exoribonuclease-resistant RNA (xrRNA) elements – RNA structures formed in the 3'-UTR of the Flavivirus RNA genome. The xrRNA element is characterized by the formation of a ring-like structure, which stalls 5'-3'-exoribonucleases like XRN1, preventing the degradation of the downstream RNA region. This property could also be useful to generate synthetic regulatory RNA elements to modulate the half live of individual RNAs. In this project, xrRNA elements are combined with aptamer structures to construct synthetic riboswitches that allow for a ligand-dependent stabilization or destabilization of a specific transcript in the cell. Besides representing a novel tool to regulate RNA stability, such riboswitches will also help to gain a deeper insight into xrRNA structure-function relation. In our pilot experiments, we could already show that the xrRNA from the mosquito-borne Aroa virus is stable against XRN1 and can be used as an actuator (expression platform) in a theophylline-dependent riboswitch. First xrRNA riboswitch candidates are currently tested by in line probing and XRN1 digestion to understand the role of individual xrRNA motifs in the switching process of these constructs.

Poster 45

Environmental endocrine disrupting chemicals bisphenol A and benzophenone-3 affect gonadal development and function with possible implications for fertility

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Endocrine disrupting chemicals (EDCs) are mostly synthetic substances that can interfere with hormonal signaling and thus impact endocrine and immune cell function. Bisphenol A (BPA) and benzophenone-3 (BP-3), two widespread EDCs, have been linked to altered endocrine signaling and immune cell profiles as well as reduced fertility, but little is known about combined effects of these EDCs. The goal of our study was to investigate the impact of perinatal exposure to BPA and BP-3 as single substances and as mixture at relevant concentrations on ovarian immune cells and ovarian function, sperm count and viability, and weight development in exposed offspring. We used flow cytometry to determine ovarian immune cell profiles and sperm viability, and analyzed folliculogenesis, ovulatory capacity and sperm count by histological means. RT-PCR was used to determine gene expression of markers of gonadal differentiation. Our results showed that exposure to BP-3 and BPA/BP-3 mixture significantly impacted offspring weight development in a sex-dependent manner. Moreover, ovarian responsiveness to exogenous gonadotropins, immune cell profile and differentiation marker expression were altered. In males, sperm count and testis differentiation marker expression were significantly different following exposure to BPA/BP-3 mixture. In conclusion, perinatal EDC exposure affects offspring gonadal differentiation and function, and alters the immune cell profile of the ovary which may affect fertility and pregnancy success.

Poster 46

Evolution and adaptation of mitochondrial EF-Tu to armless tRNAs

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tRNAs are crucial molecules in translation, carrying cognate amino acid residues to the ribosome. In all domains of life, secondary and tertiary structures of tRNAs are highly conserved. Canonical tRNAs form a cloverleaf-like shape composed of acceptor stem, anticodon stem, D- and T-arm. Interestingly, several mitochondrial tRNAs of metazoans deviate from this conserved structure, with the most severe deviation found in nematode mitochondria. In Romanomermis culicivorax, for instance, mitochondrial tRNAs lack either the D-arm or the T-arm, and in several extreme cases even both of these arms.

D- and T-arms are important for the interaction with several proteins. To bind to the armless tRNAs, these proteins must have evolved specific compensating features. One of these indispensable mitochondrial tRNA binding proteins is EF-Tu, responsible for the transport of aminoacylated tRNAs to the ribosome.

All of the tested recombinant nematode mtEF-Tu versions recognize armless tRNA. Yet, the protein from R. culicivorax shows the strongest interaction, indicating a specific adaptation for an efficient binding to these bizarre tRNAs.

Mutational studies indicate that R. culicivorax mtEF-Tu acquired an additional C-terminal domain for this interaction, compensating for the loss of D- and T-arms in the tRNA. While such C-terminal extensions are a general feature of nematode mtEF-Tus, where they contribute to the binding to tRNAs lacking just one arm, they show a considerable difference in sequence composition, indicating a specific adaptation to the individual tRNA forms.

Poster 47

Expression of liver X receptors and their transcription targets in patients with multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disease characterized by inflammation and demyelination in the central nervous system. Disturbed cholesterol metabolism impairs myelin turnover and may impact disease course. Liver X receptors (LXRs) regulate cholesterol homeostasis and some immune functions. A variant of LXRα increases susceptibility to MS.

We measured mRNA expression of LXRs alpha and beta, and their transcriptional targets, the transporter proteins ABCA1 and ABCG1, in lysed peripheral blood from MS patients, using quantitative reverse-transcription PCR.

Expression levels of LXR beta, ABCA1, and ABCG1 were higher in patients with MS (n=103) than in healthy subjects (n=29), mainly driven by patients with relapsing-remitting MS (n=68). Over two years, expression was stable in untreated patients. While LXR beta and ABCG1 expression increased on ocrelizumab and interferon β, ABCA1 also increased on interferon and decreased on natalizumab; expression levels did not change on fingolimod or fumarate, but displayed significant inter-individual variation; our sample was too small to include co-factors such as treatment response.

Expression of LXRs and their targets is altered in MS, potentially involved in its pathogenesis. Their course during treatments may be clinically useful, especially if correlations to treatment response may be identified. Prospectively collected samples from more than 1000 patients followed over up to 12 years within the Kompetenznetz Multiple Sklerose are now available; we plan to use these samples to further clarify this issue.

Poster 48

Extracellular vesicle lipid functions in inflammation

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Extracellular Vesicles (EV) are phospholipid bilayer-enclosed particles that are secreted by a variety of cell types, including macrophages. While EV nucleic acids and EV proteins have been studied extensively in current literature, the characterization and function of EV lipids is still not sufficiently understood. Particularly, bioactive lipids such as PUFAs and eicosanoids may contribute to EV functions. In our study, we first aimed at identifying changes of EV lipid profiles during inflammation. Secondly, we investigated the functional relevance of the identified changes.

EVs derived of differently polarized THP-I macrophages were purified by Sequential Filtration. PUFA and Eicosanoid profiles in the EV preparations were assessed by means of targeted quantitative mass spectrometry (LC-MS/MS). The functional relevance of the identified differences in eicosanoid profiles was investigated by means of molecular analysis. 12-LOX was inhibited by ML355.

The lipid profiles of macrophage-derived EVs differed depending on the polarization of the originating macrophages. We observed major changes in the arachidonic acid pathway. M1 macrophage-derived EVs showed decreased levels of arachidonic acid but increased levels of ARA-derived 12-LOX and 15-LOX products, particularly 8-HETE/12-HETE. Both M1 EVs and 8/12 HETE induced expression of pro-inflammatory chemokines in target cells. M1 EVs induced monocyte adhesion, monocyte migration, and endothelial dysfunction. These effects were significantly lower in 12-LOX inhibited M1 macrophage-derived EVs.

Poster 50

Glomerular-tubular crosstalk via cold shock Y-box binding protein-1 in the kidney

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Glomerular-tubular crosstalk within the kidney has been proposed, but the paracrine signals enabling this remain largely unknown. The cold-shock protein Y-box binding protein 1 (YBX1) is known to regulate inflammation and kidney diseases but its role in podocytes remains undetermined.

We analyzed mice with podocyte specific Ybx1 deletion ($Ybx1^{\Delta Pod}$). Albuminuria was increased in unchallenged $Ybx1^{\Delta Pod}$ mice, which surprisingly was associated with reduced glomerular, but enhanced tubular damage. Tubular toll-like receptor 4 (TLR4) expression, node-like receptor protein 3 (NLRP3) inflammasome activation and kidney inflammatory cell infiltrates were all increased in $Ybx1^{\Delta Pod}$ mice. In vitro, extracellular YBX1 inhibited NLRP3 inflammasome activation in tubular cells. Co-immunoprecipitation, immunohistochemical analyses, microscale cell-free thermophoresis assays, and blunting of the YBX1-mediated TLR4-inhibition by a unique YBX1-derived decapeptide suggests a direct interaction of YBX1 and TLR4. Since YBX1 can be secreted upon post-translational acetylation, we hypothesized that YBX1 secreted from podocytes can inhibit TLR4 signaling in tubular cells. Indeed, mice expressing a non-secreted YBX1 variant specifically in podocytes ($Ybx1^{PodK2A}$ mice) phenocopied $Ybx1^{\Delta Pod}$ mice, demonstrating a tubular-protective effect of YBX1 secreted from podocytes.

Our results reveal that podocytes physiologically produce YBX1, which inhibits tubular TLR4 siganlling and reduces tubular sterile inflammation. We found a YBX1-dependent molecular mechanism of glomerular-tubular crosstalk.

Poster 51

Identification of a circular RNA specifically regulated in cardiogenic shock

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Background:
Cardiogenic shock (CS) is associated with a poor prognosis and a mortality rate of about 50%. Novel biomarkers could improve patient stratification and therefore lead to an improved patient outcome. The class of non-coding RNAs includes circular RNAs (circRNA). Their function are largely unknown in CS. The aim of this study was to characterize circRNA in the blood of CS patients at time of admission and after revascularization.

Results:
Analyses by two independent bioinformatic algorithms revealed the expression of 19,182 circRNAs in immune cells, 170 of which were significantly regulated in CS. The circRNA CircShock4 was among the most upregulated. We validated the characteristics of CircShock4 by RNAse-R digestion, oligo-dT selection and Sanger sequencing of the back-splice junction (BSJ) and detected a predominant expression in monocytes. The observed regulation of CircShock4 was confirmed in a larger patient cohort with CS after AMI (n=52) compared to controls (n=38, +10.9-fold, $p<0.0001$). Interestingly, patients with AMI without CS (n=37) did not show an increase in CircShock4 compared to controls (n=31, $p=0.2$), indicating a possible CS-specific regulation of CircShock4 independent of AMI. CircShock4 levels identified patients with CS both compared to patients with AMI (AUC=0.9454, $p<0.0001$) and compared to controls (AUC=0.9378, $p<0.0001$).

Conclusion:
In summary, circRNA are regulated in CS, CircShock4 is specifically increased in CS independent of AMI. CircShock4 is enriched in monocytes and might be used as a potential biomarker.

Poster 52

Led-Seq: ligation-enhanced double-end sequence-based structure analysis of RNA

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Structural analysis is an important tool to investigate the function of RNA molecules in vitro as well as in vivo. Several procedures are available, relying on chemical modification inducing stops or nucleotide misincorporations during reverse transcription. Here, we describe Led-Seq, a new approach based on lead-induced cleavage of RNA, where both resulting cleavage products are investigated. Hydrated lead ions abstract the proton from the ribose 2'-OH group, rendering it highly nucleophilic. As non-paired RNA regions are more flexible in general, they exhibit an increased propensity to adopt an in-line conformation, where the nucleophilic 2'-O- attacks and cleaves the neighboring phosphodiester bond. In Led-Seq, the resulting RNA fragments, carrying 2′,3′-cyclic phosphate or 5′-OH ends, are selectively ligated to oligonucleotide adapters. In a subsequent RNA-Seq analysis, cleavage sites are identified as adapter ligation positions. The sequencing reads are transformed into normalized probing signals that are then converted into a probability of a position to be unpaired. With a benchmark set of E. coli transcripts, we show that Led-Seq is an improved and reliable approach based on metal ion-induced phosphodiester hydrolysis to investigate RNA structures in vivo. A great advantage of the double-end analysis is the mutual validation of cleavage sites which increases the robustness of our approach. Furthermore, it ensures that one of the libraries carries informative reads close to the transcript ends, where reads from the other library are too short for unambiguous mapping.

Poster 54

Negating Warburg mediated hypoxic resistance; A new anti-cancer therapy

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Hypoxic tumors have been shown to be resitant to conventional chemotherapuetics, which has made treatments challenging in these cases. Recent reports have shown that inhibiting the Warburgh effect with a glycolysis inhibitor, works to negate this resistance. However, the mechanism that drives these processes remain unknown. Here, we describe for the first time, a molecular mechanism that may explain this Warburg effect-mediated drug resistance, and also provide a novel target for the development of new anti-cancer drugs. We also report a new fundamental mechanism through which cells may be able to sense and correct energy imbalances by regulating mitochondrial calcium levels via ER-MAMs (ER-mitochondria associated membranes).

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Ein besonderes Charakteristikum der 1994 gegründeten und 2017 umbenannten Fakultät stellt die Zusammenführung unterschiedlicher naturwissenschaftlicher Disziplinen im Bereich Lebenswissenschaften dar. Die Studierenden, Forscherinnen und Forscher profitieren hierbei von den vielfältigen Kooperationen zwischen den unterschiedlichen Fachbereichen sowie mit anderen Fakultäten und anerkannten außeruniversitären Einrichtungen.
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Poster 55

Myelin phagocytosis leads to the development of intracellular crystals

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Demyelination in rare genetic white matter diseases such as X-linked Adrenoleukodystrophy has been associated with the development of cholesterol crystals. In rodent models of aging the connection between cholesterol crystals and a defective cholesterol clearance in demyelinating lesions was demonstrated. Leading to an activation of the NLRP3 inflammasome in the animal model, it is unclear how these crystals contribute to the disease progression in leukodystrophies. In order to establish an in vitro model studying the effects of myelin-associated cholesterol crystals, THP1-monocytes were differentiated to macrophages and treated with increasing dosages of wildtype myelin for 6 and 24 hours. Inflammatory and metabolic profiles were analyzed, and cells were examined using an electron microscope. While high dosages led to an elevation of proinflammatory cytokines such as IL1b and TNFa, metabolic changes became increasingly evident after prolonged stimulation with an increase in the expression of cholesterol exporters such as ABCG1 and ABCA1. Interestingly the expression of lysosomal proteins decreased depending on the duration and dosage of myelin treatment. Furthermore, we were able to induce the development of intracellular crystalline structures in the phagocytes after 24 hours, therefore indicating that this is a potential model to further examine the consequences of myelin-induced intracellular crystals.

Poster 56

Optimized mRNA Design and Delivery for Non-Viral Generation of Chimeric Antigen Receptor (CAR) T Cells

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CAR-engineered T cells have shown great clinical success in selected hematological cancers. However, the approved CAR T cell therapies rely on viral transduction, a time- and cost-intensive process with possible safety issues. mRNA-based CAR T cell engineering offers an efficient and safe alternative, but is still facing challenges for clinical translation due to mRNA's immunogenicity, poor stability, and low cellular uptake efficiency. This study aims to improve mRNA technology for CAR T cell engineering considering mRNA design and delivery into T cells in order to develop advanced mRNA-CAR T cells.

mRNAs with different 5' cap analogs, nucleotide modifications, and with/without untranslated regions (UTRs) were produced by in vitro transcription and analyzed regarding translation efficiency and immunogenicity. The optimal balance between CAR expression and immune activation was found in mRNA having a CleanCap cap structure, UTRs, and N1-methylpseudouridine modification. Furthermore, lipid nanoparticles (LNPs) were examined as mRNA delivery method and compared to electroporation as state-of-the-art method used in clinical trials. LNP-CAR T cells showed prolonged efficacy in vitro by up to three days as a result of extended CAR-mRNA and CAR T cell persistence. Moreover, mRNA-LNPs were able to generate functional CAR T cells from isolated cancer patients' T cells.

This study provides an improved engineering strategy for CAR T cells using an optimized mRNA design and LNPs for mRNA-delivery and should motivate to consider mRNA in further applications of cancer immunotherapies.

Poster 57

Overactivated epithelial NF-κB disrupts lung development in congenital diaphragmatic hernia

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Background

CDH is a common developmental defect (1:2500) with significant neonatal mortality and morbidity. It was recently associated with enriched pro-inflammatory processes. We analyzed the activation status and disease-related impact of the pro-inflammatory NF-κB pathway in CDH lung hypoplasia.

Methods

Fetal lung hypoplasia and CDH were induced in the established nitrofen-rat model of CDH. Dexamethasone and curcumenol served as therapeutics to antagonize the p65-signaling in affected lungs. Lungs were analysed by bright field microscopy, RT-PCR, Western Blot and Immunofluorescence. Human fetal lung sections from CDH and non-CDH control patients were assessed via immunostaining.

Results

We observed significantly impaired airway branching in nitrofen-exposed lungs with elevated activation of p65 in the airway epithelium and increased expression of NF-κB downstream targets (Tnf-α, Cxcl1, Myc). Similar, human CDH samples indicated overactivation of NF-kB, especially in the airway epithelium. Treatment with NF-κB antagonist dexamethasone partially rescued lung hypoplasia in the rat model, and normalized p65 activity. Specific NF-κB inhibititon with curcumenol also corrected the hypoplastic lung phenotype ex vivo.

Conclusion

Our results suggest an overactivation of NF-κB in human and nitrofen exposed hypoplastic CDH lungs. Pharmacological inhibition restored lung characteristics indicating a direct involvement of p65 within hypoplastic lung development. Anti-inflammatory therapeutics in CDH associated lung hypoplasia can serve as potential strategies for further investigations.

Poster 59

Simulating the age-dependent susceptibility of intestinal epithelial cells to endotoxins - a murine in-vitro model for human NEC

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Background

Exaggerated intestinal inflammation is associated with necrotizing enterocolitis (NEC) but cell culture-based models comparing specific developmental stages are limited. To gain insides into cellular function we analyzed the lipopolysaccharide (LPS) induced response of murine intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) at distinct ages reflecting human intestinal maturation.

Methods

IECs and IELs from small intestine samples of C57BL/6J mice aged 1, 2, 4, and 8 weeks mimicking the intestinal maturation of fetal, preterm, term born, and adult humans were stimulated with 1mg/mL LPS. Gene expression level of inflammation markers were determined by RT-PCR. Phosphorylation status of p65 illustrating LPS mediated signaling was detected by Western Blot.

Results

LPS treatment induced a significant increase in Tnf-α and Il-1b expression in IECs of 1- and 2-week old mice and also a rapid phosphorylation of p65 from 2-week old animals. At 4 weeks, this activation diminished. Contrariwise, IELs showed a significant response in Tnf-α and Il-1b expression at older ages and an increased phosphorylation of p65 in 4-week old mice after LPS stimulation.

Conclusion

IECs are characterized by a high degree of endotoxin sensitivity that decreases with age. The inflammatory response of IELs behaves opposite. The contrastive response of IECs and IELs to LPS correlates with an increased susceptibility of preterm infants observed in NEC patients. Our model provides an effective way to study this NEC-like inflammation in vitro.

Poster 60

The long non-coding RNA HEAT4 is increased in monocytes of patients with cardiovascular disease having an anti-inflammatory function

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Activation of the immune system has a major impact on cardiovascular disease (CVD). Single-cell sequencing identified the long non-coding (lncRNA) HEAT4, which is upregulated in the blood of patients with heart failure, acute myocardial infarction and cardiogenic shock. HEAT4 is enriched in anti-inflammatory $CD16^+$ monocytes and is induced by treatment with anti-inflammatory IL-10. Overexpression of HEAT4 in monocytes reduced the expression of pro-inflammatory cytokines, increased the number of $CD16^+$ monocytes, enhanced endothelial barrier regeneration in a monocyte-endothelial co-culture model and promoted vascular healing in an in vivo humanised mouse model. Pulldown followed by mass spectrometry identified the protein IP1 as a HEAT4 interaction partner. IP1 has been reported to play an inflammatory role and is secreted by immune cells. Overexpression of Heat4 or treatment with IL-10 resulted in reduced extracellular levels of the IP1/IP2-heterodimer, while higher levels of IP1 were found in the nucleus.

The lncRNA HEAT4, which is elevated in patients with CVD, interacts with the pro-inflammatory protein IP1, reducing its secretion while increasing its nuclear translocation. HEAT4 has an anti-inflammatory role by reducing inflammatory cytokines and increases anti-inflammatory $CD16^+$ monocytes. Understanding the molecular mechanisms underlying the function of HEAT4 may provide a new strategy for the treatment of CVD with impaired vascular function.

Poster 61

Thrombin promotes podocyte injury via PAR-4 mediated NLRP3 inflammasome activation

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Question:
Nephrotic syndrome (NS) is characterized by proteinuria and renal dysfunction. Increased thrombin generation is linked with NS, but the mechanism underlying thrombin-induced renal dysfunction in NS is not well known.

Goal:
We hypothesize that targeting protease-activated receptor 4 (PAR4) attenuates nephrotic syndrome by restricting NLRP3 inflammasome activation in podocytes.

Methods:
We performed time kinetic study for thrombin mediated NLRP3 activation in podocytes. To study the involvement of PAR4, for thrombin-induced NLRP3 inflammasome activation we used PAR4 knockout (KO) mouse podocytes. We also treated PAR3 KO mouse podocytes with thrombin. Human podocytes were treated with PAR4 inhibitors (BMS3 and P4pal-10) and PAR1 inhibitor (P1pal-12) to delineate the specific impact on thrombin-induced inflammasome activation. In nephritis mouse model, we explored the therapeutic potential of PAR4 inhibitors (BMS-3, P4pal-10) and PAR1 inhibitor (P1pal-12).

Result & Conclusion:
PAR4 inhibition reduces thrombin-induced NLRP3 inflammasome in mouse and human podocytes. PAR1 inhibition does not mitigate thrombin induced inflammasome in podocytes. In PAR3 knockout mouse podocytes treated with thrombin induced persistent NLRP3 inflammasome, suggesting PAR3 independent thrombin induced NLRP3 inflammasome activation. In NTS-induced nephritis mouse model, targeting PAR4 alleviated NLRP3 inflammasome and albuminuria. Whereas, targeting PAR-1 (P1pal-12) failed to restrict NLRP3 activation. Results suggest a promising avenue for PAR4 targeted therapies in NS.

Poster 63

YBX1 is the master regulator of the TGF-β autoinduction in endothelial-to-mesenchymal transition through stabilization of TGF-β2 mRNA

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TGF-β signaling has an important role in wound healing. The TGF-β signal is self-amplifying through the process of autoinduction. Activation by TGF-β ligand leads to TGFB mRNA expression and release of the ligand. Overactivation of TGF-β was reported in cancer, fibrosis and cardiovascular diseases. Endothelial-mesenchymal transition (EndMT) induced by TGF-β has an important role in atherosclerosis, particularly in complex and unstable plaques.

Aim: TGF-β auto-induction in the context of EndMT.

Induction of EndMT resulted in a continuous increase in TGFB2 mRNA from 24 to 96 h after addition of TGF-β2 ligand, as measured by scRNA-seq and RT-PCR. Knockdown of TGFB2 mRNA reduced the autoinduction of TGFB2 after addition of the TGF-β2 ligand. RNA immunoprecipitation (RIP) showed that the stress proteins YBX1 and YBX3 strongly (>1000-fold) interacted with TGFB2 mRNA. Knockdown of YBX1 and YBX3 resulted in a complete reduction of EndMT and prevented TGFB2 autoinduction, which was validated by YBX overexpression using mRNA transfection. Indeed, Spatial transcriptomics of atherosclerotic plaque sections showed elevated Ybx1 and Ybx3 level in plaques, as validated by scRNA-seq in human EndMT. Functionally, YBX1 knockdown prevented EndMT-induced impairment of endothelial barrier function. Mechanistically, RIP-Seq showed predominate interaction of YBX1 in the 3'UTR of the TGFB2 mRNA. RNA stability assay using actinomycin D revealed that YBX1 stabilizes TGFB2, mainly in the 3'UTR.

We identified YBX1 as a master regulator of TGF-β autoinduction in EndMT by stabilizing TGFB2 mRNA.

Poster 64

β–catenin stabilization attenuates LPS-induced p65 activation in IEC-6 cells

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Background

Impaired intestinal endotoxin tolerance is seen in human infants suffering from necrotizing enterocolitis (NEC), but the underlying mechanism remains unknown. β-catenin plays a crucial role in transcriptional activation of proliferation and cell growth in intestinal epithelial cells (IECs) and is reduced in intestinal tissue of NEC patients. Our study aims to elucidate the effects of stabilized β-catenin on the LPS-induced pro-inflammatory signaling of IECs.

Methods

To stabilize β-catenin, we treated IEC-6 cells and primary IECs of one-week-old mice with the commercial GSK3-β inhibitor CHIR99021 (10μM) and subsequent LPS stimulation (0.1μg/mL). The activation status of NFκB-p65 was assessed by Western Blot and Immunofluorescence after 30min and gene expression levels by RT-PCR after 3hrs. Co-Immunoprecipitation was performed to evaluate p65-β-catenin protein interaction.

Main results

After β-catenin stabilization, we observed a significant reduction in the phosphorylation status of p65 and a diminished p65 nuclear accumulation after LPS stimulation. We detected an attenuated transcriptional activity of p65 as seen by a reduced expression of Tnf-α, Il-1ß, Il-6, and iNos. However, a direct p65-β-catenin protein interaction could not be detected.

Conclusion

The stabilization of β-catenin diminished the LPS-induced p65 activation and nuclear translocation associated with a decreased pro-inflammatory gene expression. Thus, exogenous manipulation of the Wnt/β-catenin pathway may overcome the high LPS susceptibility as seen in NEC patients and serve as a therapeutic option.

Poster 65

Octopaminergic encoding of reward information during learning and memory in Drosophila melanogaster

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Octopamine is a neuromodulator in the invertebrate nervous system. Similar to Noradrenaline, its equivalent in vertebrates, Octopamine is involved in various physiological and behavioural processes. In this study, the role of Octopamine in reward learning and memory is analyzed. It is known that the reward system in Drosophila melanogaster is comprised of dopaminergic PAM neurons that transmit appetitive information onto the memory center, the mushroom body. Octopamine has been found to be involved in reward learning and memory, too. More precisely, dopaminergic PAM neurons receive octopaminergic input signals. Using an associative learning and memory assay, we screened and identified the octopaminergic receptors that are involved in these processes. Interestingly, the role of the octopaminergic receptors varies in different memory phases. Elucidating the working principles of the octopaminergic system in learning and memory can help to understand the different ways of how reward information is integrated in the memory process.

Poster 66

Differences in functional synaptic nanotopography as the basis for area-specificspecializations of the neocortex

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A crucial factor determining the effectivity and plasticity of synaptic transmission is the physical distance between presynaptic calcium channels and the transmitter-filled synaptic vesicles. In the mature brain, tight coupling, high synaptic reliability and short-term depression (STD) were found to prevail at synapses engaged in rapid processing of sensory information (in the primary somatosensory cortex (S1)), while at synapses engaged in higher-order functions loose coupling, lower reliability and short-term facilitation (STF) (in the prefrontal cortex (PFC)) were found. Here, we focused on M2 and investigated coupling and short-term plasticity.

We performed whole-cell patch-clamp recordings of layer 5 pyramidal neurons and stimulated extracellularly in layer 2/3. We found pronounced STF in M2 with a paired pulse ration of 1.4 ±0.04; n=48 of excitatory postsynaptic potentials (EPSCs). In order to investigate the coupling distance, we bath-applied the membrane permeant Ca2+ chelator EGTA-AM, which only interferes with release if coupling is loose. Following EGTA application in M2 and PFC we found a significant reduction of EPSC amplitudes in PFC of 46% n=7 (IQR = 60/32, $p < .00001$) compared to M2. These results indicate that synapses in M2 operate with tight coupling albeit show STF. It appears that M2 is not only connected between PFC and M1 but also that its synapses have properties intermediate between higher and lower order processing properties. Further experiments are required to clarify the mechanisms of STF in tightly coupled M2 synapses.

Poster 67

The Role of Octopamine in Bad Food Decisions

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It is important for an organism to ensure its survival by mobilizing sufficient amounts of energy. If mobilizations fails or starvation pressure further increases, the animal switches its starvation response strategy and must search for new food sources. This increase in activity costs energy. An even greater problem than this conundrum can also arise: What happens, if the animal only finds a bad quality food source? As the starvation pressure increases continuously, the organism must accept a health risk at some point in order to ensure its own survival. At that point, the animal starts to consume potential toxic or non-palatable food. Indeed, our data provide evidence that animals actively start to ingest bad food after 2 and 6 h of starvation. In detail, larvae start to eat bitter and salty food with increasing starvation pressure, which it would not consume in a satiated state as it is considered harmful. Further, we were able to show that optogenetic activation of octopaminergic neurons (OANs) increases the food intake of appetitive and harmful food. To investigate the changing factors in signal acquisition or transduction, salt and bitter sensing neurons in the periphery were analyzed using calcium imaging. Interestingly, our data suggest that starvation decreases the response of these sensing cells. Also, artificial application of octopamine (OA) elicits the same effect in non-starved larvae. Our results lead us to the hypothesis that the acceptance of "bad" food is status-dependent, and that OA is a key signaling molecule involved in the decision-making process.

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Poster 68

Prevalences and associated factors for depression, obesity and comorbid conditions in the working-age population – Results from the LIFE-Adult-Study

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Introduction:
Obesity and depression are both highly prevalent conditions with major implications for the public health system. We aim to examine the prevalences of depression, obesity, and comorbid conditions and associated factors within a population-based sample of the working-age population in Germany.

Methods:
We analyzed Baseline data from n=5784 participants (18-67 years) in the LIFE-Adult-Study, including data on depression (CES-D), obesity (BMI), socioeconomic status (SES), physical activity (IPAQ), and occupational characteristics (O*NET: e.g., freedom to make decisions). We used binary logistic regressions for data analysis.

Results:
The average age within the sample was 43.4 years (SD=12.5). 54.1% were female (n=3129). The prevalences were as follows: 4.5% (n=281) for depression, 15.3% (n=1.205) for obesity, and 1.2% (n=101) for comorbid conditions. Analysis revealed significant associations between comorbid conditions and several factors, including female gender (OR 1.8 [1.0; 3.1], p<.05), higher age groups (35-49 years: OR 6.0 [2.3; 16.1], p<.001; 50-67 years: OR 14.9 [5.2; 42.5], p<.001) compared to the young, being widowed (OR 3.4 [1.0; 11.5], p<.05), lower SES, higher freedom to make decisions (OR 1.9 [1.1; 3.2], p<.05), and lower physical activity.

Discussion:
A substantial part of people in working-age life is affected by depression, obesity and co-occurring conditions. The results underline the relevance of combined treatment approaches and implementation of long-term studies in order to increase well-being and maintain the ability to work.

Poster 69

Adherence to a lifestyle intervention – self-efficacy as key to success? Analysis of AgeWell.de as multi-domain lifestyle intervention against cognitive decline

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Background.
Better adherence to the components of AgeWell was found to effect cognition, health-related quality of life and depression at follow-up. The research question we followed was: What predicted adherence to the components?

