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Book of Abstracts: Poster Presentations

P 1  Efficient Subject Independent BCI based on Local Temporal Correlation Common Spatial Pattern method

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One of the main weaknesses of the proposed EEG-based Brain Computer interfaces (BCIs) is the requirement for specific subject training data to train a classifier. Almost all proposed BCIs in literature are custom-designed and dedicated to just one particular subject. Eliminating the calibration phase can be regarded as a good solution to enable new users to have an immediate interaction with BCIs. In addition, eliminating calibration training sessions has a vital role for those patients who have visual and audible deficiencies. Such patients do not have the mental or physical ability to perform training sessions needed for the subject dependent BCIs implementation. This study presents an efficient subject-independent approach for BCIs based on EEG signals. The main goal of this study is to develop ready-to-use motor imagery tasks based BCIs using other subjects' data, which can be utilizable for anyone, who is shown the system for the first time. To achieve this approach, we proposed a subject-independent method based on Local Temporal Correlation Common Spatial Pattern (LTCCSP) algorithm for feature selection and Genetic algorithm (GA) strategy, using Frobenious distance index, to have a simultaneous optimization of time interval and frequency. In this study, publicly available dataset IVa of the BCI competition III is used. The experimental results show similar accuracies to those of the subject dependent approaches but without the need to subject-dependent data. Therefore, our proposed system has a worthwhile practical improvement over the conventional and widely used approach.

P 2  Cardiac Support During Physical Exercise In Patients With An Implantable Left Ventricular Assist Device

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**P 3** Continuous suction monitoring reveals high probability of suction in well-adjusted VAD-outpatients.

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Study: Suction of the left ventricular (LV) wall can lead to potentially life-threatening events in left ventricular assist device (LVAD) patients. Due to limited LVAD monitoring capabilities and alarm systems, little is known about the patients' suction prevalence. This study aimed at combining 24/7 LVAD monitoring developed by our group and suction detection from estimated pump flow in stable, well-adjusted LVAD outpatients.

Methods: In a clinical observational study continuously recorded LVAD data from 10 HeartWare HVAD recipients were analyzed using a previous developed suction detection algorithm. Beginning of observation period was the patients' first outpatient follow-up and data were analyzed for the following 15 days. Patients' individual suction prevalence was calculated as the percentage of beats containing a suction event within a one hour time-window and averaged over the whole 15-day observation period.

Results: Patient characteristics of the 10 HVAD recipients (8 males, 2 females) at implant was age 58±9.6yrs, cardiomyopathy (dilated 6, ischemic 4), BMI 25±3.3 kg/m² and Intermacs profile 1-2/3-5 was n=4/6. Observation started at post operative day 78±22, mean arterial pressure was 82±13 mmHg and heart rate 78±12 bpm. Five patients showed almost no suction (<1% per hour). Five patients had a suction prevalence between 1% and 7% per hour. One of these 5 patients showed suction prevalence of over 60% lasting for 2 hours. In the other 4 patients the suction occurred mostly during daytime (7am-9pm).

Conclusion: Enhanced monitoring sheds light onto suction events in the outpatient setting. These events are unobservable during single routine follow-ups or by using algorithms based on pulsatility only.

**P 4** Evaluation of Different Turbulence Models in Simulation of Intraventricular Flow Pattern with Left Ventricular Assist Device Support

The use of centrifugal continuous flow left ventricular assist devices (LVADs) in supporting patients with end-stage heart failure mandates understanding details of intraventricular flow pattern with LVAD support. Computational Fluid Dynamics (CFD) is a powerful tool to simulate flow field that leads to access more details of interested areas where experimental approach is stretched to its limit. In this study intraventricular flow field was simulated by four turbulence models. Finally CFD results were compared to particle image velocimetry (PIV) data.

The flow field was simulated with ANSYS software. Four different turbulence models (standard k-epsilon (SKE), realizable k-epsilon (RKE), standard k-omega (SKO) and SST k-omega (SST)) were utilized to simulate the flow field inside of the ventricle. For comparison and analysis purposes various radial cuts at interested parts such as mitral and LVAD cannula position were created along the ventricle. Flow velocity was calculated at radial cuts for all simulation methods and PIV data. A validation metric \( E \) based on the velocity differences was utilized to quantify the goodness turbulence models. \( E=0 \) shows exact prediction by CFD.

Overall flow pattern inside of the ventricle demonstrate acceptable correlation between all turbulence methods and PIV results. Sum of the validation metrics at all cuts, based on the velocity profiles showed better performance of SST method, also less accurate prediction belongs to the RKE method \( \text{SKE E}=0.37, \text{RKE E}=0.47, \text{SKO E}=0.46, \text{SST E}=0.32 \). Among all created cuts, the highest value of \( E \) were related to LVAD cannula \( \text{SKO E}=0.96 \) and mitral position \( \text{RKE E}=0.59 \). Based on the computed validation metrics SST k-omega methods performed more realistic compared to the other turbulence methods. SKE method showed better agreement with PIV results on the LVAD cannula position where sudden contraction occurred. On the area with sudden expansion (mitral position) the lowest \( E \) was seen with SST.