Methods.
317 intervention group-participants with risk for dementia (CAIDE-score of≥9; mean age 68.9, 49.5% female) of the AgeWell intervention were analysed. Regression models with four blocks of predictors (sociodemographic, cognitive and psychosocial, lifestyle and chronic conditions) were run on adherence to the components nutrition, enhancement of social and physical activity and cognitive training. Adherence was operationalised as mean score of goal achievement by assessing the degree of goal achievement per component at seven time points during the intervention.

Results.
Increasing age, depressive symptoms, smoking and partwise higher body mass index were negatively associated with adherence while no effect of chronic conditions was found. Higher education, better cognition, higher self-efficacy predicted better adherence. The prediction was better for the components of social and physical activity.

Conclusion.
Results identified predictors for better and worse adherence. Particularly self-efficacy seems to be of considerable influence on adherence. Future intervention therefore should combine enhancement of the adherence with enhancement of self-efficacy as part of the intervention. This investigation strengthens the idea of more targeted interventions and gives further directions of optimization for multi-domain lifestyle interventions.

Poster 70

A qualitative evaluation of the KITA examination in the 4th year of life in Saxony – opportunities and challenges

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Introduction:
The examination of children in the 4th year of life (KITA examination) is a central part of the medical examination offer of the pediatric and adolescent medical service in Saxony. The aim of the present study is to evaluate its current practice, opportunities, barriers, and the impact of the COVID-19 pandemic from the perspective of the physicians, the daycare professionals, and the parents.

Methods:
In this qualitative study, a total of N = 13 telephone interviews were conducted with physicians (N = 5), parents (N = 4) and daycare professionals (N = 4). The interviews were conducted and recorded (audio) using a semi-structured interview guide. After transcription, the contents were analyzed computer-aided (MAXQDA) using qualitative content analysis according to Mayring.

Results:
All interviewees stated that the KITA examination plays an important role in identifying school-relevant support needs at an early stage. Examining the children in their natural environment is seen as an advantage. The presence of the childcare professional during the examination was advocated. The COVID-19 pandemic had a negative impact on the implementation (e.g. cancellation of appointments). Dissemination of information via analogue or digital media could be used, in order to increase the utilization of families who are difficult to reach.

Conclusion:
The KITA examination makes an important contribution to early intervention and represents an important additional and low-threshold offer, in addition to the regular "U" examinations in the pediatrician's practices.

Poster 71

Depressive Symptoms in Old Age during the COVID-19 Pandemic: A Comparative study of Individuals at Cardiovascular Risk and the General Population

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Aim.

We aimed to examine the association of late-life depressive symptoms with sociodemographic factors, social support and resilience, and perceptions of the COVID-19 pandemic in a cardiovascular risk group and a matched sample of the German general population during the beginning of the pandemic.

Methods.

We analyzed data of n=1236 people of old age with n=618 participants with a cardiovascular risk profile and n=618 participants from the general population. Matching of the data was conducted by applying case-control-matching based on age, gender and education. Next to sociodemographic data, we assessed worries about the Sars-Cov-2 virus, perceived threat by the virus due to preexisting conditions, supportiveness of the governmental measures, social support, resilience, depression and anxiety. Multivariate linear OLS regression models were conducted.

Results.

The cardiovascular risk sample had slightly higher levels of depressive symptoms and felt more threatened by the virus due to pre-existing conditions. In the cardiovascular risk group, social support was associated with less depressive symptoms. In the general population, high social support was associated with less depressive symptoms. Perceived resilience was associated with less depressive symptoms.

Conclusion.

Compared to the general population, the cardiovascular risk group showed slightly higher levels of depressive symptomatology at the beginning of the pandemic and could be supported by addressing perceived social support and resilience in prevention and intervention programs.

Poster 72

Changes in problematic smartphone use and quality of life in children and adolescents 2018-2023

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Objective:
The aim of this research project was to investigate changes in problematic smartphone use (PSU), quality of life, and their inter-relation from 2018 to 2023 in children and adolescents.

Methods:
Participants were 2250 10- to 17-year-old children from the LIFE Child cohort (Germany) who had reported on symptoms of PSU (Smartphone Addiction Proneness Scale) and quality of life (KIDSCREEN-27) between 2018 and 2023. Mixed-effects models were applied to assess associations (a) between PSU or quality of life and the year of assessment and (b) associations between PSU and quality of life.

Results:
Symptoms of PSU were significantly more frequent in 2021, 2022, and 2023 than in 2018 (e.g., for 2023 versus 2018: $b = 2.64$, $p < .001$). These changes decreased with increasing age of the children (e.g., for 2023 vs. 2018: $b = -0.58$, $p = .008$) and were significantly stronger in girls than in boys ($b = 2.54$, $p < .001$). Quality of life was significantly poorer in 2021, 2022, and 2023 than in 2018 (e.g., for 2023 versus 2018: $b = -2.35$, $p < .001$). PSU was significantly associated with lower quality of life ($b = -0.39$, $p < .001$). This association was significantly stronger in 2022 ($b = -0.46$, $p < .001$) than in 2018 ($b = -0.30$, $p < .001$).

Conclusion:
This study shows the increasingly problematic use of smartphones (especially among younger children) and the increasing negative association with the overall declining quality of life of children and adolescents over the last six years. The results emphasize the importance of teaching children how to use smartphones.

Poster 73

Mechanisms of change in cognitive-behavioral therapy for patients with binge-eating disorder

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Introduction:

Cognitive behavioral therapy (CBT) has shown efficacy in the treatment of binge-eating disorder (BED). However, limited understanding exists about how CBT works for BED. Main targets of CBT are among others to decrease overvaluation of shape and weight (OSW) and dietary restraint (DR) in order to reduce binge eating.

Research question:

How do changes in OSW and DR influence subsequent objective binge-eating episodes (OBEs) during CBT in patients with BED?

Method:

In this secondary analysis of a multicenter randomized-controlled trial, 84 patients (85.71% female) aged 18 to 63 years (M = 42.55; SD = 12.11), with a body mass index (BMI) ranging from 27.3 to 40.0 kg/m² (M = 34.33; SD = 3.95), diagnosed with BED or subsyndromal BED, were included. Patients were offered 20 individual sessions of CBT. At the beginning of each session, selected items from the eating disorder examination-questionnaire (EDE-Q) were used to assess OSW, DR, and OBEs over the past week. Within-patient effects of OSW and DR on OBEs were analyzed using dynamic structural equation modeling (DSEM).

Results:

Between the first and last therapy session, there was a significant reduction in OSW and the frequency of OBEs, but not in DR. DSEMs showed significant within-patient cross-lagged effects of both OSW and DR on subsequent OBEs.

Conclusion:

The findings show that improvements in both OSW and DR predict a reduction in binge eating during CBT in patients with BED. Prioritizing these factors could potentially enhance the efficacy of CBT.

Poster 74 Income, health-related behavior, and self-rated health

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Background

Aim of this study was to find out whether and to what extent various health-related behaviors affect the relationship between household income and self-rated health.

Methods

The analyses included 843 participants (average age 68.9; 52.6 % female) of the AgeWell.de study with an increased risk for dementia with CAIDE-score of 9 points or more. A regression was calculated to determine the relationship between income and self-rated health as measured by the EQ-5D-VAS. This relationship was examined using four mediation models for the influence of the following health-relevant behaviors: Fruit and vegetable consumption, social participation as well as cognitive and physical activities. The analyses were controlled for household income, level of education (using the CASMIN score), age and gender.

Results

Results show a highly significant influence of income on self-rated health. It was also found that social participation completely mediated this relationship. Both cognitive and physical activities had a partially mediating influence on the relationship between income and self-rated health. Fruit and vegetable consumption had no significant influence.

Conclusion

The outcomes can contribute to the research on the relationship between income and health. They emphasize the importance of social participation, but also cognitive and physical activities for health and could encourage reflection on strategies to make activities such as attending cultural events, educational institutions or sports courses more accessible to a broad public.

Poster 75

The worrisome State of student mental health in Saxony- why "enhance" is a chance

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Most mental illnesses begin before the age of 24. University students in particular have an increased risk of developing mental health problems and illnesses. Adding the stressors of the COVID-19 pandemic, university student's mental health deteriorated. Our team investigated the effects of the pandemic on the mental health of N= 5.510 students from six Saxonian universities. Clinical symptoms as well as social and emotional variables were assessed utilizing standardized instruments in an anonymous online survey during April and May 2022. The results were alarming: over two thirds of the students reported clinically relevant symptoms such as depressive symptoms (35.5%) and anxiety (31.5%). Even more worrisome was the high suicidality reported by almost one fifth of the students (19.6%). Alarmingly, compared to the results of 2020 (16.5%) and 2021 (14.5%) the reported acute suicidality among university students is higher than ever.

The call for action is clear: low-threshold support and mental health services should be made more easily and timely available for university students.

"Enhance" is built upon this research and a project of the University of Leipzig and it´s cooperation partner ehs (Evangelische Hochschule Dresden), aiming to fill this gap. It's goal is to strengthen the mental health of Saxonian university students and providing professional counselling support via email, chat or video call by psychologists. They may also use a mental-health app. The offer is currently being evaluated for acceptability and feasibility.

Poster 76

Associations between stressful life events and increased physical and psychological health risks in adolescents: a longitudinal study

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Background:
Stressful life events (SLE) are risk factors for mental and physical health problems, particularly in the vulnerable period of adolescence. This work investigated associations between SLE and several negative health outcomes in adolescents and considered moderating factors, using a longitudinal approach.

Methods:
Data of a healthy adolescent sample from the LIFE Child Study in Leipzig, Germany was analyzed (n=2024, aged 10-18 years). SLE were measured by a questionnaire, addressing stressful situations in the family and the peer domain, e.g., divorce of parents or being a victim of violence. Health related quality of life (HrQoL), behavioral difficulties and BMI were compared before and after a SLE had occurred. Moderator effects of socioeconomic status (SES), age, and sex were investigated using linear regression models.

Results:
64% and 40% of the participants had experienced at least one SLE from the peer or family domain, respectively. All considered health parameters had, in average, deteriorated after the occurrence of a SLE in the peer environment. SLE in the family domain appeared to be a risk factor for decreased psychological well-being and increased BMI values. Differences in HrQoL before and after a SLE were significantly stronger in girls. No moderator effect of age and a weak moderator effect of SES was identified.

Conclusion:
Findings confirm that SLE function as risk factors for mental and physical health disadvantages in adolescents. Prevention programs should seek to support adolescents affected by SLE, with a special focus on girls and young women.

Poster 77

Explorative analyses on spatial differences in the desire for social distance toward people with mental illness in a diverging city

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Introduction:

Stigma is an individual and societal process based on attitudes and power and relates to both spatial disparities and social distinction. In this study, we examined differences in desire for social distance toward people with mental illness within Leipzig using social and spatial information.

Methods:

ANOVAs and Scheffé post-hoc tests analyzed varying desires for social distance toward people with mental illness within Leipzig. Joint Correspondence Analyses (JCA) explored correspondences between desire for social distance, socio-economic status, age, life orientation, social support, duration of living in Leipzig, and shame toward having a mental illness in five city districts of Leipzig in LIFE study participants (by Leipzig Research Center for Civilization Disease, data collected 2011–2014 and 2018–2021, n = 521).

Results:

Stigma varied among Leipzig's districts ($F(df = 4) = 4.52$, $p = 0.001$). JCAs showed that a higher desired social distance toward people with mental illness corresponded with spatial differences, high levels of pessimism, high shame of being mentally ill, low social support, low socio-economic status, and older age (75.74 and 81.22% explained variances).

Conclusion:

In terms of stigma, where people with mental illness live matters. The results identified target groups that should be addressed by appropriate intervention and prevention strategies for mental health care.

Poster 78

Impact of cognitive reserve on effectiveness of cognitive behavioral therapy (CBT) for late life depression

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Background

Certain studies suggest an effect of cognitive functioning on the outcome of cognitive behavioral therapy (CBT). What is less clear, however, is whether cognitive reserve (CR) might influence effectiveness of CBT interventions for depression in older adults. We aim to analyze the impact of CR on treatment effectiveness of CBT for late life depression (LLD) in comparison with a supportive unspecific intervention.

Methods

Regression analysis were used to investigate the impact of CR, as assessed by baseline education and cognitive ability (total score for the Consortium to Establish a Registry for Alzheimer's Disease), on treatment outcome of CBT for LLD. We analyzed data from 224 participants (aged 60+), employing intention-to-treat analyses. We controlled for age, gender, anxiety symptoms, and therapy sessions, assessing depression with the Geriatric Depression Scale (GDS).

Results

Overall, individuals with lower CR seem to respond better to CBT compare to those with a higher CR. The longer period is particularly beneficial for them. At follow-up, the between-group difference in the change in GDS scores related to cognitive performance is -3.2 points (average marginal effect (AME) of CBT; 95% CI: -5.2; -1.1). In comparison, the impact of education appears to be less pronounced with a difference of -1.9 points (AME of CBT; 95% CI: -3.7; -0.3).

Discussion

To enhance CBT outcomes for LLD, therapists should address cognitive strengths and weaknesses, customizing techniques. Further research is needed to explore specific CBT elements in relation to outcomes with varying CR.

Poster 79

Mental health care utilization behavior prior to suicide in adolescence

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Suicide is a leading cause of death in adolescence. We performed a survey of all suicides below the age of 21 in the City of Leipzig and an adjacent rural district between 1996 and 2019 and compared the sample of 75 suicide cases with the medical records of the two local hospitals who grant compulsory mental health care in their child and adolescent psychiatric departments. Only 23.9% of the young adults and 20.7% of the underage suicides sought professional mental health care in hospital. Utilization of mental health care as inpatient psychiatric treatment prior to suicide was particularly low in males and hard suicide methods. Identification and reduction of barriers to treatment is mandatory in order to reach the majority of suicidal young people for a potentially life-saving treatment.

Poster 80

Risk profiles of unmet care needs in older primary care patients with depression – results of the AgeMooDe study

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Background:

An increased risk of depression in old age has been associated with a range of unmet care needs in those affected. Studies to identify possible risk profiles for depression with specific combinations of unmet care needs are still pending. The main aim of this exploratory study was therefore to investigate the profiles of care needs in old patients with and without depression.

Methods:

The sample is based on the multicenter study „Late-life depression in primary care: needs, health care utilization and costs (AgeMooDe)“ and includes 1,092 GP patients aged 75+ years. Patient needs were assessed via the Camberwell Assessment of Need for the Elderly (CANE). A latent class analysis was performed to identify the need profiles and associated sociodemographic and clinical factors were investigated. Further, a regression analysis was performed taking into account the identified need profiles and associated factors to examine the risk of depression.

Results:

The main results of the study comprised three need profiles: ‘no needs’, ‘met physical needs’, and ‘unmet social needs’. Compared to members of the ‚no needs‘ profile, members of the ‚met physical needs‘ (OR= 3.5, 95%-CI: 2.5 – 4.9) and ‚unmet social needs‘ (OR= 17.4, 95%-CI: 7.7 – 39.7) profiles were significantly more likely to suffer from depression.

Conclusions:

The study results offer valuable insights for needs-based interventions by GPs. Specifically, unmet social needs in older GP patients with depression require special attention. Innovative approaches like social prescribing can be helpful in this context.

Poster 81

Prerequisites and appropriate symptoms to use self-medication from the perspective of visitors to the „Long Night of Science“ at Leipzig University

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Objective:
While regulatory aspects for self-medication drugs are clearly regulated, little is known about the prerequisites/symptoms that scientifically interested users see to use self-medication.

Aim:
We aimed to evaluate which prerequisites/symptoms are considered appropriate for self-medication use by visitors to the “Long Night of Science” at Leipzig University.

Method:
After positive ethical approval, we invited consecutive visitors to the “The Long Night of Science” in June 23, 2023 to participate in a questionnaire survey. In order to participate, visitors had to be of legal age and to have sufficient language skills. We designed, pretested, and used a questionnaire asking for which prerequisites/symptoms the use of self-medication is considered appropriate.

Results:
A total of 189 completed questionnaires were returned. 30.7% of participants stated that they used self-medication on a daily or weekly basis. The use was more common among men than women (32.8% vs. 28.9%). 28.8% used self-medication several times a month, with those starting out in the healthcare sector using it more frequently (39.4%) than others (23.1%). As the three most important prerequisites for self-medication were considered: mild symptoms, known symptoms, and speeding up the recovery process. The top three symptoms considered appropriate for self-medication were headache, cold symptoms, and sunburn.

Conclusion:
About one third of the scientifically interested participants regularly used self-medication. Well known prerequisites/symptoms were mentioned for an appropriate use of self-medication.

Poster 82

Leuko-Expert: A guiding algorithm for detection of Leukodystophies

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Leukodystrophies (LDs) are rare genetic diseases that affect the brain white matter, with an estimated incidence of 40.000 to 100.000 [1]. LD Patients often undergo a long diagnostic journey and are frequently misdiagnosed. Furthermore, the number of LD patients is limited, and the progression of the disease can be slow or rapid. To shorten the diagnostic time and reduce the incidence of misdiagnosis, we have designed a framework to distinguish between LDs and other potential differential diagnoses.

For the classification task we use the data from three different hospitals (UK Leipzig, UK Tübingen, UK Aachen) in a coordinated registry of clinically relevant patient phenotypes. The registry is implemented in RedCAP [2] and has information about the patient, the medical history and multiple instances of examinations. The task is executed in a distributed manner through the personal health train [3], ensuring that the data stays on-site, by using the MII infrastructure and the Data integration centers. We have also implemented a synthetic data generation method, to enrich the dataset, and then used different classification algorithms to differentiate the LDs from the differential diagnosis.

We show that the used machine learning models can differentiate the group of rare diseases from the differential diagnosis and the Random Forest algorithm obtained the best result among the tested algorithms. These trained models could be used to classify patients at an early stage of the disease.

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MAX PLANCK INSTITUTE
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Poster 83

Experimental modulation of physiological force application on leg joint neurons in intact Drosophila melanogaster

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This study introduces a novel, least-invasive method to investigate the impact of mechanical force stimulation on the adhesion G protein-coupled receptor (aGPCRs) Cirl in proprioceptive leg neurons of fruit fly Drosophila melanogaster. Independently of aGPCRs, this method offers a new avenue to analyse force parameters on mechanosensory molecules in a physiological setting.

Method:
The hind leg of live adult flies were immobilised with a heptane-based glue and a single human hair. By pulling at the hair we stalled the natural flexion-extension cycle of the femorotibial joint. By employing a binary reporter system we circumnavigated the need for instant signal detection during mechano-dependent receptor activation.

Results:
The leg restraint disrupts the natural flexion-extension cycle of the femorotibial joint resulting in reduced mechanical stimulation of specialized proprioceptive leg joint neurons in Drosophila. This resulted in the robust reduction in signal intensity of a fluorescent reporter (ratio of short-lived GFP and long-lived RFP) [1] coupled to a mechano-transcriptional sensor that measures the separation of Cirl [2]. In contrast, the signal intensity ratio remained stable under mobile control conditions.

Outlook:
This protocol can be combined with different optical readouts for assessing mechanosensory features and cellular responses, and thus provides versatile options for carrying out mechanobiological inquiries.

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Poster 84

From structure to function - SRCD and SAXS analysis of (pyroglutamate) alpha-synuclein

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Alpha-synuclein (aSyn) aggregation represents a key event in synucleinopathies. Here, we present new insights into structural characteristics of aggregated full-length (FL-) aSyn and, for the first time, of recently discovered pathological pyroglutamate (pGlu-) aSyn variants of different lengths. The generation of the pGlu-aSyn post-translational modifications (PTM) requires two enzymatic activities for: (i) N-terminal truncation of aSyn and (ii) the subsequent cyclisation of the resultant N-terminal glutamine to pGlu by glutaminyl cyclase (QC).

To initiate aggregation, recombinant FL- and pGlu-aSyn variants were agitated. To gain insights into structural properties of monomeric and oligomeric states, synchrotron radiation circular dichroism (SRCD) and size exclusion chromatography coupled small angle X-ray scattering (SEC-SAXS) were performed at SOLEIL synchrotron (France).

SRCD analyses showed that soluble pGlu-aSyn variants are intrinsically disordered proteins, consistent with the known disordered nature of FL-aSyn. An amyloid pore, which was described for the FL-aSyn, could be detected using SEC-SAXS data analyses. Due to the influence of PTM, different shapes of oligomeric pGlu-aSyn variants were bioinformatically modeled. Surprisingly, data suggest the formation of a dimeric state for all proteins studied.

Our results suggest a role of QC as aSyn-modifying enzyme and highlight it as a potential target for treatment of synucleinopathies. Additionally, we here investigated the 3D-shape of the dimeric state as a key intermediate in the aggregation/ oligomerisation process.

Poster 85

Decrypting Abeta post-translational modifications in neurodegenerative diseases

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Aggregation of amyloid beta (Abeta) and post-translationally modified Abeta in particular is a neuropathological feature in brains of Alzheimer's disease (AD) patients. In other types of dementia, such as dementia with Lewy bodies (DLB) and vascular dementia (VAD), a role of Abeta in the pathology is hypothesized. We propose distinct pathogenic profiles of Abeta post-translational modifications (PTMs) in AD and other types of dementia.

We employed monoclonal antibodies for comparative analyses of different Abeta PTMs in post mortem human brain tissue of AD, DLB and VAD cases, and control subjects. Using machine learning protocols, we quantified immunohistochemical stainings of Abeta PTMs and compared the results with the quantification of Abeta variants employing ELISAs. Furthermore, histopathological findings were correlated with clinical data.

In human brain tissue, labeling with antibodies raised against Abeta PTMs isoaspartate 7 (isoD7), pyroglutamate 3 (pE3) or phosphoserine 8 (pSer8) showed a lower percentage of stained plaque area compared to labeling of total Abeta. The isoD7-Abeta variant was the most abundant among the Abeta PTMs investigated. Both total plaque load and abundance of Abeta variants were highest in AD cases, followed by DLB cases.

Abeta plaque pathology and the percentage of Abeta variants compared to total Abeta can be quantified using single labeling immunohistochemistry and machine learning protocols. Abeta PTMs, and in particular the isoD7-Abeta variant, might be considered for diagnostic and therapeutic approaches in different types of dementia.

Biophysics | Biomaterials | Translational Regenerative Medicine

Biotechnology | Biochemistry | Computational Analysis and Modelling

Clinical Research

Molecular Biology | Biomedicine I

Molecular Biology | Biomedicine II

Psychosocial Medicine and Public Health

Molecular Neurobiology

Immunology and Infectiology

Psychology and Cognition

Diseases of Civilisation | Obesity I

Diseases of Civilisation | Obesity II

Life Science Research

Computer-assisted Surgery | Digital Medicine

Experimental and Clinical Oncology

Molecular Oncology

Poster 86

Criteria for identification and accurate quantification of spinal motor neurons in healthy and disease mouse models

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Motor neuron (MN) death is the hallmark of the MN diseases spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Quantification of MN loss in mouse models is an important readout for disease progression and therapeutic assessment. The large variability in MN death reported by different groups may depend on different technical approaches to label MNs as well as investigating distinct areas of the spinal cord.

Here, we established several criteria to ensure consistent MN quantification. First, we describe a spinal cord dissection, allowing segment specific MN isolation and counting. In combination with ex vivo ventral-root back fills and immunohistochemistry, we conclude ChAT and HB9 as reliable markers for MN identification. In contrast, Nissl and SMI-32 are not suitable markers for MN quantification. Second, ventral-root back fills of MNs within select lumbar segments combined with tissue clearing and ChAT immunoreactivity showed that different spinal segments contain different numbers of MNs. Third, comparison of lumbar MNs of SMA mice revealed a progressive MN loss from L1 to L6. While a severe SMA mouse model exhibits selective MN death restricted to specific spinal segments, MN loss was evident throughout the entire spinal cord in an ALS mouse model.

Our procedural account demonstrates that a select set of criteria is required for the valid identification of MNs and their accurate quantification in mice. Furthermore, our results can be used as a reference for future studies requiring accurate MN counts as part of therapeutic assessment.

Poster 87

Cerebellar circuit dysfunction in a mouse model of severe spinal muscular atrophy

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Spinal muscular atrophy (SMA) is characterized by the degeneration of spinal motor neurons. Whether other regions of the central nervous system are affected in SMA is largely unknown. Previously, morphological alterations were found in the cerebellum of SMA. This includes a selective degeneration of Purkinje cells (PCs) and excitatory synapses of the parallel fibers onto PCs which originate from the granule cells (GCs), which get their excitatory input from mossy fibers.

To investigate active and passive properties of PCs and GCs of SMA mice, we applied whole-cell patch-clamp recordings. The stimulation of parallel and mossy fibers then let us investigate the synaptic transmission onto PCs and GCs.

These recordings revealed a decrease of capacitance and increase of membrane resistance of PCs, indicating a decreased cell size in SMA. SMA PCs exhibited hyperexcitability and broaden action potentials which resulted in a decreased spontaneous and evoked firing frequency. This indicates an output impairment of PCs in SMA mice. Recording of PCs following stimulation of excitatory parallel fibers deriving from GCs showed an increased facilitation of excitatory synapses, demonstrating weakened excitatory synaptic transmission between GCs and PCs. Recordings directly from GCs revealed hyperexcitability and broadened action potentials that resulted in decreased firing frequency.

In summary, SMNΔ7 mice exhibit severe functional impairments of the cerebellar motor circuit. This suggests a dysfunctional aspect of the cerebellum which might contribute to the overall SMA phenotype.

Poster 88

The pathophysiology of glycine metabolism in ASNS-mutation induced microcephaly

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Microcephaly is a serious medical condition with a range of severe symptoms and many different causes. In the past, our research group identified two novel mutations in the gene of asparagine synthetase (ASNS) causing microcephaly. To fully understand the pathomechanism, we established a mouse line with neuron-specific deletion of the ASNS gene (named cKO in the following, genotype ASNSflx/flx/NexCrehet). cKO mice have a decreased brain size, and an increased amount of the amino acid glycine in the cortex and hippocampus. To examine the underlying mechanism, we performed qRT-PCR in the three brain regions cortex, hippocampus and cerebellum (used as control region without Cre-expression) of cKO and control mice at 3 different age stages (p5, p28 and p90) to assess the expression of ASNS, of enzymes involved in glycine metabolism as well as of Atf4, a transcription factor known to control the expression of glycine biosynthesis enzymes. We found a significant decrease of ASNS mRNA in cortex and hippocampus of cKO mice in all 3 ages. Furthermore, mRNAs of Atf4, phosphoserine phosphatase, and serine hydroxymethyltransferase 2 were increased in the cortex and hippocampus of cKO mice at the ages of p28 and p90, but not at p5, suggesting a postnatal Atf4 dependent upregulation of glycine biosynthesis. Further experiments will reveal the relevance of this modification of glycine metabolism for the complex pathophysiology of ASNS mutation-induced microcephaly.

Poster 89

Detection of molecular markers of ferroptosis in the Alzheimer's brain

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Background:
We have previously shown that droplet degeneration (DD) signifies the beginning of neuritic plaque formation during Alzheimer´s pathogenesis. As adjacent microglia exhibited strong ferritin expression and Perl´s iron staining showed iron in microglia, droplet spheres, and Aß plaque cores, we hypothesized that DD is a form of ferroptosis [1].

Methods:
Immunohistochemical detection of transferrin receptor (TfR), ferritin and lipid peroxides in prefrontal cortex of Alzheimer's brains, investigation of spatial correlation of these with histopathological hallmarks of Alzheimer´s disease (AD), comparison of expression in Braak stages, and visualization of ferroptotic marker genes by in situ hybridization.

Results:
While lipid peroxides showed no staining, TfR was found on neurons with beaded neuritic processes that appeared to be degenerating and are exhibited typical features of droplet degeneration, but a co-localization with p-tau was a rare event. TfR-positive neurons increased with Braak-stages as did ferritin expression in microglia. mRNA of signature genes of ferroptosis were detected in pretangles and p-tau negative neurons, but less in DD.

Conclusion:
With the increased expression of TfR and ferritin in high Braak cases and the demonstration of mRNA of ferroptotic marker genes in Alzheimer´s pathologies, the hypothesis that DD is ferroptotic is strengthened. Due to the strong morphological similarity of TfR-positive structures to DD, TfR might be considered as an early ferroptosis marker, which is only expressed transient in AD pathogenesis.

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Poster 90

Defined myelin composition underlies specific functions of small caliber axons

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The peripheral nervous system (PNS) enables movement and sensation through distinct motor and sensory fibers of varying axonal size and myelination status. For unknown reasons, peripheral neuropathies often affect only a subset of fiber types, indicating specific intrinsic vulnerabilities. By combining single-cell RNA sequencing with imaging approaches and myelin proteomics, we delineate a thus far unresolved diversity of PNS fibers. In detail, we discovered that Schwann cells do not consistently segregate based on motor or sensory nerve modalities, but instead primarily cluster based on cell size. We identified marker genes and myelin proteins, including 2'3'-cyclic neucleotide-3'-phosphodiesterase (CNP), that specifically define small myelinated fibers and demonstrate, in principle, that myelin composition is functionally relevant. Indeed, CNP is specifically required for the maintenance and integrity of small myelinated fibers, which convey tactile and pain sensations.

Our study sheds new light on the diversity of Schwann cells and underlines that myelin composition is intricately linked to specific Schwann cell types. These findings have important implications for the ongoing research into peripheral nerve disorders that are characterized by distinct symptomatologies.

Poster 91

Investigation of neuroinflammation in response to high fat intake and the impact of Phytosterols

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Excessive high fat consumption is a risk factor for many health issues such as neurodegenerative disorders, including Alzheimer's disease. Microglia are resident cells of the brain's parenchyma and show age related dystrophy in humans. Activated microglia undergo morphological changes and release cytokines and chemokines to recruit other immune cells and initiate an immune response. The accumulation of certain nutrition-derived fatty-acids, biologically active lipids and their metabolites in the brain may have effects on aging and microglial cell loss. Phytosterols (PS) are plant-derived and cannot be catabolized by humans, so their nutrition-dependent distribution can be tracked by mass spectrometry. In this study, we compared the effects of normal diet (ND), high-fat diet (HFD), HFD with 2 % PS and HFD with 4 % PS in female and male C57BL/6J mice on microglial states by flow cytometry after different feeding periods (2, 12, 24 weeks). Our preliminary results show no significant upregulation of pro-inflammatory (IFNγ, IL1β, TNFα) cytokines by HFD. Similarly, we did not observe an impact of PS. However, we find evidence for an age-dependent regulation of MHC-II, CD4+ T-cells and anti-inflammatory (IL-10) cytokines. We hypothesize that PS have an impact on inflammation, but since we did not observe inflammation due to HFD, further experiments are needed to prove this theory.

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Poster 92

Alterations in Spinal Motor Circuits in CMT1A

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Charcot-Maire-Tooth (CMT) is a disease of the peripheral nervous system and despite being the most prevalent inherited neuropathy there is no effective therapy available yet. Patients suffer from both motor and sensory impairments. CMT1A is the most frequent subtype and is characterized by the duplication of the PMP22 gene which leads to developmental dysmyelination and consecutive secondary axon degeneration.

We hypothesize that the peripheral defects cause alterations in the spinal circuits that control muscle coordination and strength which in turn contribute to the motor and sensory symptoms of the disease. Moreover, we speculate that microglia mediate the synaptic changes of these circuits.

For this, we analyze the spinal cord of a model for CMT1A, the C61 PMP22 line. We aim to characterize different motor neuron populations and synaptic alterations in the proprioceptive and C-bouton circuits.

So far, we found several synaptic alterations. We observe a trend that there are more proprioceptive synapses on the motor neuron bodies of the CMT1A mice during development. Additionally, the size of the C-boutons is increased starting during development in the CMT1A mice. At the same time, microglia start contacting specific synaptic components of the C-bouton more frequently, which could hint to mechanisms of a targeted microglia recruitment to synapses. Interestingly, we also notice a selective decrease in number of γ-motoneurons at the same time. These findings hint to a change in integral components of muscle coordination and strength in CMT1A.

Poster 94

Motor neuron pathology drives spinal circuit defects and phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1 (SMARD1)

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Little is known of the motor circuit pathology in spinal muscular atrophy with respiratory distress type 1, SMARD1, caused by the deficient protein IGHMBP2. Studies performed in the SMARD1 mouse model "Nmd2J" show motor neuron (MN) loss and neuromuscular junction (NMJ) denervation. However, it is uncertain if spinal synapses are affected and what drives degeneration.

We used immunofluorescence and confocal analysis to evaluate motor circuit pathology of Nmd2J mice by quantifying MN death, NMJ pathology and spinal synaptic loss. Mice were additionally treated perinatally with adeno-associated virus 9 to selectively restore IGHMBP2 in MNs or spinal proprioceptive synapses upon Cre recombinase induction to assess the cause of motor circuit pathology.

Comparisons of proximal and distal spinal motor circuits with associated muscles reveal MN death and muscle denervation peaked to ~70% at 6 weeks of age with 50% loss of proprioceptive synapses solely in distal circuits. Viral overexpression of IGHMBP2 selectively in MNs rescues the entire motor circuit pathology and behavior phenotype of Nmd2J mice. This novel genetic-viral cell type-specific approach demonstrates that MNs drive SMARD1 pathology. These findings lay the ground for identifying novel disease markers and candidate therapeutic targets for amelioration.

Poster 95

The role of Neuregulin-1 in the development of peripheral motor circuits

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Charcot-Marie-Tooth disease type 1A affects the peripheral nervous system (PNS) of around 150,000 people in Europe but is yet untreatable. To overcome this, we aim to understand the developmental pathological events causing dysmyelination and subsequent malfunction of peripheral nerves. We use a mouse model in which Neuregulin-1 (NRG1) is specifically deleted from motor neurons. This signalling protein is expressed in developing axons, where it is essential for the survival of Schwann cells (the myelinating cells of the PNS) and myelination. To understand how deletion of NRG1 in motor axons affected myelination, we analysed the ultrastructure of ventral roots and femoral nerves by electron microscopy. Although nerves containing purely motor axons (ventral roots) were unmyelinated, the mixed sensory-motor femoral nerve had higher myelination than expected and showed post-developmental delayed myelination. These results suggest that alternative signalling pathways might contribute to Schwann cell survival and myelination. We also investigated the role of NRG1 in the formation of C-boutons on the motor neuron, which regulate the neuron's firing rate. Although NRG1 is known to accumulate on the postsynaptic site, its role there is unclear. Using the same mouse model, we used immunohistochemical analyses to study the formation of C-boutons during development. Alterations in the number and size of C-boutons in our mutants imply that NRG1 signalling is essential for the correct spatial formation and stability of C-boutons.

Poster 96

Fully-primed slowly-recovering vesicles mediate presynaptic LTP at neocortical neurons

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Pre- and postsynaptic forms of long-term potentiation (LTP) are candidate synaptic mechanisms underlying learning and memory. A classical form of presynaptic LTP has been described for layer 5 pyramidal neurons. However, how this apparent increased release probability relates to recent advances in the understanding of priming of synaptic vesicles remains unclear. We therefore performed whole-cell recordings from layer 5 pyramidal neurons in acute cortical slices of rats combined with extracellular stimulation of local excitatory inputs and analyzed the presynaptic function before and after LTP- induction. LTP increased the EPSC amplitude in half of the synapses. In those synapses, LTP increased synaptic short-term depression and slowed the recovery from depression by adding a second slow component to the time course of recovery. Analysis with a recently established vesicle priming model indicates an increased number of fully-primed vesicles that recover slowly after depletion. To further test this hypothesis, we pharmacologically stimulated the cyclic adenosine monophosphate (cAMP) and diacylglycerol (DAG) pathway, which are both known to promote synaptic vesicle priming. Both interventions mimicked features of electrically-induced LTP. Our data show that LTP at layer 5 pyramidal neurons increases the synaptic strength primarily by enlarging a subpool of fully-primed slowly-recovering vesicles.

Poster 97

Characterization of molecular heterogeneity in glycinergic neurons in the mouse spinal cord

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Chronic pain poses a major challenge for medicine, as it affects a significant proportion of the population and has so far only been treatable to an unsatisfactory degree. Glycinergic neurons in the dorsal horn have been shown to contribute crucially to the processing of potentially painful stimuli, and disturbed glycinergic neurotransmission has been shown to result in facilitated pain perception. Therefore, this cell population represents a potential target for novel therapies for pathological pain. To design therapy strategies that target specifically glycinergic neurons, a precise characterization of this cell type is essential. In this study, we have established a protocol for the isolation of genetically labeled glycinergic cells from mice by fluorescence activated cell sorting and subjected these cells to single cell RNA sequencing for characterization. Initial analysis of 1813 single cell transcriptomes revealed that 1065 cells expressed both glycinergic and GABAergic markers, 66 cells could be identified as exclusively glycinergic, while the rest appeared to be purely GABAergic. Initial cluster analysis by the SPECTRA algorithm proposed the division of the obtained dataset into 24 groups, 2 of which being purely glycinergic. A significant proportion of the remaining groups contained transcriptomes of cells with mixed transmitter phenotype. Taken together our data demonstrates that glycinergic neurons in the mouse spinal cord show significant heterogeneity. The next steps will include the identification of unique marker sets to allow for a functional analysis.

Poster 98

Proprioceptive synapses degenerate at the same time as motor neurons in a mouse model of ALS

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Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron (MN) disease in adults. It is a progressive neurodegenerative disease leading to muscle atrophy, paralysis, and death. The causes of ALS are mostly sporadic, with only about 10% of cases being inherited. Since 1993, over 200 superoxide dismutase 1 (SOD1) gene mutations have been identified in ALS patients. Past studies have focused on different molecular pathways in MNs using transgenic ALS mouse models. However, other key features of spinal motor circuits (MCs), such as proprioceptive synapses (major excitatory sensory inputs unto MNs, modulating skilled tasks, and coordinated movements of the body) are still unknown. This study applied immunohistochemistry and confocal imaging to the SOD1-G93A mouse model to investigate the time-course relation of two major ALS hallmarks: synaptic proprioceptive and MN loss. We analyzed the lumbar segments L1 which mostly innervates proximal muscles and L4/L5 innervating distal muscles. Distal L4/L5 MCs showed MN loss as early as postnatal day 80, correlating with proprioceptive synapse degeneration in these spinal regions. Proximal L1 circuits, on the other hand, reveal significant proprioceptive synapse loss much earlier than MN loss. These findings highlight an overall vulnerability in MCs innervating distal muscles and more resistance in proximal MCs, coherent with the disease phenotype in ALS patients. Our results point out the importance of proprioceptive synapses in investigating fundamental mechanisms underlying MC pathology both in ALS mice and patients.

Poster 99

Importance of Liver X Receptors in peripheral nerve myelination

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Peripheral nerve development comprises a fine-tuned sequence of events characterized by a complex network of reciprocal interactions between peripheral nerve glial cells, the Schwann cells (SCs) and axons. During the development of nerves, SCs enwrap axons by a process termed as myelination, thereby ensuring rapid and accurate impulse conduction [1]. Genetic or acquired defects that affect SC development and integrity give rise to peripheral neuropathies, common neurological diseases, characterized by invalidating sensory and motor symptoms. For the majority of peripheral neuropathies, no therapies are available. In this study, we identified an essential novel role for the Liver X Receptor β (LXRβ) [2], in early SC development. Indeed, when we conditionally deleted LXRβ specifically in Schwann cells, peripheral nerves showed a developmental arrest at early embryonic stages and dramatically reduced SC numbers along with a complete absence of myelinated axons. Consequently, mutant mice developed a severe limb paralysis and died prematurely at the age of 7 months. Elucidation of the consequences of LXRβ ablation from SCs at different developmental stages in mice will strongly aid our understanding of peripheral nerve development in the context of glial differentiation and survival as a basis for axonal support and peripheral nerve function.

Keywords: Peripheral nervous system, myelination, Schwann cells, neuropathies, LXR

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Poster 100

Histone deacetylase class I – inhibition leads to phenotypic and functional changes in peripheral CD4+ T cells comparable to CD4+ T cells in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation affecting the synovial membrane of the joints [1][2]. Autoantigen-reactive $CD4^+$ T cells play a pivotal role by contributing to pro-inflammatory milieu in RA [3]. We have previously shown a $CD4^+$ T cells population that upregulate CD8α and contribute to increased disease activity [4]. Epigenetic changes can induce the expression of CD8 lineage genes in $CD4^+$ T cells [5]. We suggest that modulation of HDAC class I in particular leads to the phenotype of RA $CD4^+CD8\alpha^+$ T cells.
We could show an increased frequency of $CD4^+CD8\alpha^+$ T cells in peripheral blood of RA patients (n=22; 4.27%) in comparison to healthy donors (HD) (n=21; 1.82%). Using an in-vitro assay, naïve $CD4^+$ T cells from peripheral blood of HD (n=5) were cultured for 3d under Th0 polarizing conditions and HDAC class I were inhibited for 24h. Afterwards, phenotypic and functional changes of $CD4^+$ T cells were investigated by flow cytometry. Our results demonstrate that inhibition of HDAC class I increases the expression of SLAMF7 (+12.66%) and CD8α (+1.67%) on the surface of $CD4^+$ T cells. In contrast, ThPOK (MFI= -70), CD27 (MFI= -3444.7), and CD40L (-17.74%) are downregulated.

In summary, HDAC inhibition in HD resulted in increased expression of cytotoxic markers on $CD4^+$ T cells and concomitant inhibition of CD4 lineage markers, which is comparable to RA $CD4^+$ T cells. This suggest that a dysregulation of HDAC class I expression/activity in RA is responsible for differentiation to pro-inflammatory peripheral $CD4^+CD8\alpha^+$ T cells.

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Poster 101

Characterization of the human humoral immune response against adeno-associated virus 2 (AAV2)

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Adeno-associated virus 2 based gene therapies (AAV2-GTx) have many benefits as long-term expression and broad tissue-tropism but deal with some therapy-limiting factors, as pre-exposure to wildtype AAV2 (estimated for up to 90 %), that excludes patients from AAV2-GTx. Pre-exposure to AAV2 results in the formation of pre-existing neutralizing antibodies (nAb) against the AAV2 capsid which minimize the therapeutic effect and might lead to severe immune reactions. Structural characterization of AAV2 epitopes targeted by nAbs therefore is essential for further capsid engineering to escape the neutralization in AAV2-GTx.

Here, we characterized the human humoral immune response by detection of anti-AAV2 titers in random blood donors and analysis of AAV2-specific memory B cells in these. We find that only 5 of 22 samples showed anti-AAV2 specific IgG titers. We optimized stability and affinity of an anti-AAV2 antibody (A20) through computational design for the following sorting experiments. The identification of AAV2-specific cells was established in fluorescence-activated cell sorting (FACS) with A20-Fab expressing cells. Afterwards, 0.01 % of the human memory B cells from anti-AAV2 positive donors were found to be AAV2-specific in FACS. We plan to sequence heavy and light chain of these using 10X genomics. In conclusion, our study will reveal epitopes that might be targeted by nAbs and thus, inform AAV capsid engineering and design in the future.

Poster 102 Computational design of epitope-scaffolds targeting pan-coronavirus neutralizing antibody lineages

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In the past 20 years, three coronaviruses have spilled over from animals to humans, with the SARS-CoV-2 virus causing devastating interruptions to global health and economics. To prevent future pandemics, a pan-coronavirus vaccine is urgently needed. Despite the large diversity of coronaviridae, a broadly neutralizing antibody, termed 76E1, has recently been described that neutralizes most α-, β-, γ- and δ-coronaviruses. 76E1 is a antibody that recognizes a well-conserved epitope in the fusion peptide of coronavirus spike proteins. The epitope is partially buried in the prefusion spike trimer, but accessible upon receptor binding during the transition to the postfusion state. 76E1 has been reported to provide sterilizing immunity in passive immunization studies, but induction of 76E1-like antibodies by vaccination is an unsolved vaccine design challenge [1]. Here, we designed epitope-scaffolds by grafting the SARS-CoV-2 fusion peptide in the 76E1-bound conformation onto a library of small thermostable protein backbones using the Rosetta sidechain grafting protocol. Well-scoring designs were further optimized in vitro using directed evolution by yeast cell surface display. We obtained several epitope-scaffolds with high affinity to 76E1. The best binding epitope-scaffold was further optimized through the introduction of glycans outside the target epitope to limit the elicitation of potentially competing off-target antibodies. This epitope-scaffolds is the lead candidate for the development of a vaccine that induces 76E1-like pan-coronavirus neutralizing antibodies.

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Poster 103 Characterization of NLRP3 Inflammasome Activation in Patients with Cardiogenic Shock

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Background:
Cardiogenic shock (CS) is a heterogeneous syndrome associated with high mortality. To improve these outcomes, tailored therapies and the identification of subgroups are needed. In this regard, systemic inflammation is of increasing interest.

Methods:
We collected serum (n=44) and PBMCs (n=7) of patients with CS at admission and 24, 48, and 72 hours post-revascularisation as well as of healthy controls (n=75 serum, n=12 PBMCs).

Results:
Gene expression of NLRP3, CASP1, ASC as well as IL1B and IL18 are consistently increased in patients with CS. We further found increased time-dependent pyroptotic cell death in CS patients with highest level 48h post-revascularisation (control 4.7% vs. CS 12.7%, p=0.004) and increased caspase-1 activity in PBMCs 24h post-revascularisation (1.7 folds over control, p=0.002). IL-1ß was also increased 24h post-revascularisation (control 0.0 pg/ml vs. CS 0.2 pg/ml, p=0.002). In serum of CS patients, ASC specks were increased and accumulate post-revascularisation (at admission 11.1 specks/µl vs. 24h post-revascularisation 19.0 specks/µl, p=0.02). Also, we saw a transient increase in circulating inflammatory particles Ex vivo. Especially MCC950, a NLRP3 inhibitor, was able to inhibit ASC speck release, indicating that this process is NLRP3 inflammasome dependent.

Conclusion:
Our data suggest that NLRP3 inflammasome activation is involved in the pathogenesis of CS. This adds a new additional parameter of inflammatory activity in CS patients and may be used to tailor anti-inflammatory therapeutic strategies to improve outcome for CS patients.

Poster 104

How specific is the Ureaplasma-driven impairment of fetal alveolar epithelial Na^+ transport?

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Background:
Ureaplasma infection is related to preterm birth and chronic lung disease in preterm infants. The mechanism of Ureaplasma-driven lung disease is mainly unknown. Epithelial Na+ channel (ENaC)-driven alveolar fluid clearance (AFC) is essential for perinatal lung transition to air breathing, a challenge for preterm infants. We previously demonstrated that Ureaplasma urealyticum infection decreases ENaC activity in fetal distal lung epithelial (FDLE) cells, impairing AFC.

Objective:
The present study aims to determine how specific the effect of Ureaplasma on ENaC activity is. We analysed, if lipopolysaccharide (LPS), mimicking gram-negative bacterial infection, also impairs ENaC activity.

Methods:
Rat FDLE cells were treated with Ureaplasma urealyticum serovar 8 (Uu8), the U parvum serovar 3 (Up3), or LPS (0.1 and 1 µg/ml) and incubated for 3 or 24 hours. ENaC activity was determined in Ussing chambers.

Results:
Compared to uninfected controls, the Uu8-infected FDLE cells showed a strongly decreased ENaC activity ($p<0.01$) after 24 h, as shown before. In accordance, Up3 also reduced Na+ transport and ENaC activity ($p<0.01$), excluding a serovar-specific effect. In contrast, LPS significantly increased ENaC activity ($p=0.02$) after 24 h. Short-term incubation of FDLE cells with either Uu8 or LPS for 3 h did not affect ENaC activity.

Conclusion:
Our findings indicate that LPS exhibits an opposing effect on ENaC activity compared with Ureaplasma. We, therefore, suggest that the impairment of ENaC is specific to Ureaplasma, contributing to preterm lung disease in colonized infants.

Poster 105

Distinct characteristics of novel immunoregulatory canine non-conventional TCRαβ+CD4-CD8α- double-negative T cells

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Conventional $CD4^+$ $FoxP3^+$ regulatory T cells (Tregs) are crucial for maintenance of immune homeostasis. Within non-conventional $TCR\alpha\beta^+CD4^-CD8\alpha^-$ double-negative (dn) T cells of dogs, we recently described a $FoxP3^+$ Treg-like subset which, similar to conventional $CD4^+$ Tregs, is characterized by high surface expression of CD25. Noteworthy, human and murine $TCR\alpha\beta^+$ regulatory dn T cells lack FoxP3.

To verify regulatory function of canine non-conventional dn T cells, the $dnCD25^+$ (enriched for FoxP3) and the $dnCD25^-$ fraction were isolated from peripheral blood and analyzed in an in-vitro suppression assay in comparison to conventional $CD4^+CD25^+$ Treg and $CD4^+CD25^-$ cells. Of note, canine $dnCD25^+$ T cells suppress the proliferation of responder cells similarly as conventional $CD4^+CD25^+$ Tregs. Albeit to a lesser extent, $dnCD25^-$ T cells are also suppressive. This is remarkable, as they have a comparable FoxP3neg phenotype like non-suppressive $CD4^+CD25^-$ T cells. Both, $CD25^+$ and $CD25^-$ dn T cells are able to mediate suppression cell-cell contact independently and, in contrast to $CD4^+CD25^+$ Tregs, do not need signals from $CD4^+CD25^-$ T cells to secrete inhibitory factors. Neutralization of IL-10 completely abrogates the suppression by $dnCD25^+$ and $CD4^+CD25^+$ Tregs in a TranswellTM system, while it only partially reduces the effects of $dnCD25^-$ cells.

Taken together, canine $TCR\alpha\beta^+$ dn T cells are potent suppressor cells in vitro even in the absence of FoxP3. Their immunoregulatory capacity is of high relevance, given the therapeutic potential of manipulating regulatory T cell responses in vivo.

Poster 106

IgM B cell receptor (BCR) cleavage by the immunoglobulin M-degrading enzyme of Streptococcus suis (Ide_{Ssuis}) impairs in vitro IgM B cell activation and leads to longer lasting impairment of B cell signaling.

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Introduction:

Streptococcus suis (S. suis) is an important porcine pathogen. Disease mostly occurs in piglets after weaning, but S. suis is also a very successful colonizer of mucosal surfaces in pigs. The cysteine-protease IdeSsuis specifically cleaves porcine IgM (1). In our previous study, we could also demonstrate cleavage of the IgM BCR and immediate impairment of IgM BCR signaling (2).

Objective:

This study tested the working hypothesis that cleavage of the IgM BCR results in an impaired IgM B cell function even after a 3-day period.

Material and Methods:

After initial treatment for 45 min with different rIdeSsuis-derived mutants, porcine PBMC were activated by TLR7/8 ligand R848 for three days and the frequency of IgM-producing B cells was investigated by ELISpot. BCR signaling after stimulation with pervanadate or an anti-IgM antibody was analyzed by flow cytometry.

Results:

Treatment with rIdeSsuis decreases the number of IgM secreting cells and results in a long-term inhibition of both BCR-independent and BCR-dependent activation of intracellular signaling.

Discussion and conclusion:

This study demonstrates that IgM BCR cleavage not only results in immediate but also in longer lasting interference with B cell function. These findings suggest modulation of antigen-dependent B cell responses by S. suis through IdeSsuis expression. Further studies are warranted to prove that modulation of B cell function by IdeSsuis contributes to infection and colonization.

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Poster 107

Interspecies analysis to dissect transcriptomic signatures of humans and hamsters in COVID-19

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Hamster species are particularly valuable for studying host-pathogen interactions in coronavirus disease 2019 (COVID-19) because they develop moderate (Mesocricetus auratus) or severe (Phodopus roborovskii) disease courses after infection. By analyzing blood leukocyte single-cell RNA sequencing datasets from hamsters along with publicly available datasets from COVID-19 patients, we identified transcriptional similarities between hamsters and humans in the early phase of SARS-CoV-2 infection. Both Mesocricetus auratus and Phodopus roborovskii showed highest transcriptional changes 2 days after infection. Inflammatory-associated genes showed highest expression levels amongst blood leukocytes of Phodopus roborovskii infected with 1 x 105 plaque forming units (pfu) SARS-CoV-2. Phodopus roborovskii infected with a lower dose of 1 x 104 pfu SARS-CoV-2 showed a less critical clinical outcome with fewer inflammatory genes expressed by classical monocytes. Further, our analysis comprised a variational autoencoder pipeline matching temporal disease states of hamsters with human COVID-19 severity ranks for individual cell types which was complemented by transcriptome-wide differential gene expression and pathway enrichment analysis. In SARS-CoV-2-infected Mesocricetus auratus, our results indicate a prominent involvement of monocytes, while our findings suggest a stronger involvement of neutrophils in SARS-CoV-2-infected Phodopus roborovskii.

Poster 108 Immunoproteomics – unbiased identification of new Aspergillus fumigatus antigens in severe equine asthma

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Severe equine asthma (SEA) is a common, chronic obstructive airway disease of adult horses. Aspergillus fumigatus (Asp f) is a common mold species in hay and has been described as a major extrinsic provoking agent of SEA, but single inducing molecules have not been identified comprehensively.

We explored Asp f as a source of candidate antigens in an immunoproteomics approach. Proteins of Asp f were extracted, separated by two-dimensional gelelectrophoresis, and immunoreactivity was tested using healthy and asthmatic horses´ serum (n= 5 each) on immunoblots. All bound serum antibodies (Pan-Ig), and the isotypes IgG4/7 (Th1-associated) and IgG3/5 (Th2-associated) were quantified by fluorescent detection.

Significant differences between antibody binding of healthy and asthmatic horses' sera were identified for several protein spots. IgG3/5 binding was higher with asthmatic horses´ sera, but Pan-Ig and IgG4/7 binding were lower with asthmatic horses´ compared to healthy horses´ sera. These spots were selected for analysis by mass spectrometry to identify the individual protein content. Of these, eight candidates were recombinantly expressed in E. coli for confirmation. Four rAsp f proteins resulted in significant Ig binding differences of asthmatic and healthy horses´ sera, which were confirmed and described as relevant antigens for the first time.

Defining specific Asp f proteins as immunoreactive antigens can form the basis for further analysis of adaptive immune responses. These might indicate the type of pathogenesis, and eventually enable specific immunotherapy in SEA.

Poster 109 IDO inhibition in a murine sepsis model

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Introduction.

In sepsis, the expression of indoleamine-2,3-dioxygenase 1 (IDO1) is upregulated and its activity correlates with the degree of hypotension and mortality. This enzyme catalyzes the conversion of tryptophan to kynurenine, a proposed systemically active vasodilator. This study aimed to evaluate the effects of IDO1 inhibitors on sepsis.

Methods.

We first assessed the IDO1-inhibitory effects of Epacadostat (Epa), Linrodostat (Lin) or PF-06840003 (PF) on in vitro human pulmonary arterial endothelial cells challenged with IFN-γ and measured the secreted kynurenine.

We then evaluated the inhibitors in a lipopolysaccharide (LPS)-based murine sepsis model: Hemodynamics were invasively monitored, IDO1 expression in lung tissue and tryptophan metabolites in serum were measured using Western Blot and HPLC, respectively.

Results.

All three IDO1 inhibitors suppressed kynurenine production in the inflammatory arterial endothelial cell model. In mice, LPS led to a reduced mean arterial blood pressure (MAP; 66±15 mmHg vs 86±11 mmHg) and an elevated heart frequency (HF; 551±55/min vs 367±81/min). Administration of Epa or Lin attenuated the decrease in MAP (85±8mmHg, 96±11mmHg) but showed no effect on HF. The up-regulation of IDO1 expression in lung tissue after LPS administration was confirmed. Serum of septic mice showed a 10-fold increase in the kynurenine/tryptophan ratio which was attenuated by all three inhibitors (Epa: 3.4-fold, Lin: 4.7-fold, PF: 4.3-fold).

Conclusion.

Our findings identify IDO1 inhibition as a therapeutic strategy to manage sepsis-associated hypotension.

Poster 110 Directed evolution of multidrug-resistant strains of Klebsiella pneumoniae

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Background

Multidrug resistant bacteria and particularly carbapenem-resistant enterobacterales such as Klebsiella pneumoniae can be considered an urgent public health threat. Klebsiella pneumoniae causes hospital acquired infections every year. Strategies to prevent the development of resistance against antibiotics require a better understanding of the underlying mechanisms. To address escape mechanisms in Klebsiella pneumoniae, we performed directed evolution through cultivation in the presence of increasing concentrations of the antibiotics and through CRISPR-Cas-based gene editing.

Methodology and Results

In addition to Klebsiella pneumoniae wild-type strains we employed a Klebsiella pneumonia strain that is resistant against commonly used antibiotics (4MRGN) but susceptible towards Polymyxin B. We performed directed evolution through cultivation of these strains in the presence of Polymyxin B concentrations that increase by square root 2 times during passages. Genome sequencing was performed during defined time points of the passaging experiments.

To simplify the directed evolution experiments, a CRISPR-Cas9-mediated knockout of the mismatch-repair-gene mutL was used. Gene knockout enables directed evolution experiments in comparison to wild-type strains with regard to the time required to obtain polymyxin B resistance and to associated mutations that have occurred.

Conclusion

We validated a protocol to systematically search for resistance mechanisms that could be developed by bacteria in vivo and that can be used with various bacteria-antibiotic-combinations in the future.

Poster 111

Immunoproteomics of Aspergillus fumigatus, candidate antigen search in severe equine asthma

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Severe equine asthma (SEA) is an immune-mediated respiratory disease in horses characterized by neutrophilic inflammation that is similar to neutrophilic asthma in humans. The SEA phenotype is based on hyperreactivity to hay dust components, e. g. molds. Our study proposes a bottom-up approach of immunoproteomics to identify causative antigens of the mold Aspergillus fumigatus (Asp. f.) for SEA. We have compared antibody (immunoglobulin, Ig) binding patterns to Asp. f. proteins between samples (serum and BALF) from six horses with SEA and from six healthy controls on two-dimensional immunoblots. A culture of Asp. f. was prepared as a source of protein. The Asp. f. proteome was prepared for two-dimensional electrophoresis (2-DE) and then transferred to a nitrocellulose membrane. Then, serum or BALF was applied and Ig bound to proteins was detected quantitatively using fluorochrome-conjugated secondary antibodies. We found different patterns of Ig binding in healthy and asthmatic horses on immunoblots indicating the presence of disease-associated antigens. Subsequently, through this study, proteins of interest from the Asp. f. proteome will be identified by mass spectrometry, expressed as recombinant proteins, and analyzed extensively in relation to equine asthma. The characterization of asthma-associated specific immune responses can improve our understanding of neutrophilic asthma and enable new therapeutic approaches using the horse as a model for human asthma.

Poster 112

The Role of Seasonal Coronaviruses and Human Leukocyte Antigen in the individual Immune Response to SARS-CoV-2

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During the coronavirus pandemic, evidence is growing that the severity, susceptibility and host immune response to SARS-CoV-2 infection is highly variable. Here, we investigated the humoral immune response against SARS-CoV-2 spike, S1, S2, the RBD, nucleocapsid moieties and S1 of seasonal coronaviruses: hCoV-229E, hCoV-HKU1, hCoV-NL63 and hCoV-OC43, as well as MERS-CoV and SARS-CoV, in a cohort of 512 individuals comprising 334 females and 178 males. A bead-based multiplex assay allowed simultaneous testing for all the above antigens and the identification of different antibody patterns. In total 737 sera were tested against several proteins of the virus and six seasonal coronaviruses. Then, we correlated these patterns with 11 HLA loci. Regarding the seasonal coronaviruses, we found a moderate negative correlation between antibody levels against hCoV-229E, hCoV-HKU1 and hCoV-NL63 and the SARS-CoV-2 antigens. This could be an indication of the original immunological imprinting. High and low antibody response patterns were distinguishable, demonstrating the individuality of the humoral response towards the virus. An immunogenetical factor associated with a high antibody response (formation of ≥4 different antibodies) was the presence of the alleles: HLA A*26:01, C*02:02 and DPB1*04:01, whereas the HLA alleles DRB3*01:01, DPB1*03:01 and DB1*10:01 were enriched in low responders. A better understanding of this variable immune response could enable more individualized protective measures.