P 5  Remote ischemic conditioning improves post-ischemic cardiac function: the role of Neuregulin-1


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Obj.: Myocardial infarction (MI) is the major contributor to mortality and morbidity worldwide. A significant number of patients develops heart failure following MI. Remote
ischemic conditioning (RIC; repeated episodes of transient ischemia/reperfusion injury (IR) in distal site) protects the heart against subsequent IR and improves post ischemic cardiac function (PICF) as well as it reduces infarct size. Impairment of Neuregulin-1 (NRG-1)/ErbB2 and 4 signalling pathway plays a crucial role of post ischemic left ventricle (LV) dysfunction. The aims of the study were to investigate the effect of RIC on PICF and whether this is associated with changes of NRG-1 expression. Meth.: Adult male anaesthetized WISTAR rats were subjected to 30 min left coronary artery occlusion followed by two weeks of reperfusion and allocated to (1) Sham operated (without occlusion; n=3); (2) IR (n=6) and (3) IR+RIC (3 x 5 min of hindlimb ischemia, 5 min of reperfusion, started at 5th min of index ischemia.; n=7). Functional parameters of the heart such as cardiac output (CO), external heart work (EHW) and LV pump function were evaluated on an isolated erythrocyte-perfused working heart model. NRG-1 expression is assessed by ELISA in plasma samples. Res.: MI resulted in a significant increase of LV/body weight ratio in comparison to Sham operated group (P<0.05). This was in line with the reduction in CO and EHW (P<0.01) and with a clear reduction in plasma concentration of NRG-1. In contrast, RIC markedly improved PICF compared to IR group (CO and EHW, P<0.05) in association with the increase of plasma NRG-1 concentration. Conc.: We demonstrated for the first time that the improvement of PICF initiated by RIC is associated and correlated with the plasma levels of NRG-1. These findings might represent a novel cardioprotective mechanism of RIC, mediated via the upregulation of NRG-1, as well as a potential therapy to attenuate adverse left ventricle remodeling following myocardial infarction.

P 6 Intermit tent Hypoxia/Hyperoxia Causes Injury in Mouse Pulmonary Endothelial Cells


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Acute respiratory distress syndrome (ARDS) is characterized by the acute onset of bilateral alveolar infiltrates and hypoxemia due to lung tissue injury. Additionally to the mechanical forces of volu-, baro-, and atelectrauma causing alveolar shear stress, non-mechanical mechanisms are thought to promote lung biotrauma, such as cyclic recruitment and derecruitment of atelectasis. It can result in varying shunt fraction and altered gas exchange, causing respiratory-dependent alveolar pO2-changes. These pO2-changes could potentially cause intermittent hypoxia/hyperoxia injury and therefore represent an additional mechanism of lung biotrauma. The aim of our study was to investigate the underlying mechanisms and involved signaling pathways of pO2-changes in primary mouse lung endothelial cells. The experiments were performed using lung preparations from C57BL/6J WT mice. Isolation and purification of lung endothelial cells was performed by magnetic separation. pO2 in control groups was constant over time (21,0.95% O2), while pO2-changes (0-95% O2) were performed at 10
cycles per hour. Phase contrast light microscope was used to assess the cells’ health and morphology. Cell viability and cytotoxicity was assessed by LDH activity assay. Apoptosis and necrosis states were distinguished by FACS. QRT-PCR was performed for candidate markers.

LDH activity assay showed increased cytotoxicity in the intervention group (0%-95% O2). FACS-analysis showed significantly more early and late apoptosis in the intervention group. QRT-PCR revealed the upregulation of lung injury markers, in particular TNFa, RAGE, tPAF, VCAM, ICAM.

Our data suggest that intermittent hypoxia/hyperoxia represents an independent mechanism of lung biotrauma potentially relevant in ARDS and obstructive sleep apnea. This work is funded by the FWF grant #P28618-B28

P 7  Ropivacaine Causes Injury in Human Umbilical Vein Endothelial Cells


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To reduce pain in obstetrics, laboring women often receive epidural anesthesia. Reduction of pain is provided by injecting ropivacaine into the epidural space. During pregnancy, the epidural space alters anatomically with an increased density of the vascular network making it more susceptible to ropivacaine.

We hypothesize that ropivacaine has an influence on human umbilical vein endothelial cells (HUVECs) by disrupting the respiratory chain in mitochondria, promoting early inflammation and apoptosis. Dysfunctional mitochondria releasing damage associated molecular patterns could promote sterile inflammation. The aim of this study is to show the toxic effect of ropivacaine in a cell culture model on HUVECs.