Poster 113 Cytokine Polymorphism in SARS-CoV2 affected households

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We addressed the question of the influence of molecular polymorphism of cytokines from different T-helper subsets on the susceptibility to SARS-CoV-2 infection. The helper T cells are divided into categories according to their action on immune response: the more pro-inflammatory helper T cells 1 (Th1: TNF-α, IFN-γ), the anti-inflammatory (Th2: IL-10) cells, and the more regulatory Th17 (IL-17, TGF-β1) and Treg (TGF-β1) cells. From a cohort of 527 samples (May 2020 to March 2022), we focused on individuals living in same household (n=58) with the SARS-CoV-2-infected person. We divided them into households with all individuals SARS-CoV-2 PCR positive (n=29 households, 61 individuals), households with mixed PCR pattern (n=24, 62) and negative households (n=5, 15), respectively. TGF-β1 and IL-6 were the only cytokines tested with a significant difference between the cohorts. We observed a shift toward Th2 and the regulatory Th17 and Treg subset regulation for households with all members infected compared to those without infection. These data indicate that the genetically determined balance between the cytokines acting on different T helper cell subsets may play a pivotal role in transmission of and susceptibility to SARS-CoV-2 infection. Contacts infected by their index persons were more likely to highly express TGF-β1, indicating a reduced inflammatory response. Those not infected after contact had polymorphism leading to a higher IL-6 expression. IL-6 acts in innate immunity, allergy and on T helper cell differentiation, explaining the reduced susceptibility to SARS-CoV-2.

Poster 114

Automatic Scoring Of Memory Content In Autobiographical Interviews Via Large Language Models

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Applying machine learning to the neuroscientific and psychological analysis of human memory, this study uses fine-tuned language models to automate the scoring of narratives in the Autobiographical Interview, a widely used tool for assessing both autobiographical memory [1] and episodic foresight [2]. Traditional scoring of these interviews is a meticulous and manual process. Experts painstakingly analyze and categorize narrative elements based on established criteria to quantify detail recovery. However, this method is labor-intensive, inherently subjective, and difficult to scale to larger datasets. Our research aims to evaluate whether large language models can provide an efficient, objective, and scalable alternative, while maintaining the integrity and depth of the traditional manual scoring process.

Methods:
Automated scoring systems were developed by fine-tuning variants of the deBERTa V3 transformer model [3] on a training dataset of 2107 manually scored, transcribed Autobiographical Interview narratives. Comparative performance analyses were conducted on a hold-out dataset of 264 narratives.

Results and conclusions:
The fine-tuned models showed a strong Pearson correlation (R=.85) and a high intraclass correlation coefficient (ICC=.9) compared to traditional manual scoring methods. These results confirm the utility of fine-tuned language models in automating the assessment of autobiographical memory and foresight, thereby streamlining research methodologies in neuroscience and psychology.

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Poster 115

BioTIP: Bio-Signal Retrieval from Thermal Imaging Processing via Face Detection and Nose Landmark Estimation.

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The Bio-TIP project focuses on thermal imaging processing to retrieve physiological signals such as heart rate, breath rate and cognitive load from human and non-human animals. We are building a set of computer vision tools for psychologists and animal behaviour researchers to retrieve bio-signals non-invasively. We aim to increase the use of thermal imaging modality in the community and avoid using more invasive recording methods to answer research questions. We built the ApeTI dataset: a Thermal Image Dataset for Face and Nose Segmentation with Apes based on chimpanzee recordings with their face and nose annotated. This dataset is used to evaluate chimpanzee face segmentation using thermal images. Several computer vision methods have been tested and evaluated on this dataset. Our proposed combination of the Tifa and Tina models, inspired by the HRNet models [1], performs the best for face detection and landmark estimation. We reach mAPs of 0.74 for face detection and 0.98 for landmark estimation.

We are also currently building a dataset with humans being filmed while cycling in a controlled environment. The RGB and thermal streams, and heart and breath rates are synchronously recorded in order to evaluate methods based on RGB and/or thermal information to retrieve physiological signals. We are currently investigating different methods to retrieve the heart and breath rates from an automatic segmentation of the face using only the thermal modality.

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Poster 116

Semantic interference effects on event-related brain potentials and pupil dilation during blocked-cyclic picture and sound naming

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Blocked-cyclic picture naming is a task frequently used in speech production research. In this task, participants repeatedly name pictures that are presented either sorted by semantic category (homogeneous context) or intermixed (heterogeneous context). Naming latencies are typically slower in the homogeneous context. Wöhner et al. (2021, JEP:HPP) showed that this interference effect is substantially larger for sound naming compared to picture naming. The authors suggested that this difference is due to early conceptual processes, because participants produced the same words in picture and sound naming so that lexical and later processing stages should be identical. We explored the differently sized interference effects using EEG and pupillometry. We predicted an enhanced ERP effect for sounds at early time windows. We found that semantic interference was associated with a centrally distributed positive ERP component that occurred between 140-180 ms and 250-350 ms for pictures and between 250-350 ms for sounds. Critically, the ERP effect was not larger for sounds than for pictures. However, there was a larger frontal negativity for sounds between 400-600 ms. The timing of the effects indicates that the difference in the behavioral effect arises at a later processing stage, close to articulation. The pupillometric data showed a stronger pupil dilation in the homogeneous context for sound naming, but not for picture naming. This indicates that sound naming might involve an additional process, self-monitoring being a likely candidate.

Poster 117

Emotional (In)stability: Neuroticism is Associated with Increased Variability in Negative Emotion After All.

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In everyday life, our emotions can change from moment to moment, and people experience such fluctuations to varying degrees. Psychologists have puzzled over the role that the personality trait neuroticism–a potent risk factor for mental illness–plays in such emotional variability. Do neurotic individuals experience not only stronger negative emotions but also more variability? This question resulted in controversy because it is methodologically challenging to separate effects of neuroticism on mean emotion from effects on variability. We suggested a new modeling approach to address the methodological issues, tested its performance on simulated data, and then re-investigated a total of 13 longitudinal data sets (2,518 individuals and 11,170 measurements in total). Findings suggest that more neurotic individuals indeed experience more variability in negative emotion in everyday life.

Poster 118

Involuntary attentional capture induced by the unexpected omission of sounds

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Salient unexpected and task-irrelevant sounds can act as distractors by capturing attention away from a task. Consequently, a performance impairment (e.g., prolonged response times, RTs) is typically observed along with a pupil dilation response (PDR) and the P3a event-related potential (ERP) component. Previous results showed prolonged RTs in response to task-relevant visual stimuli also following unexpected sound omissions. However, it was unclear whether this was due to the absence of the sound's warning effect or to distraction caused by the violation of a sensory prediction. In our paradigm, participants initiated a trial through a button press that elicited either a regular sound (80%), a deviant sound (10%) or no sound (10%). Thereafter, a digit was presented visually, and the participant had to classify it as even or odd. To dissociate warning and distraction effects, we additionally included a control condition in which a button press never generated a sound, and therefore no sound was expected. Results show that, compared to expected events, unexpected deviants and omissions lead to prolonged RTs (distraction effect), enlarged PDR, and enlarged P3a. Moreover, sound events, compared to no sound events, yielded faster RTs (warning effect), larger PDR and increased P3a. Overall, we observed a co-occurrence of warning and distraction effects. This suggests that not only unexpected sounds, but also unexpected sound omissions can act as salient distractors. This finding supports theories claiming that involuntary attention is based on prediction violation.

Poster 119

Inhibition and working memory predict rhythm production abilities in patients with neurocognitive deficits

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Deficits in rhythmic abilities have been reported in a variety of disorders. In neurotypical individuals, correlations between rhythmic abilities and cognitive functions have been demonstrated. Here, we investigate whether and how rhythmic abilities are associated with cognitive functions in 35 participants with neurocognitive deficits due to acquired brain lesions. We systematically assessed a diverse set of rhythm perception abilities, rhythm production abilities, and cognitive functions. Multiple regression analyses revealed that lower variability in aligning movements to a pacing sequence was predicted by better inhibitory control and better working memory performance. Also, lower variability of rhythmic movements in the absence of an external pacing sequence and better anticipatory timing to sequences with gradual tempo changes were predicted by better working memory performance. Importantly, all predictors remained significant when controlling for other cognitive variables (i.e., cognitive flexibility, information processing speed, and verbal learning ability) and possible confounding variables (i.e., age, symptom strength of depression, manual dexterity, duration of illness, severity of cognitive impairment, and musical experience). Sub-second time perception and beat perception were not predicted by executive functions. Enhancing the understanding of cognitive underpinnings of rhythmic abilities in individuals with neurocognitive deficits may help to further potential therapeutic implications of rhythm-based interventions in neuropsychological rehabilitation.

Poster 120 On the relevance of alpha rhythm to the generation of readiness potential

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A preparation for movement manifests itself in the electroencephalogram (EEG) as a slow cortical potential. This potential, termed the readiness potential (RP), was discovered more than 50 years ago and has since received attention both in cognitive science and philosophy. A less known indicator of movement preparation is the decrease in alpha rhythm amplitude. Since both processes occur before movement, we hypothesise that RP and alpha rhythm may be related. Namely, alpha rhythm could generate RP via the baseline-shift mechanism (BSM). BSM links evoked potentials and oscillations into a single framework, such that, when oscillations have an asymmetric amplitude and the amplitude is changing, this change should be accompanied by an evoked potential. In the current study, we analysed data from 42 participants performing Libet's task. We show that dynamics and localization are similar, although not identical, for RP and alpha rhythm amplitude. The negative RP has a steady evolution over two seconds preceding the movement, while the decrease in alpha amplitude is more pronounced right before the movement. However, the alpha amplitude change is significantly correlated with RP in several cortical regions based on linear and linear-mixed effect models. Spatially, both processes overlap, but the alpha amplitude decrease appears to be more lateralized. Lastly, the degree of asymmetry in the alpha amplitude at rest predicts the topography of RP. We suggest that demonstrating a link between RP and alpha rhythm will help to explain the neurophysiological and functional role of RP.

Poster 121

N1 suppression for self-generated sounds is unaffected by predictability of sound identity and occurrence

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Recent theories describe perception as an inferential process based on internal predictive models that are adjusted by prediction violations (prediction error). Two modulations of the auditory N1 event-related brain potential component have been interpreted as reduced or enhanced prediction error for predictable sensory input: The sound-related N1 component is attenuated for self-generated sounds compared to the N1 elicited by externally generated sounds (N1 suppression). An omission-related component in the N1 time-range is elicited when the self-generated sounds are occasionally omitted (omission N1). Interestingly, in a previous study we did not observe a modulation of N1 suppression by manipulating the predictability of sound occurrence, but a modulation of omission N1. Here, we wanted to confirm that both N1 suppression and omission N1 are sensitive to the predictability of sound identity, as reported in the literature. We manipulated the predictability of sound identity in a self-generation paradigm in which button presses in one condition always produced the same sound or in another condition produced a sound randomly selected from a large set of sounds, thereby inducing a strong or a weak expectation for a specific sound. Surprisingly, omission N1 was modulated by manipulating the predictability of sound identity but not N1 suppression. This contradicts previous reports, further challenges prediction-related interpretations of the N1 suppression and supports alternative explanations like action-related unspecific suppression of sensory processing.

Poster 122

Prevalence of Emotional Dysregulation (ED) in Adult ADHD at a Specialized outpatient Clinic for Adult ADHD, Clinical Correlates, and Implications for Assessment and Treatment

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Attention-deficit/hyperactivity disorder (ADHD) is a chronic disorder with onset in childhood characterized by attention deficit, impulsivity and hyperactivity. The prevalence in Germany is estimated at 4.7% [1]. It has been shown that 34-70% of people with ADHD in adulthood (aADHD) have difficulties with emotional regulation [2]. ED is generally regarded as a multidimensional construct, characterized by a lack of inhibition of behavior together with strong negative and positive emotions and the failure of self-regulation [3]. To date, ED has not been taken into account in clinical diagnostics. However, it is currently assumed that ED is a distinctive symptom in people with ADHD. Given that the ED presentation type is more frequently associated with comorbid disorders and responds less well to guideline-based treatment, we examined the prevalence of the ED presentation type and the frequency of comorbid disorders between ADHD types in the patient population.

The data were collected retrospectively from the medical records of a specialized outpatient clinic for aADHD. The Wender-Reimherr Interview (WRI) was used as a target variable to differentiate between the inattentive and emotionally dysregulated types of aADHD.

Results on population characteristics and their relationship to clinical correlates and associated sociobiographical factors are presented.

A future classification into the inattentive and emotionally dysregulated type in clinical diagnostics could be useful in order to be able to implement specific diagnostics and treatment of the subtypes at an early stage.

Poster 123 The parental brain in action: an emotional facial expressions task

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The parent-child relationship is pivotal for children‘s emotional and social development, relying on parents‘ ability to respond effectively to children’s needs, often communicated through facial expressions. Child faces constitute a unique category of highly salient stimuli that trigger nurturing behaviors and activate parental brain neural correlates. Previous research, primarily with mothers of infants, highlights regions within the reward, saliency, and Theory of Mind (ToM) networks as central to the parental brain.

In this pre-registered fMRI study, we sought a more comprehensive understanding of the parental brain‘s response to emotional facial cues, particularly in primary school-aged children, rather than infants. Our investigation included 89 parents, with a larger proportion of fathers than mothers, and equal proportions of daughters and sons. We examined effects of facial identity and emotion on parental neural activity. Moreover, we tested for effects of gender and parental sensitivity and/or involvement on parental brain acitvity.

Our whole-brain analyses revealed greater neural activity, specifically within the saliency and ToM networks, for viewing images of one’s own child as compared to an unknown child. Notably, no neural differences were observed between mothers and fathers during our whole-brain analyses, but our ROI analyses, which took child gender and caregiving behaviors into account, revealed distinct father-daughter interaction. Fathers of daughters exhibited with increasing caregiving sensitivity, less ventromedial prefrontal cortex recruitment.

Poster 124

Physiological underpinnings of generalizing representations of parents to peers: The role of maltreatment

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This project aims to dismantle the physiological underpinnings of how early caregiving experiences may guide social interactions with unfamiliar individuals during adolescence. During such interactions, evidence suggests that individuals may selectively draw on their parental representations as guides, but only to the extent that the target actually resembles them (Andersen & Chen, 2002). As part of a longitudinal research project, maltreated (n=45) and non-maltreated (n=63) adolescents played Cyberball (Williams et al., 2000) with two characters who were supposedly connected online: One of these (Target) was introduced to the participant as personality-wise resembling one of their parents (masked resemblance), and the other one constituted the Control. The overarching aim of the study was to illustrate that subjects would exhibit differential responses to these two co-players, indicated by their immediate transient heart rate responses (e.g. cardiac slowing), and that these responses would index maltreated and non-maltreated subjects' expectations from the Target vs the Control in the context of social inclusion and exclusion. Preliminary findings show that maltreated, but not non-maltreated, subjects display extended cardiac slowing patterns towards the Target vs Control during inclusion. We interpret this as heightened saliency of the Target due to the generalization of parental representations. These findings highlight the role of internal representations in guiding social interactions once more by showing for the first time how they operate in the physiological domain.

Poster 125

Oral health-related quality of life in adult patients with Depression or Attention Deficit Hyperactivity Disorder (ADHD)

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Objectives:
This cross-sectional study evaluated the oral-heath-related quality of life (OHRQoL) of patients suffering from affective disorders or ADHD.

Subjects and Methods:
In- and outpatients from the Department of Psychiatry, University of Leipzig, Germany were recruited. Moreover, a comparison group (HC) of mentally healthy individuals was recruited from the Department of Cariology, Endodontology and Periodontology. OHRQoL was assessed using the Oral Health Impact Profile G14 (OHIP G14). Furthermore, a questionnaire regarding oral hygiene behaviour was applied to the psychiatric group.

Results:
141 patients with psychiatric disorders (affective disorders n=94, ADHD n=47) and 145 HC individuals with a comparable age and gender distribution were surveyed. OHIP G14 median scores were significantly higher in the overall psychiatric group compared to HC (5.00 vs. 0.00, $p<0.001$). This was also found for the four dimensions of OHIP G14 ($p<0.001$). The OHIP G14 sum score of patients with affective disorders was comparable with ADHD (5.00 vs. 6.50, $p=0.302$). A significant association was found among psychiatric patients between smoking, gum bleeding, professional tooth cleaning, oral health education, interdental cleaning, and elevated OHIP scores ($p<0.001$).

Conclusion
Individuals with affective disorders and adults with ADHD show reduced OHRQoL. Interdisciplinary collaboration between psychiatrists and dentists should be fostered in order to understand and improve oral health behaviour as a facet of mental and overall health.

Poster 126

Overconfidence as a causative factor in anosognosia for hemiparesis

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Anosognosia is defined as a lack of awareness for a neurological deficit and has been conceptualized as a multi-component disorder with several co-existing deficits in memory, sensation or spatial orientation. Overconfidence as an additional factor has been identified before in a small case series. This study aims to confirm that patients with anosognosia show overconfidence in face of uncertainty and to test an additional non-verbal riddle paradigm.

We tested 31 acute stroke patients with hemiparesis, of which 13 showed anosognosia, with the ten word riddles from the case series. These riddles are designed to create an initial situation of uncertainty. Each riddle contains five clues leading to a target word. Each clue is followed by the patient's guess. The patient also rates the confidence in the correctness of his guess. In our cohort, anosognosia was associated with severer neurological deficits and impaired cognitive scores. We found a significant difference in the level of confidence for the first two clues between anosognosic patients and healthy controls, but not between patients with and without deficit awareness. All patients with anosognosia showed overconfidence. But, unlike previously described, overconfidence was also present in some patients with deficit awareness and even in healthy controls. The picture riddles were feasible, but failed to show group differences.

To conclude, overconfidence might contribute to anosognosia, but overconfidence is not specific for anosognosia.

Poster 127 Parental psychopathology and expressed emotion in children with avoidant/restrictive food intake disorder

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Background:
Family factors such as expressed emotion (EE) and parental psychopathologies play a significant role for the severity of illness and treatment outcome in anorexia nervosa (AN). However, little is known about family factors in avoidant/restrictive food intake disorder (ARFID).

Research questions:
Do parental critical comments (CC) and emotional overinvolvement (EOI), depression, and eating disorder psychopathology (ED) differ in children and adolescents with ARFID, AN, and healthy controls? Are EE and parental psychopathologies related to the severity of symptoms in children with ARFID?

Method:
A clinical interview and well-established self-report questionnaires were used. Treatment-seeking patients with ARFID (n=42) and AN (n=25) were included, and HC (n=42) individually matched to those with ARFID for age and sex.

Results:
Parents of patients with ARFID and AN showed significantly higher level of CC and depressive symptoms compared to HC, with no significant differences between ARFID and AN. EOI and food variety were positively associated and CC and illness duration were negatively correlated in those with ARFID.

Conclusion:
Both restrictive eating disorders AN and ARFID share similarities in family factors, differing significantly from healthy controls. The findings indicate that parental criticism and depressive symptoms should be considered in the treatment of children and adolescents with ARFID.

Poster 128

Children Tap Letters Faster Than Numbers In The Trail-making (B) Test In A Large Cohort Study

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Background:
The trail making test-B, where probands have to tap on randomly arranged numbers and letters alternatingly in the order 1-A-2-B etc, has been used for decades in studies and as a diagnostic tool, but so far no in depth analyses of digital variants in a large cohort of children has been published.

Methods:
The study was conducted as part of the ongoing LIFE Child cohort study. N=812 children aged 10 to 18 years performed the Trail making test. The timings of the taps on individual labels was measured, and taps on letters vs. numbers were compared using a two sample t-test.

Results:
Children tap on letters much faster than on numbers (mean difference 0.998s, $p < 0.0001$), with the difference being more pronounced after an inital training phase. The overall performance increases with age.

Conclusions:
Results of the trail making test-B depend strongly on the characteristics of letters and numbers, which suggests that they are processed differently. A possible explanation is the use of a strategy primarily focusing on the order of letters, and interrupting that flow to find the next number. The duration of the training phase gives insight into how long children take to grasp the task well enough to perform it without much hesitation.

Poster 129 Detecting Lateralized and Non-lateralized Deficits in Visuospatial Neglect Using Immersive Virtual Reality

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Patients with neglect typically exhibit lateralized deficits that manifest as a failure to report, respond, or orient to stimuli in the contralesional, often left, hemifield [1]. Additionally, neglect involves non-lateralized attentional deficits, which, despite their prevalence, are frequently underestimated [2]. This study aimed to use immersive Virtual Reality (VR) to detect and distinguish lateralized and non-lateralized deficits associated with neglect in stroke patients.

We used a VR road-crossing task (iVRoad) with the HTC Vive Pro Eye headset in 60 participants, including two groups of right-hemisphere stroke patients with (n=20) and without left neglect (n=20), and a healthy control group (n=20). We measured head movements, temporal parameters, and error patterns in the VR environment. Results revealed both lateralized and non-lateralized deficits in the neglect group. Patients with neglect showed prolonged reaction and letter insertion times, especially for left-sided traffic and letters. With regard to non-lateralized deficits, they had slower information processing speeds, which were reflected in higher experiment and reaction times.

We demonstrate the potential of VR to enhance neuropsychological assessment of visuospatial neglect. Furthermore, our findings highlight the multifaceted nature of neglect, suggesting that the syndrome encompasses not only spatially specific but also broader attentional deficits. These findings emphasize the need for comprehensive clinical assessments in the diagnosis and understanding of neglect.

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Poster 130

Childhood sexual abuse is associated with higher total ghrelinserum levels in adulthood: results from a large, population-based study

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Ghrelin is an orexigenic peptide hormone synthesized in times of stress and hunger and alterations of the ghrelin system following acute stressors could be repeatedly shown in humans. However, little data exists on long-term effects of trauma on the ghrelin system. We aimed to investigate the influence of childhood trauma on total ghrelin serum levels in a large, population-based study. Total serum ghrelin was measured in 1666 participants of a population-based cross-sectional study ('LIFE study'). The Childhood Trauma Screener (CTS) was used for the assessment of childhood trauma in the final sample (n = 1086; mean age: 57.10 ± 16.23 years; 632 males, 454 females). Multiple linear regression analyses and generalized linear models were chosen to examine the association between childhood trauma and total serum ghrelin concentrations. Childhood sexual abuse went along with significantly higher ghrelin serum levels in the total sample ($\beta = 0.114$, $t = 3.958$; $p = 0.00008$) and in women ($\beta = 0.142$, $t = 3.115$; $p = 0.002$), but not in men ($\beta = 0.055$; $t = 1.388$; $p = 0.166$). Women with severe emotional neglect in the childhood had higher ghrelin levels than those without (odds ratio = 1.204; $p = 0.018$). For the CTS Sum Score and other CTS sub-scale scores, no significant association with ghrelin serum levels was found. Our study is the first to show associations between childhood sexual trauma and total ghrelin levels in adults in a large, community-based sample. Our results should initiate further research of the role of ghrelin in human stress response in prospective study designs.

HI-MAG

The Helmholtz Institute for Metabolic, Obesity, and Vascular Research in Leipzig investigates the causes of pathological weight gain and aims to develop new therapies for obesity and its comorbidities, including metabolic and vascular diseases. The institute excels in integrating preclinical research in the laboratory with clinical trials at its outpatient clinic, facilitating the rapid implementation of the most current research findings into clinical practice. It is one of 14 Helmholtz Institutes within the Helmholtz Association, resulting from a long-standing partnership between Helmholtz Munich and the Medical Faculty of Leipzig University including the University of Leipzig Medical Center.

Our research stands on four fundamental pillars: Obesity Research, Metabolic Research, Vascular Research – each of which consists of two work groups with different scientific goals - and Clinical Studies, where our findings are tested in clinical research.

More information:
https://www.helmholtz-munich.de/en/hi-mag

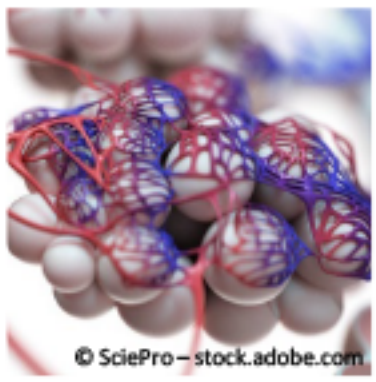
SCAN ME

Obesity Research

We are examining the mechanisms underlying adipose tissue dysfunction and their contribution to the development of obesity and related metabolic and vascular disorders. Our goal is to design novel therapeutic interventions aimed at preventing and treating obesity.

Metabolic Research

We study the interaction between peripheral organs and the central nervous system to gain a better understanding of metabolic processes, including the regulation of appetite and satiety, and to develop new drug therapies.



Vascular Research

We investigate arterial and veinous vascular diseases and create novel therapeutic alternatives to hinder disease progression and re-occlusion (restenosis) after vascular procedures.

Clinical Studies

Clinical studies at HI-MAG are a vital aspect of researching new treatments for obesity and other metabolic disorders. We collaborate with Leipzig University Medicine to perform these studies.

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Poster 131

Effects of competitive sports and obesity in childhood and adolescence on the knee joint cartilage

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Leg axis stability is crucial in the prevention and rehabilitation of knee injuries. Professional sport, obesity or malalignment of the leg axis can lead to overloading, which is associated with subclinical changes in the cartilage matrix (Crema et al., 2011). The aim of the present study was therefore to investigate empirical correlations between external load, the status of the leg axis and the reaction of the retropatellar cartilage and the knee joint cartilage.

Forty-five male subjects (15 ± 1.5 years) took part in the cross-sectional study, divided equally into volleyball players from junior professional sports, overweight children (BMI percentile > 90) and a control group. All participants performed a marker-based sports motor test battery to assess their knee stability. In addition, diffuse and regional cartilage changes were detected using T2 mapping and conventional MR sequences on a 3T MRI.

Obese individuals show increased knee valgus positions. In addition, they show significantly more focal increases in T2 times in the mapping sequences of the retropatellar and lateral-femoral overlying cartilage than the controls (p = .01). Overall, changes in the cartilage matrix are visible earlier in the T2 mapping than in the conventional sequences, which is why T2 mapping should be used to prevent damage.

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Poster 132 A new subtype of lipodystrophy due to a new variant in the adipsin/complement factor D gene

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Background:
Lipodystrophy (LD) refers to a diverse group of conditions, characterized by either partial or complete absence of subcutaneous adipose tissue (SAT). Subsequent metabolic dysregulations include conditions like diabetes mellitus and steatohepatitis. At the Lipodystrophy Center Leipzig we identified a novel mutation in the adipsin/complement factor D (CFD) gene in a family suffering from LD syndrome. The mutation provokes a frameshift and a premature stop codon. Mutations in CFD have not been described to cause LD so far. CFD as a part of the complement system (CS) is highly expressed in adipocytes and increases with ongoing differentiation.

Hypothesis:
We hypothesize that the lipodystrophic phenotype is caused by the CFD mutation rather than being a consequence of adipsin deficiency.

Methods:
A humanized transgenic mouse model carrying the frameshift mutation in CFD was used to investigate effects of the CFD mutation on adipose tissue function and metabolic profile compared to CFD-knockout mice and wild-type controls.

Results:
The metabolic phenotype of the humanized transgenic mouse model closely reflects a LD phenotype as well as the metabolic disturbances observed in the family members carrying the CFD mutation. In contrast, CFD knockout mice did not develop a lipodystrophic phenotype, which indicated that the loss of adipose tissue in the humanized transgenic model is attributable to the presence of the truncated protein rather than the absence of CFD.

Poster 133 Activation of preoptic PNOC neurons regulates energy expenditure

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The preoptic area (POA) of the hypothalamus has garnered attention due to its pivotal role in energy balance control and thermoregulation. Activation or inhibition of specific POA neurons has been shown to affect body temperature, locomotor activity and energy expenditure. To gain novel insights into this intricate regulatory mechanism, we have conducted a comprehensive analysis of a distinct cluster of neurons within the POA that express the neuropeptide prepronociceptin (PNOC).

Using chemogenetic techniques, we selectively activated PNOC(POA) neurons of mice and observed a significant reduction in brown adipose tissue (BAT) temperature and energy expenditure. Further examination of PNOC(POA) neuronal projections unveiled robust connectivity to various brain regions involved in autonomic control, including the dorsomedial hypothalamus (DMH), a key relay in BAT thermogenesis regulation. Interestingly, the activation of PNOC(POA) neurons initiated an acute inflammatory response in BAT, characterized by the upregulation of immediate early genes associated with inflammation and chemotaxis.

While extensive research has focused on investigating hypothalamic circuits, there has been limited exploration of the complex communication between hypothalamic neurons and innervated tissues, such as brown adipose tissue. By unraveling the regulatory mechanisms that are governed by PNOC(POA) neurons, we can significantly advance our understanding of the neural control of whole-body energy expenditure and adipose tissue function.

Poster 134 Change in the olfactory performance between baseline and follow-up examination in the LIFE Adult study

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Background:
In this analysis, the change in the olfactory performance after a mean period of 6.3 years between baseline and follow-up examination was investigated. In particular, we wanted to find out to what extent the responses for the individual odors changed from baseline to the follow-up examination.

Methodology:
Olfactory function was assessed in LIFE-Adult study participants using the "Sniffin' Sticks screening 12 test". Sankey diagrams were used to show the changes in olfactory performance between baseline and follow-up examination as well as changes in odour recognition within the four given choices of the individual odours.

Results:
692 individuals (54.2% males) were analyzed. The mean baseline age was 62.3 years (range 20.3-80.2 years). Olfactory performance decreased in approximately 20% of the study participants after 6 years. Strikingly, about 25% of the participants classified as hyposmics at baseline changed to a normosmic state at follow-up.

Conclusions:
The results suggest that there is a considerable random component involved in selecting the correct answer. The evaluation of the answers chosen by the participants for the individual sticks suggests that the wrong answers offered for selection in some sticks tempt them to guess. This should be taken into account when evaluating olfactory performance with the Sniffin' Sticks 12 test.

Poster 135 Deciphering the impact of novel plastic additives on human adipocytes

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Bisphenol A (BPA) is known for its disruptive effects on the endocrine system, as well as the metabolism. This includes interference with adipocyte function, causing insulin resistance, which in turn can lead to a variety of metabolic illnesses. Due to recent restrictions on its use in the EU, BPA alternatives are now prevalent in various consumer products, and their effects on the endocrine system and metabolism remain poorly understood, rendering the hazard assessment of these novel plastic additives nearly impossible. This research project aims to evaluate eight BPA alternatives for their impact on human adipocytes. The analysis will focus on lipid accumulation, cell toxicity, binding effects to cellular proteins using thermal proteome profiling, and alterations in the proteome via mass spectrometry-based global proteomics. These findings will be incorporated into adverse outcome pathway (AOP) frameworks to identify key mechanisms leading to adverse health outcomes. First results of this study indicate the harmful effects of halogenated bisphenols, specifically regarding increased lipid accumulation at environmentally relevant concentrations. Overall, the results from this study could unveil molecular mechanisms triggered in human adipocytes and simultaneously aid in the hazard assessment, thereby supporting necessary restrictions for chemicals, we are permanently exposed to.

Poster 136

Exploring the role of lysine acetylation and phosphorylation in adipocyte differentiation and contaminant exposure

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Obesity is a major public health burden worldwide. Factors beyond genetic predisposition and modern sedentary lifestyle may facilitate the development or progression of obesity. Metabolism-disrupting chemicals (MDCs) are external factors that can promote obesogenic onset by interfering with hormonal regulation yet causing metabolic conditions. The mechanisms underlying the exposure to MDCs are incompletely understood, especially for emerging contaminants, fundamental knowledge about their modes of action is lacking. Proteins critically control biological processes, with their post-translational modifications (PTMs) serving as proxies of cellular signalling.

Hence, we analysed the PTMs acetylation (AcK) and phosphorylation (PP) during adipocyte development and during exposure to the emerging plastic additive DINCH, to gain insight into molecular events triggered upon exposure. We combined mass-spectrometry based proteomics with PTM enrichment strategies on human SGBS adipocytes. We present a resource of time-resolved data on AcK and PP during adipogenesis, that revealed distinct time-dependent profiles. AcK was dominantly involved in core metabolism while PP was found important for cell structure and insulin signalling. Chemical exposure altered PTM profiles of glycolysis/gluconeogenesis (AcK) and insulin signalling (PP) and pointed to the Rab effector WDR44 as relevant phosphorylation target.

This data contributes to a better understanding of the ways MDCs affect adipogenesis, thereby aiding the development of adverse outcome pathways (AOPs) and regulatory risk assessment.

Poster 137

Gene expression changes in adipose tissue in metabolic syndrome predict successful weight loss

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Aims:
Lifestyle-induced weight loss (LIWL) is regarded as efficient therapy to reverse or ameliorate metabolic syndrome (MetS). Here we investigate the effect of LIWL on gene expression in subcutaneous adipose tissue (SAT) before and after 6 month LIWL.

Methods:
The study is embedded in a prospective, controlled, monocentric, 6-month LIWL intervention trial in individuals with MetS (ICTRP Trial Number: U1111-1158-3672). Before and following LIWL, 43 participants underwent SAT biopsy. Differentially expressed genes (DEGs) were analyzed in paired tissue samples by microarray analysis. DEG changes were correlated with parameters defining the MetS and fitted into a regression model predicting successful weight loss (according to Wing and Hill, 2001).

Results:
We identified 642 DEGs. Pathway enrichment analysis revealed upregulation of genes related to cholesterol metabolism. Spearman correlation indicated that DEGs correlate with the parameters characteristic for the definition of the MetS (BMI, HDL cholesterol, triglycerides (TGs), blood pressure and fasting plasma glucose (FPG)) in LIWL. The significant DEG changes after LIWL were fit into a regression model. We found a fat tissue signature of three DEGs, namely SUMO3, PRKG2 and ADAP2, predicting successful weight loss (AUC 0.963 (95 % CI: 0.906- 1.0)). Interestingly, the three DEG changes correlate with changes in FPG and HDL cholesterol changes in LIWL.

Perspective:
We want to predict successful weight loss by using an interdisciplinary approach with imaging and laboratory parameters, instead of the rather invasive biopsy.

Poster 139

It's all about thyroid hormones - driving white adipose tissue to a thermogenic fate

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Adaptive thermogenesis in brown adipose tissue (BAT), a process to dissipate excess energy, is synergistically regulated by thyroid hormones and norepinephrine (Silva, Front Biosci 2011). Brown-like adipocytes can also be induced in classical white adipose tissue (WAT). This so-called WAT beiging may open new avenues to treat obesity and related metabolic disorders. Zinc finger protein 423 (Zfp423) is an anti-thermogenic factor that is enriched in white vs. brown adipocytes and suppressed upon cold exposure leading to profound WAT beiging (Shao et al., Cell Metab 2016). We previously reported that thyroid hormones directly regulate the expression of Zfp423 in adipose tissue (Roth et al., Cell Rep 2023). Here, we studied the role of thyroid hormones for the formation and activation of beige adipocytes that were generated in response to genetic Zfp423 ablation.

Therefore, mice with an adipocyte-specific Zfp423 knockout were rendered hypothyroid (thyroid hormone deficient) by feeding iodine-free chow diet supplemented with 0.15 % propylthiouracil for 4 weeks. Euthyroid mice were kept on regular chow diet. During treatment, mice were either held at 30°C (thermoneutrality), 22°C (room temperature) or 18°C (mild cold stress) to examine the effect of chronic norepinephrine release due to sympathetic stimulation. Besides, acute stimulation of adaptive thermogenesis was evaluated by treatment with 1 µM norepinephrine.
Our work demonstrates that thyroid hormones are indispensable to enable adrenergic activation of Zfp423-ablated beige adipocytes and unleash full thermogenic potential.

Poster 140

MEDISCO - synthetic obesity transcriptome data for fast-paced analysis development

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Obesity is a global health concern. Accelerating obesity research, for example using machine learning and artificial intelligence, needs diverse and comprehensive datasets that can be slow to acquire (1). We present MEDISCO (Metabolic Diseases Clinical Data Synthesizer for Obesity), which uses existing patient clinical, RNA sequencing, proteomics and other data to create synthetic data sets which faithfully mimic the characteristics of real-world obese populations (2). MEDISCO offers Realism and Diversity – MEDISCO captures the richness of real-world patient data, enabling the development of robust and adaptable machine learning models for accurate predictions and improved treatment outcomes; Customizability – Researchers can fine-tune synthetic data generation by controlling parameters such as age, sex, genetics, and environmental factors. This allows for targeted scenario simulations and deeper insights into obesity-related outcomes; Ethical Data use – MEDISCOs synthetic data preserves patient anonymity by using neural networks; Accelerated Research and Development – MEDISCO streamlines data generation, minimizing time and resource requirements. Researchers can focus on critical tasks like model development and algorithm optimization, expediting progress in obesity research (3). MEDISCO empowers researchers to develop innovative obesity interventions using modern algorithms and is available as a responsive R package.

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Poster 141 MINCH causes metabolic rewiring towards lipid accumulation and adipogenesis

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The steadily rising prevalence rates of obesity are leading to a massive health and societal burden worldwide. At the same time, humans are ubiquitously exposed to an increasing amount of metabolism-disrupting chemicals (MDC), i.e. substances that alter metabolic processes by interfering with the endocrine system. Recent studies have shown that exposure to MDCs, such as the phthalate plasticizer DEHP, can promote obesity in humans. Due to the ban of DEHP in the EU, it has been replaced by safer declared substances such as DINCH. However, it has been previously shown that the primary metabolite of DINCH, MINCH, induces the differentiation of human adipocytes and affects enzyme levels of key metabolic pathways.

Thus, we aimed to analyze the effects of DINCH and MINCH on major metabolic pathways by metabolomics. Human SGBS cells were exposed to varying amounts of each chemical and the effects were compared to cells differentiated with the PPARG agonist rosiglitazone and untreated control cells. While DINCH had no effect on metabolism, MINCH induced lipid accumulation similar to rosiglitazone by upregulating the pyruvate cycle, which has recently been identified as a key driver for de novo lipogenesis. In addition, MINCH-treated cells showed elevated lactate secretion, suggesting altered glucose homeostasis.

In conclusion, MINCH rewires the metabolism towards lipid accumulation and adipogenesis similar to rosiglitazone, suggesting that exposure to MINCH could potentially lead to a weight-promoting effect and other adverse side effects observed with thiazolidinediones.

Poster 142 The role of macrophages in adipocyte degradation - extracellular digestion via lysosomal exocytosis

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Macrophages are essential for the clearance of dead cells, usually via phagocytosis. Because adipocytes can reach sizes about five times of macrophages, they cannot be phagocytized easily. One way of degradation is the accumulation of adipose tissue macrophages (ATMs) around dead adipocytes, forming crown-like structures (CLS). So, extracellular digestion via lysosomal exocytosis is proposed as a degradation method. Lysosomal exocytosis is calcium-dependent, and the main calcium channel in the lysosome is the TRPML1 channel.

We aimed to modulate lysosomal exocytosis, so we blocked TRPML1 with the PIKfyve inhibitor Apilimod. To enhance lysosomal activity, we used the TFEB activator C1.

To observe the degradation of adipocytes by ATMs ex vivo, we cultivated small pieces of the epididymal white adipose tissue (AT explants) of CalMac-mice (Ca2+-sensitive GFP in macrophages) over seven days. After stimulation, we studied AT explants over time via live-imaging and analyzed the culture medium for secretion of lysosomal enzymes. After collagenase digestion of the explants, ATMs were stained and examined via FACS.

We found that the number of CLS increased over the time of cultivation, as did the amount of secreted lysosomal exocytosis markers. By applying Apilimod we could inhibit lysosomal exocytosis in AT explants. For instance, we observed an increased number of ATMs under stimulation and a moderate impact on the ATM phenotype. Currently, we are studying ATMs after stimulation of lysosomal exocytosis. In perspective, we are aiming at verification of our data in human adipose tissue.

Poster 143

Transcriptomics on the Tip of Your Tongue: The contribution of salivary extra-cellular vesicles to taste-cell transcriptomics and eating behaviour in obesity

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Research Question:
Can extracellular vesicles (EVs) serve as carriers for targeted therapeutic and diagnostic purposes in obesity, influencing taste perception on a cellular level?

Objectives:
Explore the correlation between metabolic changes in obesity, alterations in taste perception, and the distribution and composition of salivary EVs in a cohort of N=111 subjects from the Obese Taste Bud Study.

Methods:
Isolate salivary EVs using ÄKTA, a size- and molecular weight-based separation system, from 2 ml saliva per subject. Employ Nanosight technology and western blot analyses for EV quantification and characterization. Conduct RNA extraction with the miRNeasy Micro Kit and perform sequencing on the NovaSeq6000 platform for qualitative assessment of microRNA content in salivary EVs.

Results:
Study links EV size to lower taste buds (P=0.0074), nutritional markers (all P<0.05). Higher EV concentration ties to lower body weight (P=0.0495), reduced arm (P=0.025), and calf circumference (P=0.035). Body composition, not just obesity, affects salivary EVs. Vesicle isolation integrity, confirmed by EV markers, and PPARg presence suggest a potential origin from fat cells, highlighting nuanced interplay between EVs, taste perception, and metabolic health.

Conclusion:
These findings shed light on the intricate interplay between EVs, taste perception, and obesity, offering insights for effective interventions in addressing this health concern. Ongoing analyses of the microRNA composition of salivary EVs may further enhance our understanding of how these particles influence taste buds.

Poster 144

LRP1 in Brown Adipose Tissue: Unraveling its Role in Metabolism and Thermogenesis

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The multifunctional lipoprotein receptor LRP1 is crucial for numerous physiological processes. In the adipose tissue (AT), LRP1 is important for lipid uptake and adipocyte differentiation (Masson 2009) and AT-specific LRP1-deficient mice are resistant to diet-induced obesity with preserved glucose tolerance (Hofmann 2007). The metabolic advantage was explained by increased muscle shivering to maintain body temperature (BT), compensating for limited non-shivering thermogenesis (NST) due to decreased BAT mass. Yet, in the long-term lack of LRP1 in AT leads to increased AT inflammation and atherosclerosis (Konaniah 2017).

In this work, we aim to dissociate the relevance of LRP1 specifically in thermogenic adipocytes from its role in adipocyte differentiation, using siRNA-mediated knockdown (siKD) in mature brown adipocytes and BAT-specific Lrp1 KO mice.
siLrp1 KD at day 6 of differentiation reduced LRP1 expression by ~75% but did not affect adipogenesis and lipid content. However, siLrp1 KD significantly increased PKA activity and lipolysis after adrenergic stimulation. In vivo, compared to WT littermates BAT-specific Lrp1 KO mice exhibit lower body weights albeit increased food intake during 7 days of cold exposure, while BAT and BT were not different. Upregulation of Glut4 and Fatp4 expression in Lrp1 KO mice may indicate compensatory glucose and fatty acid uptake to make up for lack of LRP1-mediated lipid uptake to fuel thermogenesis.

Together, we reveal LRP1 as a potential double-edged sword in fueling and throttling BAT lipid metabolism and NST during adrenergic activation.

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Sonderforschungsbereich 1052
„Mechanismen der Adipositas“

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Obesity Mechanisms

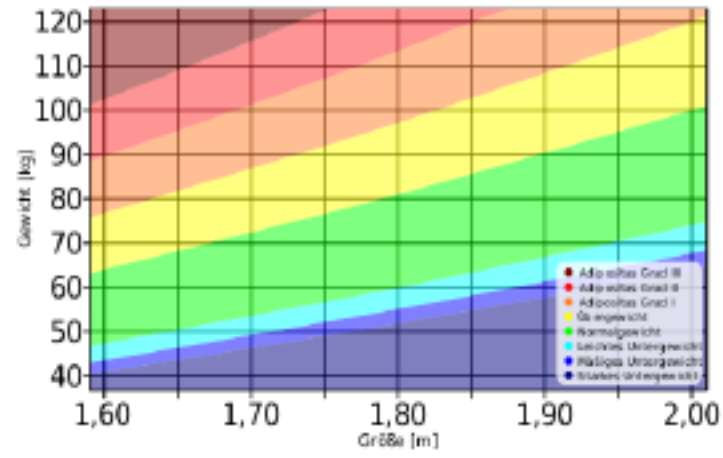
DURCH DICK UND DÜNN – EIN BLICK HINTER DIE KULISSEN DER ADIPOSITASFORSCHUNG

Was ist Adipositas?

Adipositas (von lateinisch „adeps“ = Fett) ist eine Ernährungs- und Stoffwechselkrankheit. Kennzeichen ist ein starkes Übergewicht mit einer starken Vermehrung des Körperfetts und krankhaften Auswirkungen. Nach Definition der Weltgesundheitsorganisation liegt ab einem Körpermasseindex von 30 kg/m² Adipositas vor. Ergänzend werden der Bauchumfang und das Taille-Hüft-Verhältnis zur Beurteilung herangezogen.

Die Adipositas wird unterschieden in drei Schweregrade: Grad I (BMI 30 – 34,9), Grad II (BMI 35 – 39,9) und Grad III (ab BMI 40).

In Deutschland ist fast ein Viertel der Bevölkerung von Übergewicht betroffen. Mit Adipositas werden verschiedene Begleiterkrankungen assoziiert, wie etwa Diabetes Typ 2, Bluthoch-druck, Fettleber, koronare Herzerkrankungen.



Was erforscht der Sonderforschungsbereich (SFB)?

Krankhaftes Übergewicht ist eine multifaktorielle Störung, die durch eine Kombination von Genetik, Verhalten, Lebensstil und Umweltfaktoren beeinflusst wird. Unser Anliegen ist es, neue Ziele und Mechanismen für therapeutische pharmakologische Herangehensweisen in der Behandlung von Adipositas und ihren Begleiterkrankungen zu identifizieren.

Der Sonderforschungsbereich konzentriert sich dabei auf drei Schwerpunkte: **Überernährung, Fetteinlagerung und Entzündung sowie Adipokine** (Hormone im Fettgewebe).

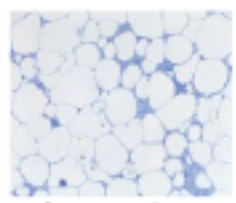
Überernährung

Aus evolutionärer Perspektive war es für unsere Vorfahren wichtig, dass in nahrungsreichen Zeiten überschüssige Kalorien in den Fettzellen gespeichert werden konnte. In Mangelzeiten konnte der Körper darauf zurückgreifen. Heute kann diese Speicherfunktion zu Adipositas führen, weil der Körper diese Reserve nicht in Anspruch nehmen muss. Wir wollen die evolutionäre Vergangenheit dieser genetischen Veränderungen verstehen. Und welche Unterschiede es zwischen Populationen gibt und in welchem Umfang diese Unterschiede für die Anfälligkeit für Krankheiten des Energiestoffwechsels vorhersagbar sind.

Fetteinlagerung und Entzündung

Es gibt verschiedene Arten von Fettgewebe: weißes, braunes und beiges Fettgewebe.

Die Speicherung von Fett in Fettzellen geschieht vorrangig über **weißes Fettgewebe**, das nur schwer abgebaut werden kann.



Weißes Fettgewebe.

Vor allem die viszerale Fetteinlagerung (in der Bauchhöhle) wird mit der Neigung zu Komplikationen assoziiert. Uns interessiert, welche genetischen Faktoren die Anzahl der Adipozyten (Fettzellen), Unterschiede in der Fettverteilung (viszeral oder subkutan) und ihre Assoziation mit Stoffwechselstörungen bestimmen. Mit Hilfe der Fettgewebebank in Leipzig sollen Gene identifiziert werden, die darin involviert sind.

In den Fokus der Forschung gerät derzeit das **braune Fettgewebe**. Es ist an der Thermogenese beteiligt, bei der weißes Fettgewebe in braunes umgewandelt wird. Wenn der Mensch leichter Kälte ausgesetzt wird, kann so die Körpertemperatur erhalten werden. Es kann auch durch Sport aufgebaut werden. Braunes Fettgewebe wird in der Forschung mit Gewichtsverlust und der Unterdrückung von Stoffwechselerkrankungen (z. B. Diabetes) in Verbindung gebracht.

Adipokine (Fettgewebshormone)

Das Fettgewebe sondert zahlreiche Hormone ab. In den letzten Jahren wurden zahlreiche Adipokine identifiziert. Unser Anliegen ist es, für ausgewählte Adipokine ihre Funktion, ihre molekularen Ziele und ihre potentielle klinische Relevanz als Biomarker oder in der Behandlung von Adipositas und deren Begleiterkrankungen zu definieren.

Struktur des Adipokins SerpinA12 (Vaspin) pdb:4FI8

Was ist der SFB?

Der Sonderforschungsbereich 1052 vereint Wissenschaftlerinnen und Wissenschaftler aus den Bereichen Anatomie, Biochemie, Biophysik, Chemie, Dermatologie, Endokrinologie, Genetik, Kardiologie, Labormedizin, Neurologie, Pädiatrie, Physiologie.

Beteiligte Einrichtungen sind die Universität Leipzig (Medizinische Fakultät; Fakultät für Lebenswissenschaften; Fakultät für Chemie und Mineralogie), das Universitätsklinikum Leipzig, das Max-Planck-Institut für Kognitions- und Neurowissenschaften und die Ben-Gurion-Universität in Beer-Sheva, Israel.

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Poster 145 Time-resolved transcriptomic and metabolomic signature of muscle polyamine homeostasis in health and obesity

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Metabolic disorders such as obesity have been linked to circadian disruption of metabolites in a tissue-specific manner but the impact on cellular and whole-body homeostasis remains largely elusive. In this study, we combined a targeted, comprehensive mouse muscle transcriptomic profiling of important genes related to polyamine biosynthesis with re-analyses of published databases for time-resolved single-cell transcriptomic (muscle injury) and metabolomic (exercise, obesity) adaptions in human and mice, to generate a tempoal and tissue-specific signature of polyamine homeostasis. In mice, we provide first evidence for a muscle fiber type-specific and clock-dependent regulation of Odc1 (ornithine decarboxylase 1) and Srm (spermidine synthase). We show that the regulation of Odc1 and Srm is highly dynamic in mouse muscle tissue and depends timely on physiological and cellular challenges. Strikingly, transcriptomic adaptations to short-term and long-term exercise training in healthy sedentary men revealed an acute induction of ODC1 post-exercise, suggesting that the polyamine metabolism is a crucial modifier of muscle cellular and metabolic homeostasis. Finally, we uncovered a so far unknown diurnal oscillation of polyamines and the polyamine precursor in muscle of lean mice, which was abolished upon nutritional stress in diet-induced obese mice. Together, we here shed new light on muscle polyamine homeostasis, with great potential to utilize this knowledge for developing new strategies to prevent or treat metabolic complications and to evaluate nutritional recommendations.

Poster 146

The Integrin CD11c affects Adipose Tissue Inflammation and Macrophage Fusion

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Obesity is associated with a low-grade inflammation in visceral adipose tissue (vAT) leading to diabetes type II.[1] Due to adipocyte death, the number of AT macrophages (ATMs) rises, ATMs accumulate to form crown-like-structures (CLS) and switch their phenotype to a pro-inflammatory state.[2] MGCs are huge fat-eating macrophages, which are beneficial for a more efficient phagocytosis of adipocyte cell debris supporting AT repair.[3] The mechanism of MGC formation in AT needs to be clarified, but CD11c is known to participate in bone marrow MGC formation.

For investigation of CD11c participation in AT inflammation and MGC formation, we tested the effect of global CD11c-KO in lean female mice. We reconstruct AT inflammation by an ex vivo model using explants from visceral AT of CD11c+/+ (WT) and CD11c-/- (KO) mice and 7d cultivation without any interventions. Afterwards, stroma-vascular fraction was isolated and i) stained for pro- (CD9) and anti-inflammatory (CD301) ATM markers for flow cytometric analysis or ii) seeded onto cover slips prepared for MGC identification by epifluorescence microscopy. Paraffin sections where used for comparison of adipocyte size, CLS formation and ATM number of both genotypes.

Flow cytometry data showed a significant reduction of CD11c expression in ATMs, but KO has no impact body weight, organ weights or adipocyte size. Flow cytometry data indicates a trend towards a more pro-inflammatory AT state. Of note, MGCs formation is impaired in CD11c-KO supporting the hypothesis of the beneficial role of AT MGCs.

References:

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Poster 147

Obesity and pancreatitis: effects on pancreatic immune cell subpopulations

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One of the most aggressive cancer types is pancreatic ductal adeno carcinoma (PDAC) which is often associated with a preceded chronic pancreatitis. Obesity is a well-known risk factor for various diseases and cancers, such as PDAC, and is associated with a state of chronic inflammation within several tissues, most preferentially within the adipose tissue.

Here, we investigate whether obesity affects the pancreatic immune profile in mice. Therefore, genetically-induced obesity models (ob/ob and db/db mice) as well as a high fat diet (HFD)-induced obesity model have been used. Immune cell populations within the pancreas have been analyzed using histology and flow cytometry. Thereby, we focused on three different immune cell subpopulations: myeloid derived suppressor cells (MDSCs), tissue resident macrophages, and T cells. For MDSCs we observed a significant increase in ob/ob mice as well as in male C57BL/6 mice after a short-term HFD (2 weeks). Interestingly, however, this increase could not be confirmed for later stages of obesity (12 and 24 weeks of HFD). For macrophage subpopulations, we exclusively observed an increase in pro-inflammatory (M1-like) macrophages in ob/ob mice. Regarding T cells, neither T-helper, cytotoxic nor regulatory T cells did show differences in abundance.

These data indicate that in wild-type mice, obesity only leads to a marginal immune cell reaction within the pancreas. To investigate the effect of obesity on pancreatic carcinogenesis, we plan further studies with diet-induced obesity in cancer susceptible KRAS mice.

Poster 148 Transformation of environmental contaminants by lean and obese specific gut microbiota

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The globally increasing prevalence of obesity has massive negative health and societal consequences. One of the systems in the human body influenced by obesity is the gut microbiome, which plays an essential role in human health and has a great impact on metabolic health. Thus, understanding the impact of obesity on the composition and function of the gut microbiome and how the microbiome affects obesity in return is a crucial part in gaining insight into the mechanisms of obesity. We aim to analyze what differences occur in the gut microbiota derived from stool samples collected from lean individuals and individuals with obesity. The bacterial composition will be characterized with 16S amplicon sequencing and functional properties with metaproteomics and metabolomics.

By analyzing the taxonomic and functional profiles of microbiomes from lean and obese individuals, we aim to uncover the underlying mechanisms contributing to microbiome dysbiosis in obesity. Furthermore, we will explore the effects of specific environmental contaminants on the microbiome‘s functionality, providing valuable insights into their potential to modulate microbial activity and impact human health. The ultimate goal of this research is to advance our understanding of the complex relationship between the gut microbiome, environmental chemicals, and obesity, paving the way for targeted interventions and microbiome-based therapies.

Poster 149 Unbiased identification of sucrose-responsive neuronal circuits in control of glucose metabolism

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The central nervous system (CNS) plays an essential role in regulating glucose metabolism and energy homeostasis. Alterations within the CNS can be correlated with metabolic dysfunction, potentially leading to diseases such as obesity or diabetes. The consumption of added sugars has been linked to the development of these disorders. Nevertheless, there is limited understanding of the specific neurocircuits responsive to consumption of foods with high sucrose content.

Therefore, we employed an unbiased experimental approach to profile molecular signatures that define the neuronal populations activated after the ingestion of a hypercaloric palatable high-sucrose diet (HSD). We found significant activation of galanin (GAL)-expressing neurons in the mediobasal hypothalamus after 1-day access to HSD. In-situ hybridization further confirmed an activation of GAL neurons in the arcuate nucleus (ARC) of the mediobasal hypothalamus. Galanin is a neuropeptide widely distributed in the CNS and peripheral nervous system. It has pleiotropic biological effects and is involved in a plethora of physiological and pathophysiological functions, e.g., in feeding behavior, insulin secretion and inflammation. However, little is known about the galaninergic signaling in the regulation of glucose homeostasis.

Expanding on these initial findings, we will investigate the role of GAL neurons in the arcuate nucleus in glucose control to improve our understanding of energy balance mechanisms and potentially uncover novel therapeutic targets for metabolic disorders.

Poster 150

Coagulation FXII induces persistent uPAR-integrin β1 signaling promoting DNA damage and senescence in diabetic kidney disease

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Background:
Diabetic kidney disease (DKD), the leading cause of kidney failure worldwide, is linked to altered coagulation protease signaling. Whether FXII signaling, known to be associated with several diseases, plays a role in DKD is currently unknown.

Aim:
To identify the mechanistic role and signaling of coagulation FXII in DKD.

Methods:
Persistent diabetes was maintained for 24 weeks in wild-type (WT) and F12 knockout mice (F12-/-). Kidney bulk RNA sequencing, computational modeling, ex vivo analyses of human and mice samples and in vitro work were conducted to obtain mechanistic insights.

Results:
Urinary FXII correlated with kidney dysfunction in DKD cohorts. FXII expression was detected in renal tubular cells, which increased in human and mouse DKD. F12-/- mice were protected from diabetes-induced kidney injury. RNA sequencing revealed negative enrichment of pathways related to cell cycle arrest and fibrosis, in F12-/- mice kidneys. FXII induced tubular expression of urokinase plasminogen activator (uPAR), linking senescence and DKD. Structural modeling and functional assays revealed FXII-uPAR interaction in tubular cells via integrin β1 signaling. This signaling axis promoted oxidative stress, DNA damage and senescence. Blocking uPAR or integrin β1 ameliorated FXII-induced tubular cell injury

Conclusion:
FXII-uPAR-integrin β1 signaling axis promotes tubular DNA damage and senescence in diabetic kidneys. Inhibition of FXII or the related signaling pathway could be a promising therapeutic approach to prevent senescence in DKD and to halt the progression of the disease.

Poster 152

Activated Protein C maintains efferocytosis to ameliorate diabetes induced atherosclerosis

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Defective phagocytic engulfment of the dead cells by the macrophages is known to promote inflammation and atherosclerosis. Macrophage efferocytic capability is impaired in diabetes-induced atherosclerosis. Plasma levels of aPC declines in atherosclerosis and diabetes.

BMDMs were isolated from wild type mice or PBMCs (from healthy individuals) were exposed to glucose conditions with or without aPC. Additionally, PBMCs were isolated from diabetic patients and ex vivo treated with aPC. After 24 h, fluorescently labelled apoptotic Jurkat cells were added to BMDMs and efferocytosis was assessed. ApoE-/- mice were made diabetic and treated with exogenous aPC with or without MerTK inhibition and analysed.

Macrophages efferocytic ability was reduced in cells exposed to glucose ex vivo or isolated from diabetic patients as reflected by decreased surface expression of proteins involved in phagocytosis (MerTK), proteins involved in the efferocytosis pathway. aPC treatment prevented glucose induced reduction in efferocytosis. Moreover, ex vivo aPC treatment was sufficient to restore efferocytosis in PBMCs (from diabetic patients). Exogenous aPC treatment restored macrophage efferocytosis and ameliorated diabetes induced atherosclerosis. Inhibition of MerTK abolishes aPC`s restoration of macrophage efferocytosis in diabetes induced atherosclerosis.
These data suggest that high glucose impairs macrophage efferocytosis and hence promote diabetes induced atherosclerosis, which can be reversed by aPC. This suggests that reversal of glucose-induced macrophage efferocytic ability is feasible.

Poster 153

Identifying GPCRs involved in adipose tissue function using the innovative RNA-seq database FATTLAS

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Objective

G protein-coupled receptors (GPCRs) modulate the function of adipose tissue (AT) in general and of adipocytes, specifically. Although it is well-established that GPCRs are widely expressed in AT, their repertoire, regulation and function in (patho) physiological conditions (e.g. obesity) are not fully resolved. Here, we speculated that publicly available RNA-seq data could be used to identify hitherto unrecognized GPCRs relevant for adipogenesis and AT function.

Method

Collating publicly available RNA-seq data from various cohorts of mouse and human AT we established FATTLAS, an interactive public database, for improved access and analysis of gene expression data.The GPCRome of lean individuals and individuals with obesity was extracted before identifying highly expressed and/or differentially regulated GPCRs. Following, we analyzed their physiological relevance in a (pre) adipocyte cell model using receptor activation and siRNA-mediated knockdown.

Results

Our analysis revealed four receptors either highly expressed in AT (GPR146) or differentially regulated in obesity (MRGPRF, FZD5, and PTGER2). Besides all receptors being involved in adipogenesis, MRGPRF is essential for adipocyte viability and regulates cAMP levels, while GPR146 modulates adipocyte lipolysis via constitutive activation of Gi proteins. FZD5 and PTGER2 did not affect the analyzed adipocyte functions.

Conclusion

Taken together, we implemented an innovative RNA-seq database, called FATTLAS. Using FATTLAS, we described four hitherto unrecognized GPCRs associated with AT function and adipogenesis.

Reference:

Kaczmarek, I., Wower, I., Ettig, K., Kuhn, C.K., Kraft, R., Landgraf, K., Körner, A., Schöneberg, T., Horn, S., and Thor, D. (2023). Identifying G protein-coupled receptors involved in adipose tissue function using the innovative RNA-seq database FATTLAS. iScience 26, 107841. 10.1016/j.isci.2023.107841.

Poster 154 Lactylation-dependent GAPDH activity underlying altered glycolysis in hyperglycemia

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Chronic hyperglycemic-induced endothelial damage was associated with inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity. Post-translational modifications (PTM) of GAPDH regulates GAPDH activity and thereby impairing glucose metabolism, whether lactate-induced lactylation on GAPDH underlying molecular mechanisms in hyperglycemia remain unresolved.

Using endothelial cells, we show here that chronic hyperglycemia (normal glucose 5.0 mM v.s. high glucose 30.0 mM medium, 96 h) activates three major upstream glycolytic metabolites pathways (Advanced Glycation End products (AGEs), Protein kinase C, and O-GlcNAcylation) by inhibiting GAPDH activity. Hyperglycemia triggers increased lactate production along with vascular barrier damage. Immunoprecipitation assay reveals lactylation on GAPDH was found to be a consequence of hyperglycemia or lactate stimulation. Specifically, increased GAPDH lactylation attributes to inhibition of GAPDH activity. Seahorse assay shows impaired glycolysis in chronic hyperglycemia treated endothelial cells. Increased GAPDH lactylation and lower GAPDH activity were found in vascular tissue of STZ diabetes mice, indicating a detrimental role of GAPDH lactylation in hyperglycemia-induced endothelial dysfunction. Of noted, both the hyperglycemia-induced decreased activity of GAPDH and its upregulated lactylation were prevented by co-treatment of activated protein C, which subsequently alleviates endothelial impairment.

Taken together, lactylation-dependent GAPDH activity under hyperglycemia conditions attributed to endothelial dysfunction.

Poster 155

Myoglobin-mediated lipid shuttling and brown adipocyte metabolism

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Brown adipose tissue (BAT) is a dynamic tissue that efficiently burns glucose and fatty acids to generate heat, a process known as thermogenesis. This mechanism demands a heightened and effective supply of energy substrates and oxygen to the BAT. Myoglobin (MB), predominantly found in cardiac and skeletal muscle, stores and supplies oxygen during exercise. Our previous studies have analyzed how MB expression influences BAT function, establishing MB as a crucial regulator in BAT metabolism.

Presently, our focus lies in examining how MB‘s interaction with lipids influences molecular and metabolic processes. We mutated MB‘s predicted lipid-interacting residues and created brown adipocyte cell clones, overexpressing non-lipid-binding MB or wildtype MB. High levels of MB improve adrenergic activation of brown adipocyte metabolism, while mutant MB does not affect lipid uptake, lipolysis, or mitochondrial respiration. Utilizing the Seahorse Analyzer, we show that MB is important for rapid availability of specific fatty acid for mitochondrial respiration. Together, our data demonstrates a new role for MB as a lipid shuttle within BAT enhancing substrate flow and mitochondrial respiratory capacity.

To fully understand the role and regulation of MB in thermogenic adipocytes, we now aim to uncover the impact of myoglobin expression on whole body energy expenditure, body weight and thermoregulation by comprehensively phenotyping BAT-specific MB knockout mice. Modulating adipocytes MB expression could be an intriguing approach to improve adipocyte function and metabolic health in obesity.

Poster 156 The consequence of ADGRL1/LPHN1 dysfunction in adipose tissue and its contribution to obesity

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G protein coupled receptors regulate several different cellular functions and, thereby, are important regulators of adipocyte function and lipid content. The physiological function for many receptors expressed in adipose tissue (AT) is still unknown. Among the highly expressed receptors is LPHN1, which has been mainly described for its involvement in neuronal and developmental processes.

Interestingly, when using a knock-out (KO) mouse model we observed an increased weight gain in KO mice compared to wildtype (WT) littermates.

First results are pointing towards LPHN1 being necessary for different mechanisms involved in regulation of glucose and energy homeostasis. Gene expression analysis of adipose tissue from KO and WT animals show significant difference especially in the visceral AT. The most pronounced results showed the levels of Perilipin 4 and HSL, where expression is downregulated indicating that the lipolysis in the KO mice can be impaired. In the cell model 3T3-L1 LPHN1 knock-down prior to differentiation showed no effect onto adipogenesis, however, its impact onto function of mature adipocytes has not yet been evaluated. Therefore, I will knock-down the receptor in fully differentiated 3T3-L1 cells to analyse adipocyte function.
My results will answer the question how LPHN1 modulates adipocyte function by using the KO mouse model as well as the model cell line 3T3-L1. Furthermore, I will identify the underlying mechanisms leading to obesity in mice lacking LPHN1. These data will help to understand which impact loss of receptor function might have in humans affected.

Poster 157 Therapeutic potential of CHOP-ASO in atherosclerosis

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Background:
Atherosclerosis is a leading cardiovascular complication, which is characterized by vascular endothelial cell dysfunction, macrophages accumulation, and sterile inflammation. Despite having decades of research there is still a need for better therapeutic candidates. Activation of the maladaptive endoplasmic reticulum (ER) stress-induced CHOP promotes atherosclerosis.

Research question:
To evaluate the efficacy of silencing CHOP in the pathogenesis of atherosclerosis.

Objective:
To study the role of CHOP antisense oligonucleotide (CHOP-ASO) in the prevention and reversal of experimental atherosclerosis.

Methods:
To prevent atherosclerosis development, ApoE (-/-) age-matched (8 weeks) old mice were fed high-fat diet (HFD) or chow diet (control mice). A group of HFD mice was treated with CHOP-ASO (1mg/kg, alternate days (once) intraperitoneally) for up to 12 weeks. Mice were then sacrificed and atherosclerotic plaque morphology and plasma lipid profile were determined. In parallel, the efficacy of CHOP-ASO on endothelial barrier integrity and macrophage function was evaluated.

Conclusion:
Morphological analysis of the blood vessels revealed larger plaques in HFD-fed mice versus chow-diet mice. Importantly, markedly reduced atherosclerotic plaque size was observed in CHOP-ASO-treated mice versus HFD-fed mice. In vitro, CHOP-ASO reduced sodium palmitate-induced endothelial cell dysfunction and inflammatory and apoptotic markers. Taken together, these results suggest that CHOP-ASO may act as a potential therapeutic entity for reducing atherosclerotic complications.

Poster 158

Dermal white adipose tissue induces interleukin 4/13 expression in myeloid cells during inflammation in an IL-33 dependent manner

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Recent research has highlighted the multiple functions of dermal white adipose tissue (dWAT) beyond its traditional role as an energy reservoir. Since the balance between induction and resolution of inflammation is critical for effective tissue responses to infection and injury, we investigated the impact of dWAT on the control of the activation of inflammation. In the present study we show, that inflammatory activation of the skin triggers the expression of alarm signals, adipokines and chemokines in dWAT. These soluble mediators of proinflammatory activated dWAT regulate the expression of genes in myeloid cells important for the control of inflammation such as genes regulating myeloid cell migration, response to chemokines, macrophage activation, organisation of the extracellular matrix.Interestingly, soluble mediators of the act-dWAT upregulated the typical Th2 cell cytokines interleukin-4(IL-4) and -13(IL-13) in inflammatory-stimulated bone marrow cells (BMC). Proinflammatory activation of dWAT is crucial since BMCs treated with supernatants of dWAT from healthy, untreated mice did not show upregulation of IL-4, IL-13 expression. Consistently, myeloid cells isolated from inflammatory skin showed a significant upregulation of IL-4 and IL-13 expression. Using function-blocking antibodies, IL-33 was identified as soluble mediator from activated dWAT responsible for the stimulation of IL-4 and IL-13 expression in BMC. This induction is impaired in obesity due to lower IL-33 levels, which could contribute to the persistent inflammation and delayed wound healing.

Poster 159 A novel 3D model of bone marrow hematopoiesis

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Introduction:

The control of normal and neoplastic hematopoiesis involves close interaction between hematopoietic stem cells and the stromal cells in the bone marrow niche. Co-culture models hold the key to characterizing such interactions, to understanding leukemogenesis and to preclinical drug screening. Traditional plastic-adherent long-term bone marrow culture systems take many weeks to become established and often display low and variable activity. To overcome these limitations, we are combining primary cell types into a 3D aggregate to recreate a hematopoietic niche rapidly and reproducibly.

Methods:

Mesenchymal stromal cells (MSCs), human umbilical vein endothelial cells and MSC-generated osteoprogenitor cells are combined in a defined in-house medium in ultra-low adherence 96-well plates in the presence of CD34+ hematopoietic stem and progenitor cells from the bone marrow of healthy donors- (HD) or patients with myelodysplastic neoplasms (MDS). The medium is changed weekly, and hematopoietic activity is assessed by microscopy, colony-forming assays and histochemical staining of cells released from the aggregate into the supernatant.

Results:

Spheroid formation occurs within 24 hours. Hematopoietic activity can be observed as a continuously growing halo around the spheroid. Both HD- and MDS-derived cultures are stable for up to 2 months.

Conclusions:

We have successfully generated a novel 3D bone marrow culture system that maintains in vitro hematopoiesis over long periods. We are currently mapping the fate of neoplastic cells in this system.

Poster 160

Analyzing homogenous siRNA loading distribution in bovine milk derived extracellular vesicles for their use as modern oral drug delivery system.

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All living cells release extracellular vesicles (EVs) [1]. They play a crucial role in intercellular transport and communication. Bovine milk-derived EVs are a viable option as oral drug delivery system due to their abundance, accessibility and stability in the gastrointestinal tract [2]. In a prior study [3], we established a siRNA loading strategy yielding an average of 150 siRNA copies per EV. However, we assume the siRNA distribution is uneven. A precise and reliable analytical method to distinguish between loaded and non-loaded EVs is needed. This study systematically investigated the suitability of Nanoparticle Tracking Analysis (NTA) equipped with a fluorescence detection option to answer this question.

EVs were isolated with a combination of differential centrifugation, size exclusion chromatography and ultracentrifugation [4]. We loaded EVs with fluorescently labeled siRNA and dual asymmetric centrifugation. NTA was utilized to measure particle size and particle concentration.

NTA results revealed a dilution-dependent EV distribution. For reliable determination of particle concentration, a higher dilution was necessary. We hypothesize that this effect is caused by larger particles that scatter light with higher intensity. Upon dilution, larger particles become less dominant and smaller particles are more reliably detected. However, undiluted samples had to be used for fluorescence analyses because of low fluorescence intensity. To solve this, higher fluorescence intensity is necessary, either by the fluorescence label or by the fluorescence detection sensitivity.

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Poster 161

Detection of the Peptides Influencing Appetite in the Brain following Intranasal Administration

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Peptides are emerging as promising candidates for drug development, but their therapeutic application faces significant challenges. Their limited oral bioavailability and difficulties in crossing the blood-brain barrier hinder their use in addressing conditions related to energy homeostasis in the hypothalamus. Therefore, we explored direct intranasal delivery as a method of bypassing these obstacles.

We focused on four peptide types: Y1R Agonists and Ghrelin Receptor Agonists, which can stimulate food intake, Ghrelin Receptor Inverse Agonists and Melanocortin receptor 4, known for its role in suppressing food consumption.

In our study, we aimed to track the distribution of TAMRA-labeled Peptides in the brains of C57/BL/6N mice following intranasal administration. We perfused the mice at different time points (15 min up to 24 h), then collected and stained brain sections with Anti-Tamra Antibody and DAPI to visualize the peptides. Our goal was to detect the presence of these peptides in the olfactory bulb, cortex, and hypothalamus using a fluorescence slide scanner.

Our results demonstrated that the administered peptides reached these brain regions within 15 min and remained stable for up to 24 h. We now start to invest food consumption in mice of the most promising peptides over 14 days with daily nasal application. These findings hold promise for the development of more targeted therapeutic interventions for conditions like obesity, anorexia, and cachexia, potentially improving the quality of life for individuals worldwide.

Poster 162 Diffusion Tensor Imaging as a Method to Assess Muscle Architecture

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The structural properties of a muscle determine its performance and preventive capabilities. To determine muscle fibre architecture, the vast majority of studies to date have used 2D ultrasound imaging, a convenient technology that suffers from standardisation and a limited field of view (Blazevich et al., 2006). In contrast, MR-based diffusion tensor imaging of muscle (mDTI) is an innovative approach to quantify the fibre arrangement for the entire muscle volume (Damon et al., 2002). For accurate calculation of fibre metrics, muscle segmentation prior to tractography is considered necessary but reduces the convenience of mDTI. As segmentation is known to be operator dependent, it is important to understand how segmentation affects tractography. The aim of this study was to compare the results of deterministic fibre tracking based VOIs extracted by two independent operators. In addition, this study compares the results with a VOI-free approach. For this purpose, 15 healthy male volunteers underwent mDTI of the shoulder supraspinatus muscle. The results show that different VOIs had no effect on tractography and fascicle length, fibre volume, fractional anisotropy, axial diffusivity, radial diffusivity and mean diffusivity showed excellent intraclass correlation estimates (>0.97). In contrast, the VOI-free approach revealed significant differences in fascicle length. From these results we conclude that tractography is not sensitive to variations in segmentation. However, automatic segmentation approaches need to be considered to further enhance the potential of mDTI.

Poster 163

Evaluating keratinocyte model systems for treatment of Epidermolysis bullosa simplex by the multi-kinase inhibitor PKC412

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We are currently investigating a therapy approach for the blistering skin disease Epidermolysis Bullosa Simplex (EBS), caused by mutations in keratin genes KRT5 or KRT14. Such mutations trigger the collapse of the cytoskeleton into cytoplasmic protein aggregates, followed by skin fragility and blistering, diminished cell adhesion, inflammation and itch. Embarking on drug repurposing, we identified the multi-kinase inhibitor PKC412, which is already in clinical use for several diseases, and in cultured immortalized keratinocytes reduces keratin aggregation and increases cell adhesion. Here we evaluate PKC412 on primary keratinocytes in comparison to our existing model of immortalized keratinocytes with regards to cytoskeletal structure, desmosome formation and target phosphorylation. Furthermore, we present data based on a physiologically relevant organotypic model of EBS keratinocytes to validate PKC412 action and targeting in a near-physiological setting. This model is a crucial step towards a clinical trial together with our collaboration partners.

Poster 164

Evaluation of modified polyethylenimine (PEI)-based polyplexes and lipopolyplexes for the pulmonary delivery of siRNAs in 2D cell culture and in air-liquid interface systems

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Introduction:
Lung tumors are among the leading causes for cancer related deaths worldwide, and new therapeutic approaches are needed. In this context, the exploration of RNAi via direct delivery of siRNAs has gained increasing interest. For complexation, the cationic polymer polyethylenimine (PEI) and its chemical derivatives have shown promising activities for siRNA delivery in cell culture and in preclinical therapeutic intervention in vivo.

Methods:
Herein, we show the use of different PEI/siRNA complexes, including defined chemical PEI modifications, for transfection in 2D cell culture including hard-to-transfect cells (A549-, 16HBE-, Calu3 reporter cells). Furthermore, studies were extended towards a more complex air-liquid interface (ALI) system (A549-eGFP/Luc cells). Transfection efficacies were determined via eGFP knockdown in flow cytometry. Additionally, the solution containing a promising polymer/siRNA complex was nebulized and the activity of the complex in the aerosol was evaluated.

Results:
Based on different tyrosine-modified PEIs showing distinct differences in biological activity, optimal nanoparticles with highest efficacy were identified. The high performer also achieved gene knockdown in hard-to-transfect cell lines, like 16HBE or Calu3. Although some of the siRNA was lost in the process, these nanoparticles also retained their activity upon nebulization prior to transfection, thus allowing for their inhalation. Knockdown efficacies in the ALI models indicate the potential of modified PEI/siRNA complexes for local lung disease treatment by inhalation.

Poster 165

Exploring a potential vasodilative pathway of kynurenine in human pulmonary smooth muscle cells

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Introduction

Impairment of hypoxic pulmonary vasoconstriction (HPV) leads to severe hypoxaemia in patients with acute lung failure. The tryptophan metabolite kynurenine (Kyn) acts as a systemic vasodilator and its synthesis by endothelial indoleamine-2,3-dioxygenase 1 (IDO1) is induced by inflammatory cytokines. It may therefore contribute to HPV disruption. This study aims to explore potential Kyn-induced signalling pathways leading to vasodilation in human pulmonary artery cells (hPASMC) in vitro.

Methods

We evaluated IDO1 expression and activity in hPAMSC after stimulation with interferon γ using Western Blot. Tryptophan metabolites were quantified by photometric analysis of cell supernatants. To characterize vasoactive signalling, cyclic nucleotide-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was assessed by Western Blotting.

Results

Inflammatory challenge led to an increase in IDO1 expression in a time dependent manner and displayed consecutively increased Kyn equivalent concentrations in cell supernatants (4-fold increase IFN vs. control). Stimulation with Kyn increased phosphorylation of VASP at serine 157 (5-fold increase Kyn vs. control), but no significant changes at other phosphorylation sites were detected.

Conclusion

IDO1 is induced by an inflammatory stimulus in hPASMC, leading to increased kynurenine levels. VASP phosphorylation implicates the contribution of cAMP dependent protein kinase to the kynurenine pathway, cGMP dependent protein kinase does not seem to be involved.

Poster 166 Exploring putative peptide-activated orphan GPCRs in the cardiovascular system

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G protein-coupled receptors (GPCRs) are involved in the regulation of many physiological and pathophysiological processes. This is also reflected in the fact that they are targeted by over 30% of all FDA-approved drugs [1].

Despite the wide abundance of drugs addressing this system it is evident that not all GPCR classes are targeted equally and that many subgroups remain underrepresented. This is especially evident for orphan GPCRs that lack an assigned endogenous ligand. Nevertheless, recent evidence shows that the expression pattern of many orphan GPCRs is severely altered in pathophysiological conditions suggesting important physiological functions.

In this project, we want to develop peptide-based ligands for a subset of putative peptide-activated orphan GPCRs that are upregulated in the cardiac system upon hypoxic conditions.

Among the most interesting targets is GPR68 which belongs to a small family of proton-activated receptors, but also shares typical features of peptide-activated receptors [2]. We found that GPR68 is upregulated in heart tissue from patients with ischemic cardiomyopathy and that activation of this receptor by the positive modulator ogerin reduced hypoxia-induced cell damage in human induced pluripotent stem cell-derived cardiomyocytes. However, the underlying signaling pathways are unknown. To resolve this, we successfully established assay systems to monitor the activity of the receptor in its main signaling pathways including Gs, Gq, G12/13, as well as arrestins which provides the required technical background for the development of new ligands.

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Poster 167

Introducing a Navigated Platform-Independent 3MP-FUS System for the Stimulation of Deep Brain Regions

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Transcranial focused ultrasound has become of great importance for neurological treatments.

Magnet Resonance-imaging guided Focused Ultrasound (MRgFUS) is of interest due to real-time co-registration of targeted brain regions with ultrasound transducer, as well as possible direct visualisation of FUS-effects. Based on the need for versatile mobile MRgFUS systems for any MRI, this study presents first steps towards a clinical MRgFUS neuronavigated system.

MRgFUS research system consists of rolling table for flexible transportation with monitor and amplifier for connection of MR-compatible and beam steering capable mobile FUS-system with a 256-channel matrix transducer (MRInstruments). Transducer size of 10x10x11 cm fit into most head coils. System is equipped with MR-compatible optical tracked navigation system (Localite). For MR-based transducer tracking semi-active resonant fiducial markers were improved at the transducer housing and investigated for directionality and visibility in T1- weighted gradient-echo sequences.
MR measurements were performed in Biograph mMR PET/MRI (Siemens Healthineers) based on modified ASTM sequences. Treatment planning and transducer placement was tested on a phantom using ACCESS head coil, and DuoFlex Quadrupole coils (MRInstruments).

The semi-active MR markers showed a >12 times increased brightness compared to Gd-filled markers. The feasibility of the mobile versatile MRgFUS-system was shown and various measurements are being performed to verify that a safe operation on human subjects is possible in the particularly difficult area of the brain.

Poster 168 Local search in Drosophila melanogaster larvae

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Orientation and navigation within the environment are essential key features that are important for an animal's survival. To realize navigational and orientational behaviours, animals utilize on several, predominantly, external cues such as the sun or landmarks. However, if external cues are rare or absent, animals' resort to internal representation of their environment for their navigation. This neuronal process is defined as internal path integration. Therefore, animals measure self-motion cues during outbound trajectories to calculate a direct path back to their start position, a behaviour fist described in desert ants[1]. It is known that the central complex (CX), a mid-brain structure consisting of the ellipsoid body, the protocerebral bridge, the fan-shaped body and the paired noduli, integrate these cues and mediate the navigational behaviour[2][3].

Recent studies in Drosophila melanogaster demonstrated that flies locally search around and revisit a previous food source even in the absence of external cues[4]. It is suggested that this behaviour was mediated by the CX. An equal behaviour we observed in Drosophila larvae, searching locally around a previous odour source. Despite the equivalent behaviour, larvae evolve no functional central complex and should, therefore, not be able to show such a behaviour. To identify and analyse involved brain compartments in future studies, we established a larval local search paradigm by determining optimal test conditions. We identified larval parameters indicating a local search by evaluating tracking data via FIM[5].

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Poster 169

Stabilization of β-catenin in CD97 overexpressing IECs attenuates LPS-induced pro-inflammatory signaling

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Background

Necrotizing enterocolitis (NEC) as seen in human preterms is characterized by exaggerated inflammation along with a decrease in β-catenin levels affecting enterocyte proliferation. Tissue specific overexpression of CD97 has been shown to stabilize β-catenin, thus reducing experimentally induced colitis. We studied the role of CD97 overexpressing in intestinal epithelial cells (IECs) with respect to intestinal inflammatory signaling.

Methods

Primary IECs were isolated from wild-type (Wt) or transgenic CD97 (TgCD97) mice at four different stages of intestinal development (1-8 weeks) and stimulated with 1µg/ml lipopolysaccharide (LPS) for 3 hours. Gene expression of Tnf-α and Il-1β was analyzed by RT-PCR. Phosphorylation status of p65 and total β-catenin was determined by Western Blot. The commercial inhibitor iCRT3 was used to suppress β-catenin signaling.

Results

TgCD97 IECs showed a significantly reduced gene expression of Tnf-α and Il-1β after LPS stimulation especially at younger ages. In Wt IECs, we observed an increase in phosphorylation of p65 after LPS treatment, which was absent in TgCD97 IECs. Additionally, β-catenin was negatively regulated by LPS in Wt but unchanged in TgCD97 IECs. Treatment with iCRT3 restored LPS susceptibility of TgCD97 IECs partially.

Conclusion

These results provide additional evidence that stabilization of β-catenin attenuated LPS-induced pro-inflammatory signaling and thereby may be a promising therapeutic approach for the treatment and prevention of NEC in the future.

Poster 170

Targeting phosphorylation of the keratin-desmosome complex for the treatment of Epidermolysis Bullosa Simplex

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Mutations in K5 and K14 cause the skin disorder Epidermolysis Bullosa Simplex (EBS), associated with a collapse of keratin filaments into cytoplasmic protein aggregates. This results in fragility of basal keratinocytes and skin blistering upon mild mechanical trauma. Current treatment of EBS is only supportive and consists primarily of wound care and avoidance of mechanical stress.

Numerous post-translational modifications (PTMs) such as phosphorylation or acetylation occur on keratins, controlling the re-organization of keratin networks. We and others have found that EBS-associated mutations such as K14.R125C affect keratin phosphorylation and acetylation at distinct sites and thereby can aggravate keratin aggregation and EBS severity. We identified the multi-kinase inhibitor PKC412 as a drug that promoted reformation of filaments from mutated aggregates and stimulated formation of stable desmosomes, thereby strengthening intercellular cohesion. At the molecular level, global phospho-proteomic analysis revealed key phospho-sites in keratins and desmoplakin that were reduced upon PKC412 treatment. Subsequent PamGene-based kinase profiling of PKC412-treated EBS cells identified a rapid decrease in upstream tyrosine kinase activity that reduced several serine/threonine kinases. Given that PKC412 is already in clinical use, our data advance the route toward a clinical trial using PKC412 for patients with EBS.

Poster 171

The influence of epigenetics and metabolism on TET2 activity in hematopoietic stem cells

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Imbalances between self-renewal (SR) and differentiation (diff.) of hematopoietic stem cells (HSCs) caused by clonal hematopoiesis (CH) and non-genetic factors can lead to inflammatory disease, leukemia or exhaustion of the bone marrow niche. One of the genes most frequently mutated in CH is TET2, encoding a dioxygenase that promotes HSC diff. via DNA-demethylation. This involves hydroxylation of methylcytosine using vitamin C (vit.C) as a cosubstrate and α-ketoglutarate (α-KG) as a cofactor. TET2 activity and HSC diff. may therefore be linked to the metabolic activity and environment of HSCs.

We aim to characterize the influence of cellular metabolism on HSC fate via TET2 activation. Using the murine multipotent HSC line FDCPMix we determine effects of vit. C and α-KG levels on the balance between SR and diff.

The SR potential of cells, a surrogate of stemness, is measured as the proportion of cells with colony-forming ability. Treatment effects on lineage-specific maturation are evaluated based on morphological examination by cytospin microscopy. We are measuring lineage and maturation-specific surface markers via flow cytometry. To examine the link between supplementation levels, TET2 activity and diff. we will quantify 5-hydroxymethylcytosine in genomic DNA by dot blot.

Vit. C treatment is associated with accelerated differentiation rate while α-KG treated samples have reduced proliferation. These results suggest that treatment-mediated TET2 activation enhances HSC diff. and leads to a loss of stemness, so HSCs may be maintained within a metabolically limiting niche.

Poster 172

Towards a framework for secure automated linkage of personalized data across data sources — A first example: Linking LIFE Adult study participants with the Leipzig Cancer Registry

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Medical data which has been collected in hospitals is increasingly used for medical research. The most prominent challenge in these scenarios is to overcome the heterogeneity of used data structures. This changes dramatically when different data holding organizations (DHOs) are needed to answer a medical research question. Due to the high degree of autonomy, each organization uses local identifiers to identify single persons. The reuse of present global identifiers, such as social insurance numbers, is often prohibited. A similar global identifier for research purposes does not exist yet. This hinders data integration.

We suggest a framework for linking data between independent DHOs in a privacy-preserving way. We explore the feasibility of our approach in an ongoing use case by linking identification data from the LIFE Adult study with the Leipzig cancer registry. LIFE Adult is a long-term cohort study which collects medical and self-reported data of randomly selected subjects. Cancer diagnoses fall into the category of self-reported data. LIFE Adult seeks to validate this data by linking personal information from participants who consented to record linkage with the cancer registry.

We developed several components to enable privacy-preserving record linkage on tabular data. We evaluate the linkage quality by comparing the results of our suggested PPRL workflow with proven standards. If successful, our proposed framework eliminates the transmission of identification data between data sources entirely to produce reliable linkage results without leaking sensitive data.

Poster 173

The Drosophila neck connective: bridging connectomes with light level data to solve the bottleneck between the brain and the ventral nerve cord.

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In insects, the neck connective represents a signalling bottleneck between the brain and the ventral nerve cord (VNC, spinal cord analog), with most sensory processing taking place in the former and motor control in the latter. Systematic descriptions of the diverse populations of descending neurons (DNs) sending their axons through the neck connective in Drosophila have hitherto been incomplete surveys constrained to light microscopy (LM).

With the emergence of whole brain electron microscopy (EM) datasets a dense survey of the entire fly neck connective is now possible. Here, we present the first complete description of DNs in three EM datasets: the full adult female brain, and both female and male adult nerve cords (FAFB, FANC and MANC, respectively). This comprehensive search revealed 47% more DNs than estimated in previous LM-based studies.

To date, a dataset consisting of the brain and VNC has not been available to the Drosophila community. Through systematic comparison with LM images we were able to bridge the gap between the brain and the VNC connectomes for more than half of the DN population.

In combination this data allows us, for the first time, across all available Drosophila EM and LM datasets to investigate:
1. Trace sensory-motor processing circuits from sensory input in the brain to motor output in the VNC.
2. Run comparative connectomics between individuals (hemibrain vs FAFB).
3. Investigate sexually dimorphic circuitry (FANC vs MANC).

Poster 174

3D mapping of parvalbumin interneuron-derived cortico-striatal axonal projections

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Fast-spiking parvalbumin-expressing interneurons (PV-IN) provide robust GABAergic inhibition in cortical networks, a major prerequisite for synchronized network activity tightly linked to learning and memory. PV-IN follow a protracted trajectory during brain development, including complex axonal outgrowth, and (partial) axonal myelination. Combined with a substantial energy demand due to their fast-spiking behavior, these features render PV-IN vulnerable to various cell-autonomous and external insults during neurological disease conditions. While the local axonal arborization and integration of PV-IN into cortical microcircuits is well documented, there is an emerging appreciation of a subpopulation of cortical PV-IN with long-range axonal projections to subcortical regions, including the striatum. Neuregulin (NRG)-1, an EGF-like ligand for the receptor-tyrosine kinase ErbB4, is a key regulator of peripheral myelination and GABAergic functions in PV-IN of hippocampus and neocortex, but if NRG1/ErbB4 signaling serves a function in the formation or maintenance of PV-IN-derived cortico-striatal axonal projections has not been studied. This research aims to bridge this gap in the current state of knowledge by a detailed mapping of PV-IN-derived cortico-striatal axonal projections, their local arborization, and synaptic terminals in the striatum which would serve as a framework for studies into regulatory mechanisms of axonal connectivity in the cortico-striatal pathway using mouse mutants lacking the EGF-like signaling factor NRG-1.

Poster 175

Intracranial aneurysm rupture prediction: A deep learning based approach

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Objective:
Intracranial aneurysms, found in 3-4% of the population, represent a common vascular pathology associated with a significant risk of rupture. The increasing availability of angiography has contributed to the higher detection rate of these aneurysms. Precise rupture risk assessment is crucial due to the substantial disability and mortality associated with ruptures and the notable surgical treatment risk. Our objective was to develop an automatic machine learning-based model for predicting the rupture risk of intracranial aneurysms, addressing the limitations of human evaluation and existing methods, due to the potential high disability and mortality associated with rupture.

Methods:
Analyzing 386 CTA scans (500 aneurysms, 250 ruptured, 250 unruptured), we utilized automated vessel and aneurysm segmentation to extract features. Our model incorporated deep learning-derived vessel and aneurysm shape features, along with demographic and morphological parameters. An ablation-type study assessed feature importance, and eight machine learning models were trained to identify ruptured aneurysms.

Results:
The best-performing model, utilizing a random forest algorithm with feature selection based on Spearman‘s rank correlation thresholding, achieved an area under the receiver operating characteristic curve (AUC) of 0.851. This robust performance in classifying intracranial aneurysm rupture status highlights the significance of our approach as a valuable tool for managing rupture risk.

Poster 176 A Concept for short-range wireless Biomedical-Data-Transmission via LiFi

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Research Question

The 5G-Compass Project aims to explore different wireless data-transmission technologies in various areas. Medical environments benefit from such technologies in a way that data can be provided over the whole infrastructure or directly at the point of care. In this work we present a concept for short-range wireless data-transmission of a patient monitor using Light Fidelity (LiFi).

Objective

We aim to implement a wireless data-transmission of biomedical patient data using LiFi. LiFi is a short-range data-transmission technology using infrared waves emitted from LED-lights. Since we are trying to transmit critical data, a fast and reliable connection is important.

Methods

To test the transmission for biomedical data via LiFi, we connect a patient monitor (Dräger M540) with a computer where the data is converted via service-oriented device connectivity (SDC). The PC is connected to a LiFi-Transmitter. The data is sent to a LiFi access point (AP) which has another PC connected to it. Here a SDC-Client consumes the data and it is shown in a graphical manner. The quality of the transmission will be evaluated by latency, used bandwidth and packet loss. Furthermore, the change of quality at different distances and angles of the LiFi equipment has to be evaluated.

Conclusion

In this work we describe a concept for biomedical data transmission using LiFi. We describe a demo setup using the Dräger M540-Patient Monitor. The concept will be further evaluated after the first implementation. In first tests, simulated SDC-data has been successfully transmitted via LiFi.

Poster 177 A Digital Twin Model for Evidence-based Clinical Decision Support in Multiple Myeloma Treatment

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Substantial progress has been made in the treatment landscape for multiple myeloma (MM) in the last decade [1]. To address the increasing complexity of the treatment decision, we present the development of a similarity-based multiple myeloma digital twin (MMDT), that enables simulation of probable responses based on retrospective data.

We defined and formalized a graph model with clinical parameters associated with the characterization of MM patients as well as evaluation of therapeutic regimens using the resource description framework. With the instantiation of the model, a snapshot of one particular patient state in the context of a specific therapy line can be defined. To do so, treatment response and disease course is simulated through a twin cohort derived from a real-world evidence database. To test the model and present the resulting evaluation panel, we defined an initial evaluation scenario using the MMRF CoMMpass database [2].

Our derived knowledge graph is defined by 475 unique entities connected through 438 edges. The evaluation of the test cases resulted in a graphical panel showing a representative distribution of potential outcomes from multiple perspectives in first-line MM treatment.

The development of DT models faces significant challenges, including the availability of clinical data and the trustworthiness of evaluations [3]. Focusing on explainability and interpretability, we propose a collaborative approach to address regulatory and ethical concerns associated with incorporating automated decision-making tools into clinical routines.

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Poster 178 Synthetic Data for Applications in Precision Medicine

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The adoption of electronic health records (EHR) increased highly during the last decade. The exploitation of the emerging data motivates the use of data-driven techniques from the fields of statistics and machine learning as integral parts of many healthcare applications.
However, the access to the EHR data is often limited due to privacy concerns, ethical considerations, and the sensitive nature of patient information.

This lack of data availability constitutes an obstacle for the development of robust and reliable models and obstructs the reproducibility of published results
and scientific exchange in general. Synthetic data generation techniques are one way to overcome accessibility restrictions and can further increase the robustness of the models to out-of-distribution data.

We generate synthetic EHR data with generative Variational Autoencoders, making use of several extensions like the Importance Weighted Autoencoder Objective and Inverse Autoregressive Flows.
We try to improve data quality by post-hoc filtering techniques and evaluate the resulting data by model-specific techniques such as Marginal Likelihood Estimation via Importance Sampling and model-agnostic approaches such as Manifold Estimation Techniques and machine-learning based techniques for feature prediction and discrimination between real and synthetic data.

We use the generated data for the training of further downstream algorithms for decision support and personalized medicine, such as mortality prediction of colorectal and larynx cancer patients.

Poster 179 KAIT - a clinical decision support platform for multiple myeloma

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Background

In a time of increasing amounts of scientific data it becomes more and more difficult for medical professionals to stay up to date. The human brain is not capable of processing gigabytes of data and is prone to bias. Many institutions gather patient data that could be evaluated and used to improve the treatment of future patients. The KAIT platform aims to make use of this data and alleviate problems by supporting hematologists in making treatment decisions. [1] [2]

Methods

Users are able to add structured patient data to the KAIT platform. In return, the treating physician receives a list of relevant publications from the PubMed database. KAIT also processes the input data using clinical practice guidelines and searches a real-world evidence (RWE) database of myeloma patients for similar cases. KAIT then calculates a result object with a list of treatment options suitable to the input patient and highlights conflicts and warnings if a treatment option contradicts pre-existing conditions. A Sankey diagram visualizes the decision path from input data to the resulting therapy evaluation.

Results

KAIT as an IT platform helps manage processes of diagnostics and success evaluation of treatments. It uses the HL7 FHIR standard, helps streamline literature search and data management, and allows planning of decentralized tumor board meetings.
The long term goal is to improve and simplify the decision making process for treating physicians.

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Poster 180 AI-based white-matter lesion detection in multi-protocol brain magnetic resonance images

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Background:
Magnetic Resonance Imaging (MRI) plays a crucial role in detecting White Matter Lesions (WML) associated with diseases like Multiple Sclerosis (MS) and Leukodystrophies. Integrating Artificial Intelligence (AI) enhances MRI analysis and disease diagnosis. However, the emphasis of the literature on using data from the same scanner and acquisition protocol limits the AI-based analysis of the data available in medical centers. Existing MRI harmonization methods often demand extensive MRI data and lack generalizability across diverse diseases. A universal harmonization approach is yet to be established.

Method:
This work introduces an innovative approach for WML detection in multi-protocol brain MRI data using intensity clustering. The approach is scanner-independent, allowing flexible analysis of diverse datasets. FLAIR images undergo preprocessing, and a part of the white matter is used to train a convolutional neural network model. The model, as a binary classifier, identifies brain MRIs containing WMLs. The applicability of the method is demonstrated on datasets with limited data, to simulate rare diseases.

Results:
Utilizing MS and control MRI data from 9 datasets with over 30 protocols, the model achieves an average classification accuracy of 90% when trained on 102 MS MRIs. By reducing the MS data to one-third, this accuracy decreases by 15%.

Outlook:
The presented method unlocks new possibilities for analyzing multi-center and multi-protocol MRI data. Future work involves applying a similar approach to classify a rare disease versus its differential diagnoses.

Poster 181

Towards a Treatment Recommendation System for Patients with Liver Cancer - AI-based Liver Cancer Segmentation in Computed Tomography Scans

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Liver cancer is one of the most prevalent global cancer diseases. Germany has about 5000 hepatocellular carcinoma (HCC) yearly. While in large tertiary care companies such as university hospitals, procedures incl. surgeries and other minimally invasive treatments are prevalent for these diseases, hospitals in the landscape often lack the knowledge and ability for the most helpful therapy approaches. Recommending the right therapy for a specific cancer setting requires first to build profiles taking different features into account, including current therapeutic approaches and, secondly, to classify a single patient to one of these profiles. We are currently working on the profiling. A step necessary in this process is to find liver cancer compartments in computer tomography (CT) scans. The goal is to use the layout as a feature set and group them into classes (profiles) with common properties. Finding and describing cancer compartments requires segmentation. We applied different deep-learning methods to detect and segment cancer compartments automatically. These include Unet, DenseUnet, and ResUnet. Our current test uses publicly available data sets, including LiTS'17, MEDSEG, and 3D-ircadb-01. Altogether, these data sets include 19,156 CT images in DICOM format. Our first results are promising, particularly on average dice coefficient score (according to the masked image compartments on a voxel basis) of 88% to segment the liver in the image and 71% to segment cancer compartments. While it is feasible for most classification tasks later on, it shows room for improvement.

Poster 182 Evaluation of telemedic supported preclinic performed ultrasound

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Only a small number of paramedics are trained in emergency sonography, but mobile ultrasound devices offer new possibilities for initial assessments. This work aims to evaluate if preclinical ultrasound exams supported by tele medics generate a benefit for the preparation of trauma rooms and what tools are necessary for telemedic supported ultrasound exams in rescue operations.

We used a Clarius C3 HD3 to transmit ultrasound video data from ambulance to trauma room dashboard with controls for zoom, gain and image freeze. For the communication between paramedics and trauma team we used a one directional video feed of the ambulance interior and bidirectional audio.

A rescue operation of a road accident was simulated. Paramedics checked for critical injuries, established communication with the hospital, transmitted patient information and did a sonography for pneumothorax as well as free fluid in abdominal cavity with the help of a practitioner from the trauma team.

In the subsequent expert discussion and survey out of 8 paramedics and practitioners 85% fully agreed with: “I can imagine contacting a tele-emergency doctor in difficult situations”, 85% tended to agree that the live transmission of an ultrasound image from the ambulance to the hospital offers added value and 57% fully agreed that an audio/video connection between the ambulance and the clinic would offer added value, while 43% tended to agree. The expert feedback was overall very positive. One major critic point was, that the usefulness of ultrasound exams strongly depends on the rescue scenario and time constrains.

Poster 183

Analysis of Current Statistical Models for Reliable Uncertainty Quantification in Predictive Medicine

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With the growing adoption of electronic health records (EHR), medical decision making becomes an increasingly complex process reaching the limits of human comprehension. The expanding application of AI promises remarkable strides in terms of improved medical care and efficiency. Next to raw predictive performance, the critical nature of medical decisions makes reliability and trustworthiness of automated inference methods a crucial requirement. This hinders widespread AI adoption due to safety and ethical concerns.

Among the latter is the overconfident behavior under data shift widespread Deep Learning architectures suffer from. This can lead to an unacceptable false sense of security when extra- or interpolation beyond the scenarios well supported by evidence. While this is known for simple architectures, purpose built stochastic deep learning methods implement the estimation of local uncertainty with any given prediction.

We analyze the uncertainty estimation using Bayesian neural networks (BNN), Ensembles (ENN) and Gaussian Processes (GP). Our method centers around the exemplary use case of mortality prediction utilizing Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial data.

Our study shows that safety-critical overconfidence remains an often-undetected problem in BNN and ENN while showing the favorable behavior of GP. Our study highlights the special considerations needed for medically sound uncertainty prediction helping to foster trust within the medical community.

Poster 184 Laparoscopic Hyperspectral Imaging (HSI) of peritoneal lesions

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The assessment of the extent of the tumor burden during a surgical intervention remains a challenging task. In order to be able to make a precise statement about this, it is crucial to distinguish between malignant and physiological tissue, which can sometimes be difficult even for experienced surgeons. To assist surgeons in their evaluation, new diagnostic tools are required. Hyperspectral Imaging (HSI) appears to be a promising novel intraoperative method for this purpose as it combines non-invasive tissue spectroscopy and digital imaging. This study examines the suitability of HSI for the detection of peritoneal carcinomatosis and therefore the discrimination of malignant peritoneal nodules from non-malignant lesions.

By performing HSI measurements on in-vivo peritoneal tissue with the TIVITA®Mini system (Diaspective Vision GmbH, Am Salzhaff, Germany), data from 29 patients and 73 different lesions were acquired during laparoscopic or open abdominal surgery. Four different methods of machine learning (SVM, LDA, LR, and MLP) were evaluated for the classification of physiological and malignant tissue in a Leave-One-Patient-Out Cross-Validation (LOOCV). These assessments are compared against the surgeon's evaluation of the findings, and with histopathology serving as the gold standard.

Hyperspectral Imaging combined with machine learning is suitable for automatic tissue classification of peritoneal malignancies and could therefore support surgeons in tissue assessment and clinical decision-making.

Poster 185

Individual modelling of haematotoxicity with NARX neural networks: A knowledge transfer approach

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Cytotoxic cancer therapies frequently result in severe haematotoxic side-effects. Predicting a patient's haematologic response to treatment is of high clinical relevance but is difficult due to between-patient heterogeneity. While several (semi-) mechanistic models of bone marrow hematopoiesis have been developed to solve this task [1][2][3], the established models could not sufficiently describe certain patients exhibiting irregular dynamics [1][2]. Here, we propose a data-driven hypothesis-free machine learning approach to model individual patient's time courses. We apply recurrent neural networks based on non-linear autoregressive exogenous (NARX) models to describe the highly non-linear dynamics of haematologic lineages under chemotherapy. To cope sparsity of individual patient data, we implement a transfer learning approach and employ several model reduction techniques. We evaluate this framework on a virtual patient population generated with a semi-mechanistic model of Friberg et al. [3]. First, the dynamics of an index patient are learned. From this, for each virtual patient a personalized prediction model is derived via finetuning. This transfer closely resembles possible real-world applications. We provide an example of predicting individual haematotoxicities of selected patients. We observe good generalization performances in the virtual patient population and for the selected patients. We conclude that NARX modelling can provide robust predictions of an individual patient's response to treatment, and therefore, can serve as an alternative to mechanistic modeling.

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Poster 186 Prediction of unplanned intensive care unit readmission

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Hospital readmission is a crucial measure of hospital performance and quality of care. High readmission rates can adversely affect hospitals, posing significant challenges for the healthcare system. These include negative impacts on patient health outcomes, such as increased morbidity and mortality, as well as personnel overload and financial resource depletion. Hospital readmission rates in the US can reach up to 22%. However, research suggests that many hospital readmissions can be prevented, highlighting the importance of predicting such events. The abundance of electronic health record (EHR) data in the intensive care unit (ICU) has facilitated the use of machine learning (ML) models to address readmissions to the ICU.

This study employs ML techniques to predict unplanned ICU readmissions within a 30-day period after the initial discharge for all ICU patients, without emphasizing specific diseases. The publicly accessible Medical Information Mart for Intensive Care IV (MIMIC-IV) dataset has been used. Specifically, the aim is to develop conventional ML models that employ commonly available patient demographics, vital signs, and laboratory measurements collected within 48 hours prior to discharge for prediction. Furthermore, to enhance the model performance deep learning methods are utilized that incorporate temporal details embedded in the sequential data frequently recorded during hospitalization. The findings demonstrate that conventional ML models achieved promising results, while complex deep learning models required further data preparation.

Poster 187

An Automatic Pipeline for Efficacy Evaluation of Brain-MRI Defacing

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Sharing medical imaging data, such as structural magnetic resonance imaging (MRI), for research purposes presents significant privacy risks due to the potential for facial recognition of patients, which could potentially violate the General Data Protection Regulation (GDPR). Advancements in scan quality and facial recognition software further increase the risk of re-identifying patients via 3D rendering of MRI scans, and highlight the inadequacy of removing sensitive metadata to protect patient privacy. Defacing algorithms are tools to mitigate the risk of re-identification in MRIs by removing facial elements. Nonetheless, despite their complexity, they are susceptible to errors and may occasionally leave certain facial features detectable.
This study aims to develop machine learning models that can assess the effectiveness of defacing algorithms by identifying similarities between scans obtained before and after defacing.

This method can automatically classify a defaced MRI scan as either accepted or rejected. The model was implemented on around 200 MRI scans from the Leukodystrophy registry of the clinic and polyclinic for neurology at the University of Leipzig Medical Center, utilizing three commonly used defacing software programs. The results demonstrate the promising performance of ML models in distinguishing between successful and improperly defaced MRIs, achieving 80% accuracy, 82% sensitivity, and 79% specificity. The model can be integrated into the image processing pipeline for subsequent analysis, such as segmentation.

Poster 188

MARS: Multiuser Augmented Reality System. Identifying Requirements to enable Multiuser capabilities among AR Applications

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This work is dedicated to the analysis of augmented reality (AR) system requirements to identify elements that must be appended to existing multiuser engines (MEs) to enable colocated multiuser AR applications. The focus lies on synchronizing user and hologram locations among multiple participants in the same room.

Method

The methodology focused on identifying key elements across AR and multiuser networking to synchronize holograms and user positioning. The architecture of a prototypical AR application for the Microsoft HoloLens 2 and based on the Unity game engine 2020.3.48f1, along with the Photon ME and Mixed Reality Toolkit 2.8.3, was evaluated to determine features necessary for a shared experience.

Results

Two key features of colocated AR were identified: the persistence of objects in space and the shared user position among all participants. Holograms persisted in their position in space, and QR-Code tracking was perceived as accurate. However, the spatial maps of distinct users acquired via SLAM varied substantially due to differences in user movement, as did the origin of the coordinate system. Current AR devices and MEs set the origin at application launch and do not yet support its adjustment at runtime.

Discussion

It was concluded that establishing a common reference point, which is to act as the origin of the virtual room in real space, is the basis for enabling shared AR experiences. This dynamically adjustable reference point can be shared via QR code detection. User movements and hologram positions will then be synchronized with regard to this reference point.

Poster 189 Deep learning in acute stroke care: predicting outcomes of thrombectomy

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The introduction of mechanical thrombectomy has significantly improved outcomes for stroke patients with proximal vessel occlusion. In the extended time window (> 6h after onset), patient selection primarily relies on thresholded CT perfusion maps. While this approach is the current clinical standard, it might not fully exploit the potential of multimodal CT data. Machine learning techniques, in contrast, have proven their ability to capture complex patterns in high-dimensional data and promise more accurate predictions.

Our work aims to develop and validate a deep learning approach for improved prediction of final infarct extent depending on thrombectomy success. We present a convolutional neural network (CNN) featuring a 3D multi-path hybrid-fusion architecture and attention units. Once trained, this model utilizes acute patient data to predict individual tissue outcomes in both successful and unsuccessful thrombectomy scenarios. To train and evaluate the model, we deployed a multi-center dataset of 405 stroke patients with acute proximal vessel occlusion in the anterior circulation who underwent mechanical thrombectomy.

Compared to two methodological baselines (clinical thresholding and generalized linear model), our CNN demonstrated significant superiority with a mean Dice similarity coefficient of 0.50, versus 0.31 and 0.34, respectively. Given its accuracy, discriminative power, and generalizability, our approach may provide an individual biomarker in acute stroke care, aiding in the future in selecting patients for thrombectomy in the extended or unknown time window.

Poster 190

Evaluation of different techniques for uncertainty quantification in machine learning for medicine

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Machine learning, particularly in the realm of medicine, has emerged as a transformative method, offering new insights and efficiencies in patient care and disease diagnosis. However, this innovation is not without its challenges; the adoption of machine learning in medicine, a field fraught with safety-critical and ethical considerations, necessitates precise uncertainty quantification to ensure reliable and ethical decision-making in such high-stakes environments. This underscores the critical need for effective uncertainty quantification, ensuring reliable and transparent decision-making in healthcare applications. In recent literature, many methods have been proposed to tackle the problem of uncertainty quantification in machine learning methods [3] [2] [1] . In this work, we evaluate a specific set of requirements for uncertainty quantification in the medical domain. We compare different methods from literature using a set of suited benchmark evaluations designed to measure how effectively the requirements can be fulfilled by the various methods. The evaluation makes use of toy-, synthetic medical-, as well as publically available medical datasets.

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Poster 191

Machine Learning for Assessing Gene-Environment Interactions in Parkinson's Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease and the one with the fastest-growing incidence. So far, a wide range of risk factors for PD have been identified. Among them are both genetic defects and environmental factors, to which a person is exposed over the course of her life. Due to the complexity of the disease, the risk for PD cannot be sufficiently described by individual factors alone. Instead, it is determined by the interaction of a wide range of factors. New algorithms in the field of machine learning provide the opportunity to identify patterns within complex structures, offering great potential to gain new insights into how various factors interact and influence the risk of developing a disease.

To understand what insights new algorithms can provide, we explore a range of state-of-the-art ML and deep learning (DL) models in assessing the risk of PD. We employ shapley values, a mathematical framework based on cooperative game theory, to estimate the impact that one or more factors have on the prediction outcome of a model to show gene-environment interactions in the UK Biobank, a large research resource encompassing over 500.000 individuals from the UK.

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Poster 193

CT texture analysis of Klatskin tumors – associations with tumor grading, tumor markers and clinical outcome.

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Purpose:
Texture analysis derived from computed tomography (CT) might be able to provide clinically relevant imaging biomarkers and could be associated with histopathology features in tumors. The present study sought to elucidate possible associations between texture features derived from CT images with grading, tumormarkers and survival in extrahepatic, perihilar cholangiocarcinomas.

Materials and methods:
22 patients (n=10 females; 45%, mean age of 71.8 ± 8.7 years) were included into this retrospective study. Texture analysis was performed using the free available Mazda software. All tumors were histopathologically confirmed.

Results:
In discrimination analysis, "S(1,1)SumVarnc" was significantly different between patients with long-term survival and non long-term survival (mean 275.80 ± 32.57 vs. 239.65 ± 26.03, p=0.01). The first-order parameter "Skewness" showed a statistically significant association with the tumormarker Carcinoembryonic antigen (r=-0.66, p=0.01). Also, a significant correlation of the texture parameter "S(5,0) SumVarnc" with tumor grading (r=-0.587, p=0.004) was identified. Several other texture features were correlated with the tumormarkers "Ca-19-9" and "AFP" and with T- and N-stadium of the tumors.

Conclusion:
Several texture features derived from CT were associated with tumor characteristics and survival in patients with extrahepatic cholangiocarcinomas and therefore could provide novel biomarkers in clinical routine.

Poster 194

An immunohistochemical analysis of Basal Cell Carcinoma in younger patients

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Basal cell carcinoma (BCC) is the most common skin cancer, mainly occurring in the elderly. At the same time, BCC in younger patients is rare, however, incidence is rising and studies reflecting alterated cell cycle and immunogenity are scarce.

We analysed 57 BCC of 50 patients with a median age of 35 years. Besides histomorphological evaluation, we performed immunohistochemistry focusing on cell cycle, immunogenity and HER2-expression. BCCs were subclassified in aggressive and non-aggressive growth pattern.

26% belonged to the aggressive, 74% to the non-aggressive subtype. P53-alteration was found in 42%, p16-alteration in 12%. P16-alteration lead to higher expressions of Cyclin E1, stronger Ki67-staining and was significantly more enriched within the aggressive subtype. P53-affected BCC did not show any significant correlation. Aggressive BCC with p16 alteration showed higher intra-tumoral staining of Cyclin E1, but lower rates of T- and B-cells in the surrounding tumor stroma. 28% were Her2-negative and 72% showed Her2-low expression, which was underlined by in-situ hybridization. Her2-low BCC showed stronger expression of Ki67 and Cyclin E1 in the tumor and higher rates of T-cells in the surrounding tumor stroma.

This is the first IHC-analysis in BCC of the younger patient. Aggressive BCC in the younger seem to be p16-enriched. Its tumorigenesis might be inter alia explained by an over-activation of CyclinE1 and a low immunogenity. Our preliminary data might suggest Her2 as a good discriminator, as Her2-low BCC show higher proliferation rates and T-cell enrichment.

Poster 195 Detection and Characterization of Disseminated and Circulating Tumor Cells in Cervical Cancer

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Introduction:
Despite initial therapy, 30% of cervical cancer patients experience recurrence.[1] While breast cancer research links disseminated and circulating tumor cells (DTCs, CTCs) to poor outcomes, their prognostic significance in cervical cancer remains uncertain.[2][3] Our study addresses this gap by detecting and characterizing CTCs and DTCs, including cells undergoing epithelial-mesenchymal transition and those expressing therapeutic markers.

Methods:
Blood samples from 43 patients were obtained pre-therapy and during two years of follow-up, while bone marrow was aspirated during surgery. Epithelial CTCs and DTCs were detected using immunocytochemical staining (ICC) against cytokeratin (CK). For DTC characterization, multi-parameter immunofluorescence (IF) staining was developed, allowing simultaneous analysis of various markers on the same cell, including epithelial (CK), mesenchymal (vimentin), HPV-associated (p16) and therapeutic markers (PD-L1, VEGF), along with CD45 leukocyte and DAPI nuclear staining. Matching tumor tissues, obtained at diagnosis, were also stained against p16, VEGF and PD-L1.

Results:
ICC staining revealed a 55% positivity rate for epithelial DTCs (n=42), 16% for epithelial CTCs pre-therapy (n=43), and 16% during follow-up (n=38). Positive control specimens for IF were established using CaSki (CK, PD-L1) and HeLa (VEGF, p16) cervical cancers, and T98G glioblastoma cell lines (vimentin).

Outlook:
IF analysis of patient samples is ongoing. We aim to correlate distinct cellular profiles with clinical data to identify potential prognostic DTC markers.

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Poster 196 Genome wide high resolution SNP array analyses of matched pairs of brain and liver metastases in primary colorectal cancer

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In colorectal cancer (CRC) patients, brain metastases formation is a rare and late event and is associated with poor survival. Deeper insights on chromosomal aberrations in brain metastases are still very limited compared to other metastatic sites. Therefore, we performed high resolution single nucleotide polymorphism (SNP) array using OncoScan® FFPE Assay Kit and the software Chromosome Analysis Suite (ChAS). The cytogenetic analyses were performed on matched primary CRC and brain metastases of four patients as well as on liver metastases of three patients. Remarkable, we identified more chromosomal aberrations in brain metastases compared to primary tumor and liver metastases. The detected gains on 8q11.1-q24.3 (primary CRC), gain of 13q12.13-q12.3 (liver metastases), and gain of 20q11.1.-q13.33 (brain metastases) are commonly occurring aberrations. Furthermore, we revealed more copy-neutral losses of heterozygosity (cn-LOH) regions in brain metastases (23 cn-LOHs) compared to primary tumor (one cn-LOH) and liver metastases (3 cn-LOHs), whereby 26 of these cn-LOH aberrations are not previously described. Interestingly, these cn-LOH results could provide more information on the processes of brain metastases formation in CRC. Nevertheless, further genetic analyses on primary CRC tumors and their matched metastases including liver, lung, and brain metastases are necessary to learn more about the significance of cn-LOH regions and the potential affected genes.

Poster 197 Investigation of the desmoplastic peritumoral microenvironment in cervical cancer

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Background:
Desmoplastic alterations in the tumor microenvironment, characterized by increased collagen deposition from cancer-associated fibroblasts (CAF), are often linked to poor outcomes[1]. While in other solid cancers the role of the Angiotensin-II-type 1 receptor (AT1R) in CAF regulation has been highlighted, desmoplasia in cervical carcinoma has barely been described[2]. This retrospective study aims to investigate desmoplastic remodeling in cervical cancer.

Methods:
To quantify the extent of desmoplasia, we performed HE and Pikro-Sirius Red (PSR) staining of FFPE tumor tissue from 100 patients with stage I or II cervical cancer. Immunohistochemistry is used to quantify AT1R expression. Stained tissue slides are digitalized and subsequently subjected to image analysis. The extent of desmoplasia and AT1R expression will be linked to clinical outcomes.

Results:
Of the 76 squamous cell and 24 adenocarcinomas, the presence and degree of desmoplasia was determined in 67 cases by trained pathologists using HE stained tissue. Digitalized PSR slides and deep learning methods were used to develop an algorithm that quantifies desmoplasia based on stained collagen. Protocols for AT1R and CAF marker staining were established using validated controls such as liver tissue.

Outlook:
Artificial intelligence based quantification of desmoplasia could improve manual assessment by pathologists. Objective estimation gains significance, particularly if our research shows a correlation between AT1R expression and the extent of desmoplasia, providing a basis for targeted therapeutic intervention.

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Poster 198

Molecular classification of vulvar cancer: Comparing pre-surgical biopsy and surgical specimen of radical vulvectomy from the same patients

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Background:
Classification of vulvar carcinoma (VC) into molecular subtypes has significant prognostic and therapeutic impact [2]. An HPV-associated lesion (p16+/p53wt) represents improved prognosis compared to other subtypes and better response to chemoradiation. It has been shown that patients with HPV-independent VC, mostly associated with lichen sclerosus (p16-/p53mut) may benefit from more radical surgical approach [1]. A third subtype is characterized by p16-/p53wt staining. The aim of this study was to determine the concordance of molecular subtyping on diagnostic biopsy compared to the surgical resection specimen.

Methods:
56 matched pairs of VC biopsies (Bx) and its surgical resection specimens (vulvectomies) were blinded and immunohistochemically evaluated for the expression of p16 and p53. Doubtful staining results were molecularly analysed for HPV-DNA and/or TP53-mutation.

Results:
Matched pair analysis represented a high agreement for molecular subtyping of VC comparing Bx and vulvectomy: 93.7% (16/17 cases) for the p16+/p53wt cases; 94.4% (34/36) for p16-/p53mut. Within the group of p16-/p53wt VC, there was a lower concordance (50%; 2/4 cases).

Conclusion:
There is a high concordance rate (~94%) between Bx and surgical resection specimen for the two major subtypes when using expression of p53 and p16. By accurate subtyping of the VC before curative treatment, the prognostically best treatment approach can be chosen for the patients. So, molecular subtyping of VC within diagnostic biopsies represents a robust tool to tailor the treatment approach of patients with VC.

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Poster 199 Multiparameter Immunofluorescence Analysis of Disseminated Tumor Cells in Triple-Negative Breast Cancer

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Background:
Despite advances in the treatment of breast cancer, 30% of patients face recurrence. Disseminated tumor cells (DTCs) migrating to bone marrow (BM) are potential relapse causes. Current detection methods mainly target epithelial DTCs, but our previous research in hormone receptor-positive breast cancer revealed mesenchymal DTCs associated with poor prognosis[1]. Triple-negative breast cancer (TNBC) is especially challenging due to its aggressiveness and adverse outcome. To identify (non-)epithelial DTCs as well as potential therapeutic markers, we use a sequential staining and detection approach.

Methods:
BM aspirates were collected from primary (n=67) and recurrent (n=13) TNBC patients. We established a multiparameter immunofluorescence (IF) staining method using releasable antibody-fluorochrome conjugates to determine multiple markers simultaneously on the same cell, including epithelial (cytokeratin (CK)), mesenchymal (vimentin) and proliferation markers (Ki67) next to therapeutic targets (HER2, estrogen receptor (ER)).

Results:
Immunocytochemical staining showed DTC positive rates of 44.8% in primary and 46.2% in recurrent TNBC patients. For the IF, positive controls were established using the epithelial breast cancer cell line ZR75-1 (CK, Ki67, ER, HER2) and the mesenchymal glioblastoma cell line T98G (vimentin).

Outlook:
We will analyze bone marrow aspirates from 80 TNBC patients. By correlating our findings with clinical data, we aim to identify potential prognostic DTC features, such as subpopulations and therapeutic targets, for the challenging-to-treat TNBC.

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Poster 200

NANOVISION: Development of HNSCC-specific markers for visualization and surgical removal in head and neck oncology

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This work aims to establish a workflow of removing tumor tissue from HNSCC (Head and neck squamous cell carcinoma) patients during ENT surgery with the help of tumor-specific surface markers. Of particular interest are FAP (fibroplast-activating protein) and EphrinA2 to determine a suitability for intraoperative tumor imaging. Both are known overexpressed receptors/targets on the HNSCC-cell-surface and give additional information about the EMT.

Method

Specimen collection took place during surgery in 27 ENT-patients pre-diagnosed with HNSCC. Samples were dissected and enzymatically digested before examination using Flow Cytometry and qRT-PCR. The tissue was dissected using a combination of mechanical dissociation and enzymatic degradation. After dissociation, cells were washed, counted and checked for viability using propidium iodide staining.

Results

In samples, there are differences in choosing digestion media. Using Miltenyi kits resulted in higher viability (up to 90%). Using a Kollagenase IV digestion, FAP was expressed in approximately 10% of tumor cells, whereas EphA2 was expressed at RNA but no longer detectable at protein level. Results with Miltenyi Kits are incoming.

Discussion

We established a reproducible workflow including patient identification, sample collection, processing and cell analysis. Cell viability with the new dissociation method is higher. Contrary to expectations, EphA2 was not overexpressed as a surface protein, whereas FAP was at 10% of the cells. These findings help, having more viable cells to develop binding methods enabling tumor cell detection.

Poster 201

Oncogenic and tumor microenvironmental role of INHBA and TREM2 in esophageal adenocarcinoma

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Background:
It has been reported that basement membrane (BM) significantly affects clinical outcomes of patients with esophageal adenocarcinoma (EAC). This study aimed to explore the BM related genes (BMRGs) signature to predict the prognosis of EAC.

Methods:
A series of bioinformatics analyses were conducted based on TCGA and GEO dataset (GSE19417). Lasso regression, ROC curve, ssGSEA, CIBERSORT and ESTIMATE algorithm were employed to understand the relationship between the tumor microenvironment (TME) and risk scores. Finally, INHBA was explored in EAC cells in vitro.

Results:
A total of 48 prognostic BMRGs were identified, and a novel of 2-genes (INHBA and TREM2) prognostic model was constructed to predict the survival of EAC patients. Patients in the high-risk group had poor prognosis and TME analysis showed that the stromal score, immune score and Estimate score in the high-risk group was significantly higher than that in the low-risk group. Single cell clustering analysis showed that INHBA was mainly expressed in fibroblast and myeloid cells and TREM2 was mainly expressed in myeloid cells in tumors. In vitro experiments showed that inhibition of INHBA expression significantly reduced the viability of EAC cells.

Conclusion:
INHBA and TREM2 were found to be significantly in predicting the prognosis of patients with EAC, and correlated with immune cell infiltration especially for M2. Inhibition of INHBA expression significantly reduced the viability of EAC cells.

Keywords:
Esophageal adenocarcinoma; Basement membrane; Signature; Immune microenvironment; Single-cell

Poster 202

Validation of four miRNAs as reference in human plasma samples from prostate, breast and colorectal cancers patients

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MicroRNAs (miRNAs) are promising biomarkers for the diagnosis and prognosis of various diseases. Quantitative PCR is the most frequently used method of measuring expression levels of miRNA. To our knowledge, there is no widely accepted reference to use miRNAs as biomarkers for diagnostic, prognostic or predictive qualities in human plasma samples.

Four hundred and thirty plasma samples from prostate, breast and colorectal cancer patients were used. Isolation of RNA (miRNeasy Serum/Plasma Advanced Kit, Qiagen) and synthesis of cDNA (miRCURY LNA RT Kit, Qiagen) were performed accordingly to the manufacturer's protocols. Four candidate miRNA reference genes including miRNA-16-5p, miRNA-484, miRNA-222-3p and miRNA-107 were measured by qPCR (miRCURY LNA Probe PCR Kit, Qiagen and miRCURY LNA miRNA Probe PCR Assays, Qiagen) and their expression stability was verified by five algorithms (geNorm, Normfinder, BestKeeper, comparative deltaCt, and RefFinder) using qPCR, which allowed a ranking of miRNA expression stability.

All reference miRNAs could be detected in all 430 human plasma samples used. MiRNA-484 was the most stable one across all 430 samples, followed by miRNA-222-3p, miRNA-16-5p, and miRNA-107. MiRNA-484 was found to be the most stable miRNA in all analyses, except using BestKeeper.

The used algorithms are powerful tools to validate the stability of the reference gene expression for miRNA analysis in human plasma. Our analyses reviled miRNA-484 as the most stable reference miRNA. However, it is recommended to use a panel of two to three miRNA for normalization.

Poster 203

Prediction of QoL of head and neck cancer patients after intervention based on clinical parameters

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Survivors of head and neck cancer (HNC) treatment often confront unique challenges linked to the cancer‘s specific location, impacting their quality of life (QoL) and increasing the likelihood of mental disorders. This raises the question of whether certain therapies affect both physical and psychological well-being.

For a proof of contept-study, we analyzed data from 960 HNC patients obtained through the „OncoFunction“ tool during post-treatment surveillance. The dataset included patient-reported outcome measures (PROMs) on physical and mental states, clinical parameters, and lifestyle variables. The PROMs included mental aspects (depression, anxiety, fatigue, and QoL) and physical parameters such as pain, swallowing issues, and tracheotomy. Correlations between PROMs, demographic, and clinical parameters (such as gender, age, HPV-status, type of treatment, TNM-stages, ICD- and UICC-classification) were analyzed. Using a Deep Bayesian Network (DBN) to predict PROMs of QoL post HNC treatment, our method yielded AUC values of 0.79 and 0.80 for poor and good overall QoL, respectively. Notably, AUC values of 0.95 and 0.85 were achieved for predicting depression and pain levels, respectively.

Thus, our study confirms relationships between clinical parameters and treatment decisions, and overall QoL of patients during follow-up.

Poster 204

Incidence of mental illness following cancer diagnosis: A nationwide population-based cohort study in Denmark

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Background:
Psychiatric morbidity is prevalent among cancer patients. However, evidence on the downstream impact of a primary incident cancer diagnosis on the development of mental illness is limited. Population-based data encompassing all tumor entities with real-world psychiatric diagnoses are needed in order to draw valid conclusions about the dimension of this clinical problem.

Methods:
Incidence rates of mental illness, calculated by psychiatric diagnosis and use of psychotropic medication, will be determined in a Danish nationwide sample of primary cancer patients diagnosed between 1996 and 2015. Cox proportional-hazard models will be used to estimate the hazard ratio (HR) for mental illness in cancer patients compared to matched cancer-free controls, adjusted for age, gender, socioeconomic status and comorbidity. Incidence rates and comparisons will be analyzed across different cancer sites and subtypes of mental illnesses.

Results:
Data linkage between registries and data analysis will be executed by access to data via Statistics Denmark and is projected to be completed by December 2023.

Conclusion:
This nationwide population-based study will provide real-world data on new-onset mental illness following a cancer diagnosis and will thus contribute to gaining new insights on the actual risk for cancer patients to develop psychiatric disorders. Stratification by tumor type will enable the identification of high-risk cancer patient groups, informing clinical oncologists and enhancing psychotherapeutic and psychiatric treatment planning in oncological institutions.

Poster 205

How does dyadic coping of cancer patients and their partners affect quality of life? – a longitudinal study

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Purpose

Coping of couples with one partner facing the diagnosis of cancer is considered as a mutual dyadic process (dyadic coping, DC). The different dimensions of DC can potentially influence the quality of life (QoL) of patients as well as their partners. We investigate the relationship between coping in patient-partner dyads after newly diagnosed cancer and QoL of those couples six months after.

Methods

Data were collected as a part of the prospective multi-center cohort study "Prevalence of mental disorder, psychosocial distress and need for psychosocial support in cancer patients and their relatives stratified by biopsychosocial factors". We report the first measurement time point within < 8 weeks after diagnosis of a solid cancer (t1) and the second 6 months after t1 (t2). Validated questionnaires (Dyadic Coping Inventory, dimensions of DC: positive, negative, delegated, common; Short-Form Health Survey SF-8, subscales of QoL: physical, mental) were used. Data were analyzed using actor-partner interdependence models (APIM).

Results

Our sample comprises the results of 182 couples (patients: 64.3% men; average age 60.5 years; 81.3% married). Most prevalent cancers were prostate (34.6%), breast (14.8%) and skin cancer (7.7%). Data are currently being processed. Relationships between QoL and the dimensions of DC will be presented at the festival.

Conclusion

We aim to determine the extent to which different dimensions of DC of cancer patients and their partners have an impact on the QoL of the couple. Implications to improve psycho-oncology care will be discussed at the festival.

Poster 206

Prognostic significance of Measurable Residual Disease assessed by a novel NGS-based assay in Acute Myeloid Leukemia patients undergoing allogeneic stem cell transplantation

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Relapse is the main reason for poor outcomes of patients (pts) with acute myeloid leukemia (AML). Allogeneic hematopoietic stem cell transplantation (HSCT) is the preferred post-remission treatment in pts at high relapse risk. Measurable residual disease (MRD) during and after therapy is an important predictor of relapse.

We established a targeted NGS-based MRD assay using single-molecule molecular inversion probes (smMIPs) covering commonly mutated genes in AML. Computational error correction allows a sensitivity of ≥0.5% allele frequency.

We evaluated MRD status on pre-HSCT bone marrow samples from 98 pts who underwent HSCT in 1st or 2nd remission. Overall, we detected 190 variants in 71 samples. The most commonly affected genes were DNMT3A (37%), TET2 (21%), PPM1D (12%), IDH2 (10%) and TP53 (5%). While 29% of pts had only variants affecting clonal hematopoiesis-related genes (DNMT3A, TET2 or ASXL1; 'DTA'), 44% were MRDpos defined as ≥1 variant in a non-DTA gene. MRDpos and neg pts had similar baseline and HSCT-related characteristics.
MRD positivity associated with significantly inferior overall survival (OS; 5y rate, 49% v. 85%, P=.011) and non-significant higher cumulative incidence of relapse (46% v. 24% at 5y, P=.14), while non-relapse mortality was similar in both groups. Multivariate analysis confirmed a significant association of MRD with inferior OS (P=.035).

MRD detection using smMIP-NGS provides prognostic information in AML pts undergoing HSCT. This method is applicable to a large proportion of AML pts and cost-efficient for broad clinical use.

Poster 207

Characterization of the sodium-hydrogen-exchanger subtype 3 (SLC9A3) in esophageal adenocarcinoma cells

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While the incidence of the esophageal adenocarcinoma (EAC) is rapidly increasing, it is diagnosed mostly in advanced stages and its prognosis remains poor. Recently, SLC9A3 (solute carrier family 9 member A3) has been identified as a biologically risk gene for Barrett's esophagus (BE) and EAC. SLC9A3 encodes the epithelial brush border Na/H-exchanger NHE3, which is responsible to keep the sodium ion gradient to regulate the intracellular pH. Importantly, an increase in SLC9A3 expression has been correlated with the severity of gastroesophageal reflux disease, which is a major risk factor for BE.

An in vitro cell culture model of BE and EAC was used, to verify the SLC9A3 expression by qRT-PCR. In siSLC9A3 transfected cells, proliferation and dead cells measurements were performed by live cell imaging, Additionally, live cell imaging was performed using standard medium and acidified media (pH 6.8).

SLC9A3 is highly expressed in the investigated EAC cell lines (FLO-1, OE19) compared to metaplasia and dysplasia cell lines. A sufficient siRNA mediated knockdown of SLC9A3 was performed. First results indicate that siSLC9A3 transfected cells have a lower proliferation rate and an increased amount of dead cells. Acidification lowered the proliferation rate of EAC cells. However, siSLC9A3 transfected cells have a better growth surviving in acidified media compared to physiological pH.

Nevertheless, follow-up studies are warranted to generate further evidence for the involvement of SLC9A3 in BE/EA development and to elucidate exact mechanisms of SLC9A3 in BE/EAC development.

Poster 208

Development of CAR-T cells targeting TMEM158 or PTPRZ1 for treatment of Glioblastoma

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Glioblastoma (GBM) is the most common and deadly form of brain tumor. Current treatment options show only little improvement in patients, so new therapies are urgently needed. Immunotherapies based on chimeric antigen receptors (CARs) show great promise for treatment of GBM. However, antigen heterogeneity still limits effective treatment, and the discovery of new GBM antigens is needed. The aim of this study was to develop CAR-T cells against two potential GBM targets, TMEM158 and PTPRZ1.

The expression of both targets was analyzed using public databases and a cohort of local patients. A CAR against TMEM158 was designed based on a natural peptide ligand, and the FNIII-domain of tenascin-C was used as binding domain for a separate CAR construct against PTPRZ1. CAR-T cells against both targets were produced and their function was investigated using cytotoxicity assays.

Overexpression of TMEM158 and PTPRZ1 was confirmed in patient samples and cell lines. The CAR-T cells were produced and showed cytotoxicity against several GBM cell lines. Mutated versions of the anti-TMEM158-CAR enhanced cytotoxicity against some GBM cells and allowed targeting of tumor-associated integrins in parallel with TMEM158 by a single CAR.

In this study, we confirmed that TMEM158 and PTPRZ1 are suitable targets for a CAR-based immunotherapy against GBM. Thus, we have expanded the repertoire of antigens that might be used to overcome the antigen heterogeneity that currently impedes effective treatment of GBM by immunotherapies.

Poster 209

Effects of HDAC-Inhibitors on oxidative stress and the NRF2 pathway in gastric cancer

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Objective:
We aimed at investigating the role of oxidative stress in gastric cancer cells upon treatment with HDAC inhibitors. A particular focus was on the role of the NRF2 pathway, which can mediate resistance to reactive oxidative species (ROS).

Methods:
Using fluorescence-based ROS sensors, oxidative stress was measured in human gastric cancer cell lines. Activation of the NRF2 pathway was monitored by luciferase reporter assays and mRNA expression analyses of NRF2-regulated genes. Furthermore, the effects of ROS scavengers and NRF2-knockdown on HDAC-dependent antiproliferative effects were investigated using the colorimetric WST assay.

Results:
HDACi treatment led to increased oxidative stress levels and increased expression of a luciferase reporter under the control of an antioxidative response element. The latter finding suggests activation of NRF2 pathway as an adaptive response to oxidative stress. Accordingly, the expression of the NRF2 target gene HMOX was upregulated upon HDAC treatment and this effect was abrogated by NRF2 knockdown. In addition, treatment with antioxidants reduced cytotoxicity of HDAC inhibition, whereas downregulation of NRF2 led to enhanced sensitivity of cancer cells towards HDAC inhibitors.

In conclusion, oxidative stress induced upon HDAC inhibition contributes to the antitumor effects of HDAC inhibitors and activation of NRF2 represents a potentially important adaptive response of gastric cancer cells in this context.

Poster 210

HDAC Inhibitor entinostat induces the EGF receptor (EGFR) and its ligands in gastric cancer cells, providing a rationale for combination therapies

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The antitumor effects of HDAC inhibitors (HDACi) are generally based on the downregulation of oncogenic signaling molecules and the upregulation of tumor suppressor genes. However, the relatively unspecific effects of HDACi can also lead to the stimulation of pro-tumorigenic pathways. As acquired vulnerabilities, these may be exploited for combination therapies.

This study investigated the effect of the class I HDACi entinostat on the expression of the EGF receptor (EGFR) and EGFR ligands in gastric carcinoma. A dose-dependent induction of EGFR and classical EGFR ligands was shown on the mRNA level in various tumor cell lines as well as in tissue slice cultures ex vivo. This profound upregulation was confirmed on the protein level, with a particularly profound increase of amphiregulin. HDACi effects were also associated with stimulation of the ERK1/2 pathway. On this basis, we tested the combination of entinostat with the EGFR inhibitor erlotinib, both in vitro and in a tumor xenograft-bearing mouse model in vivo. In cell culture, the combination therapy showed stronger tumor cell-inhibitory effects compared to the single drugs. More importantly, the combination of entinostat with erlotinib led to pronounced inhibition of tumor growth in vivo, at dosages where neither entinostat nor erlotinib alone were able to produce any inhibitory effect. Our findings and the above-mentioned acquired vulnerability concept indicate that the use of HDACi may lead to the sensitization of gastric cancer to EGFR inhibitors via functional stimulation of EGFR signaling.

Poster 211

Identification and analysis of novel pharmacological agents to restrain glioblastoma invasion in different test systems

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Glioblastoma (GBM) is the deadliest primary brain tumor in adults with a median survival time of about 15 months post-diagnosis despite best available treatments. The poor prognosis is attributed to the high invasiveness of GBMs. Since ion channels facilitate cell migration, we hypothesized that inhibiting ion channel-mediated signaling would reduce GBM invasion independent of cytotoxicity. Thus, the aim of the current study was to investigate the effects of multiple agents on GBM viability and invasion. Inhibitors included the bumetanide derivative AqB011 blocking aquaporin 1-mediated ion flow, the neurotoxin Apamin blocking Ca2+-activated K+ channels, and three novel semi-synthetic indole alkaloids.

Cytotoxicity of the agents was assessed by cell viability assays using alamarBlue and WST-8. Drug effects on invasion were examined in 3D-transwell invasion assays using U87MG, U251MG, DBTRG0.5 and KNS42 GBM cell lines, and in a patient-near tissue slice co-culture model composed of murine brain and U87MG or G55T2 tumor xenograft slices.

The agents did not significantly impact GBM cell viability but significantly reduced invasion in 3D-invasion assays at micromolar doses by about 20-60% compared to the DMSO vehicle control. These inhibitory effects were confirmed in the co-culture model.

These results support the hypothesis that reduced ion channel flow restrains GBM invasion in vitro and ex vivo. All tested agents showed promising effects in controlling GBM invasion, which could prolong patient survival and extend the time to eradicate the tumor through complementing therapies.

Poster 212

Investigation of changes in lipid metabolism during the malignant transition from hepatic steatosis to hepatocellular carcinoma

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Hepatocellular carcinoma (HCC), the most frequent primary hepatic malignancy, arises in majority in chronically damaged liver. In the context of steatosis, HCC development is initiated already in early disease stages. Our research has shown that premalignant HCC stages were accompanied by molecular changes in hepatic energy metabolism.

The aim of this study is to investigate changes in energy metabolism during different stages of malignant transition induced by hepatic steatosis.

Primary-like human hepatocytes genetically engineered to obtain proliferation competence (HepaFH3) were used as an in vitro model of a premalignant HCC stage. In parallel isolated primary human hepatocytes (PHHs) and primary human hepatoma cells (PHCs) represent benign and malignant stages, respectively. Steatosis is induced by cultivation with free fatty acids in HepaFH3.

Our results show that the lipid accumulation in vitro is comparable to the in vivo situation. The characterization of the different phases of lipid metabolism (lipid uptake, excretion, fatty acid beta oxidation, de novo lipogenesis) by RT-qPCR, Western blot analysis and functional assays is work in progress.

We anticipate that our models will be useful to track down relevant signaling and metabolic components which contribute to the development of HCC in steatosis.

Poster 213

Investigation of the mechanisms responsible for the antineoplastic effect of metformin on malignant glioma

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Metformin has an antineoplastic effect on several cancers, including gliomas. To date, the mechanisms or targets underlying this effect have not been identified. The aim of our study is to investigate the glycerol-3-phosphate shuttle (G3PS) as a potential target of metformin in different glioblastoma (GBM) cell lines.

To investigate the effect of metformin on cell viability, cells were exposed to different concentrations of metformin. After 24 hours, viability was determined by measuring ATP in cell lysates using the CellTiter-Glo assay. Cells were then exposed to metformin at the IC50 for several days while incubated in a live cell imager. RT-qPCR and Western blot were performed to quantify the expression of enzymes responsible for G3PS, namely GPD1, GPD1L and GPD2.

Most GBM cell lines showed a sigmoidal response to 24-hour incubation with metformin, but with variations in IC50 and total viable cell number. Long-term incubation resulted in impaired cell growth in 9 of 10 cell lines in the presence of metformin. Expression at the RNA and protein levels correlated significantly for both GPD1L and GPD2. The protein GPD1 was expressed at such low levels that it was undetectable by Western blot.

Currently, we investigate the contribution of G3PS to the antineoplastic effect of metformin, performing enzyme assays and siRNA knockout experiments.

Since the antineoplastic effect of metformin differs in different cell lines, we anticipate that a more comprehensive knowledge of the mechanisms and cellular targets may increase the usefulness of metformin for stratified tumor treatment.

Poster 215

Nme2 in Hematopoietic Stem Cells: Bridging Metabolism and Signaling Pathways

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Myeloid leukemias arise from cumulative alterations in hematopoietic stem and progenitor cells (HSPCs) that affect both their intrinsic control and the interaction with their bone marrow niche. The niche supports the balanced maintenance and differentiation of HSPCs by providing appropriate structural, signaling, and metabolic environments. The interplay between metabolic and signaling pathways is of particular importance since this is likely to link specific cell fates (quiescence, self-renewal, differentiation) to distinct environments. Non-metastasis protein 2 (Nme2), a nucleoside diphosphate kinase that exchanges the gamma-phosphate group between NTPs and NDPs, has a potential function in this interplay. Our previous research revealed accumulation of Nme2 mRNA in metabolically stressed AML cells and co-localization of Nme2 protein with the Bcr/Abl oncoprotein in CML cells. Additionally, we have found Nme2 to be required for JAK/STAT signaling in stem/progenitor cells at low energy states. Based on this, we propose that Nme2 maintains the activity of ATP-dependent kinases using GTP to regenerate ATP, thus bridging metabolism and signaling in stem/progenitor cells. We are currently manipulating Nme2 expression in murine multipotent FDCP Mix cells, transduced with a FLAG-tagged Nme2 construct, to identify potential binding partners via co-immunoprecipitation. We intend to focus on interactions that change in response to metabolic environment and differentiation state, to unravel the role of Nme2 in coordinating hematopoiesis in the niche.

Poster 216 Oncogenic role and target properties of the lysine-specific demethylase KDM1A in chronic lymphocytic leukemia

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Aberrant expression of the T-cell leukemia 1A (TCL1A) oncogene is a hallmark of chronic lymphocytic leukemia (CLL). High TCL1A levels are associated with aggressive disease features. A better understanding of functional networks around TCL1A would likely catalyze new treatment approaches in CLL. In our mass-spectrometry based proteome-screens, we show that the lysine-specific demethylase KDM1A interacts with the TCL1A protein in non-malignant B-cells and CLL cells. Furthermore, KDM1A is overexpressed in B-cell lymphomas and is implicated in the development and sustenance of several other hematological malignancies. We show that TCL1A interacts with KDM1A in the nucleus of B-cells and increases KDM1A's demethylase activity. Higher KDM1A expression correlated with adverse clinical characteristics and a shorter PFS in CLL. Pharmacologic inhibition of KDM1A with the compound C12 induces apoptosis and affects H3K4/9 target methylation levels in B-cell lines and primary CLL samples. Moreover, this genetic Kdm1a knockdown in Eμ-TCL1A mice (iKdm1aKD;Eμ-TCL1A vs Eμ-TCL1AKdm1aWT) inhibits leukemic cell growth in peripheral blood and spleen. This leukemic deceleration was accompanied by upregulation of p53 and pro-apoptotic pathways. Additionally, KDM1A expression in the microenvironmental has an impact on its support for CLL progression. Furthermore, RNA-seq analysis of differentially expressed genes upon Kdm1a knockdown suggests that Kdm1a might act as a transcriptional repressor.

Poster 217

Therapeutical potential of HDAC targeting in T-PLL and other hematological malignancies

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HDACs represent promising therapeutic targets in cancer, particularly in T-PLL and other hematologic malignancies, given their pivotal role in epigenetic regulation and gene expression, prompting further investigation to optimize treatment strategies. We systematically evaluated a range of novel and established HDAC inhibitors using primary T-PLL cells, healthy T-cells and PBMCs. The screening was further expanded to 38 T-cell leukemia/lymphoma cell lines, aiming to delineate HDAC inhibition‘s therapeutic potential across various hematological entities. Co-culture experiments with NKtert cells mimicking stromal support assessed the impact of HDAC inhibition in the T-PLL microenvironment. Additionally, combination strategies were explored, investigating the potential synergy of HDAC inhibition with MDM2 inhibition, DNA methylation agents, BCL2 inhibition, and purine analog in primary T-PLL cells. RNA-seq analysis of HDAC isoform expression, combined with an assessment of HDAC enzymatic activity and correlation with cell death induction, aims to reveal the therapeutic suitability of isoform-selective HDAC inhibitors in T-PLL. The identified promising combination(s) and their respective treatment schedules will be tested in a patient-derived xenograft (PDX) mouse model, providing a preclinical platform for further validation. Overall, we aim to explore the therapeutic potential of HDAC inhibitors in T-PLL and extends the investigation to other hematological malignancies.

Poster 218

TRPA1-Mediated Activation of MARCKS: Implications for a Non-Canonical Chemotherapy Defense Mechanism

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Chemotherapy resistance is a major factor in tumor recurrence, and understanding the underlying mechanisms is crucial for improving cancer therapy outcomes. The redox-sensitive, non-selective cation channel Transient Receptor Potential Ankyrin 1 (TRPA1) plays a significant role in cancer progression and response to oxidative stress. TRPA1 activation by e.g. an increase in intracellular reactive oxygen species (ROS) levels mediates extracellular calcium influx, which can engage a non-canonical antioxidant defense program and support cancer hallmarks such as hyperproliferation, survival against pro-apoptotic stimuli, and invasive behavior. The protein „Myristoylated Alanine-Rich C-Kinase Substrate“ (MARCKS) is known for its ability to bind and sequester the phospholipid PIP2, influencing cell movement, proliferation, and protein internalization. Abnormal MARCKS signaling has been observed in the development and progression of various cancer types.

In multiple cancer models with robust TRPA1 expression, we could identify a new mechanism of Calcium-dependent MARCKS activation, initiated by TRPA1 activation and altering the response to chemotherapy. We employed various methods like imaging experiments, assays for cellular viability and classical methods of molecular biology, combined with the pharmacological activation and inhibition of TRPA1 and MARCKS.

Targeting TRPA1 and MARCKS might offer a new approach to treat metastatic diseases by modulating migratory traits of tumors in the presence of chemotherapy and hypoxia.

Poster 219

Phenotype Switching of Breast Cancer Cells upon Matrix Interface Crossing

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In the process of metastasis, cancerous cells migrate from the primary tumor tissue into the surrounding healthy tissue. The invading cells are challenged by a defined interface between these two tissues due to a rapid change in microenvironmental stiffness and extracellular matrix density. Previous research showed that MDA-MB-231 breast cancer cells that migrated across clearly defined interfaces between two differently porous collagen-I matrices exhibit changes in phenotype and gene expression [1, 2].

In this study, 3D collagen-I matrices of different porosity were used as biomimetic tissue interfaces to analyze the influence on cancer cell metastasis.

To reveal insight in the underlying process, we now examined mechanotransduction activation of cells as well as the heterogeneity of the cell populations before and after transmigration across matrix interfaces. We found an induction of the formation of focal adhesion sites after transmigration. Furthermore, the comparison of the heterogeneity of cell populations prior and after transmigration in terms of proliferation showed no changes in heterogeneity, indicating an instructive phenotype change during transmigration.

In sum our new findings suggest that transmigration of breast cancer cells across tissue interfaces instructs a phenotype switch towards a more aggressive behavior, triggered by mechanotransductional signaling.

[1] J. Sapudom et al., Advanced Healthcare Materials 2016, 5, 1861-1867.
[2] P. Riedl et al., ACS Applied Materials & Interfaces 2023, 15, 24059–24070.

Poster 220

Growth limitation of cancer cells by mechanical properties of the extracellular matrix

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The extracellular matrix (ECM) primarily serves to provide tissue structure and, depending on its composition, shapes the stability and stiffness of various tissues. Through specific interactions between cells and the ECM, the ECM plays an important role in mechanotransduction and thus specifically influences cell behavior. According to previous work, the proliferation of different cell types can be limited by increasing stiffness of the surrounding ECM and is sometimes completely stopped without inducing cell apoptosis. However, the causes of this and the connections to the regulation of the cell cycle remain poorly understood.

In our work, we target this issue by developing 3D poly(ethylene glycol) (PEG) hydrogels at a defined stiffness, that allow to investigate the proliferation behavior of cancer cells, focused on an ECM stiffness-induced cell cycle arrest. For detailed analysis we use HCT-116 wild type (WT) and HCT-116 double knockout (DKO) cells with depleted genes for RB and LIN37 that exhibit ˝unlimited˝ proliferation behavior. PEG hydrogels were prepared using vinyl sulfone and thiol functionalized PEG precursors with different ratios to adjust the hydrogel stiffness between 1 to 20 kPa. In first experiments, HCT-116 wild type cells were encapsulated into the hydrogels and cultivated over several days. The size of evolving cell clusters was quantified from 3D image stacks of fluorescence microscopy. The results indicate small differences in cluster sizes that suggest a stiffness-dependent cell growth limitation.

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