HUVECs were isolated from human umbilical veins of placentas after childbirth. The cells were then incubated with ropivacaine 0.2% (intervention group) or with 0.9% NaCl (control group) for 24 hours. Tests were performed at 1,4,24 hours to investigate short and long-term effects of ropivacaine. Phase contrast microscopy was used to assess health, morphology and cell count. Cell viability and cytotoxicity was addressed by LDH activity and trypan blue staining. Early apoptosis and necrosis was distinguished by FACS. ELISA was used to detect the release of cytokines such as IL1ß, IL6, IL8, TNFa.

Results show that at all time points (1,4,24hours), significantly more early apoptosis occurred in the intervention group. Late apoptosis occurred significantly more often in the intervention group after 24h. LDH activity was significantly increased in the intervention group after 24h. ELISA showed increased release of pro-inflammatory cytokines in the intervention group.

We conclude that ropivacaine induces apoptosis and inflammation in HUVECs. This mechanism might trigger sterile inflammation and potentially may harm the unborn fetus.
**Early identification of patients at very low risk of acute coronary syndrome using triage-information and ECG only**


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**INTRODUCTION:** Numerous algorithms exist for the exclusion of acute coronary syndrome (ACS), usually including laboratory test results, such as troponin. However, many patients who visit an emergency department have very low pre-test probability for ACS. With a steady increase in the volume of patients, a rising number of laboratory tests are inevitably associated with an increase in costs for the health system. The information collected at initial triage in many emergency medical facilities together with the ECG provides almost all the information necessary to calculate risk scores such as the GRACE score. We aimed to investigate whether patients with a very low risk of ACS can already be identified at the triage in order to minimize subsequent laboratory tests.

**METHODS:** All patients treated at the department of emergency medicine of a tertiary care hospital due to chest pain during a one year period were included. Cases in which the diagnosis of an ACS was already made by emergency medical services or other hospitals were excluded. Using triage information and ECG, the Mini-GRACE score without laboratory parameters was calculated. Data was compared with the ACS registry from the same period and measures of diagnostic test accuracy were calculated.

**RESULTS:** In total 2,755 patients (1199 (44%) female, age 44 ± 17 years) were included. Acute myocardial infarction was diagnosed in 103 (3.7%) patients (45 (44%) STEMI). A total of 2,562 patients (93%) had a GRACE score <108 and a normal ECG, and four (0.2%) of these patients had myocardial infarction. This results in a sensitivity of 96.1%, specificity 96.5%, positive predictive value 51.3% and negative predictive value 99.8%.

**CONCLUSIONS:** Patients with a very low risk of ACS can be identified with high certainty using triage information and ECG. Cardiac biomarkers might be avoided in many cases, leading to a significant cost reduction.

**Correlation between circulating micro RNAs levels with perfusion-metabolism imaging by 18-FDG PET-MRI**


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Introduction. In a porcine reperfused myocardial infarction (MI) model, 18-FDG PET-MRI were performed 3 and 30 days after MI to assess the area at risk, infarction and left
ventricular function (LVF). We have investigated the levels of myocardial related circulating miRNA biomarkers in relation with myocardial injury.

Methods. Reperfused MI was caused by balloon occlusion of the left anterior descending artery for 90 min followed by deflation in anaesthetised pigs. Blood samples were taken from each pig: before MI, directly and one hour after reperfusion start, and one month later. RNA was isolated from plasma using TRIzol and column based extraction, succeeded by cDNA transcription. The levels of two cardiac microRNAs were examined (miR-21, miR-122) using qPCR. miRNA abundance was normalized using a spike-in reference. A two-tailed t-test was used to determine significance.

Results. Seven of 20 pigs showed unusually high tracer uptake in the infarct area causing a mismatch to the MRI data (high FD-glucose uptake in severely hypo-akinetic areas, a currently unexplored phenomenon) (mismatch MI group) 3 days post MI, in contrast with the usual lack of tracer uptake in infarcted area (MI group). MRI at 30 days showed severely reduced LVF in the mismatch group. The levels of miR-21, which is typically up regulated in heart failure and fibrosis, were increased 10fold one month after MI over baseline values in the mismatch MI group, while in the MI group it only increased two-fold. Shortly after MI, a diminution of miR-122 was detected in both groups (2.5-fold in the mismatch MI group and 1.25-fold in the MI group).

Conclusion. This pilot study showed altered plasma levels of cardiac miRNAs in pigs showing mismatch at 3 days post-infarction in the MI area. Further investigations are necessary to examine the roles of the individual miRNAs and their value as potential predictive biomarker for MI severity.

P 10 The regulatory role of Tenascin C on matrix metalloproteases expression induced by hypoxia and reoxygenation in H9C2 cardiomyocytes cell line


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Tenascin C (TNC) expression play a role in the maladaptive signalling cascade involving left ventricle remodelling following myocardial infarction and hypertension. Of importance, increase in TNC expression is associated with Matrix Metalloproteases (MMPs; MMP2 and MMP9) upregulation in left ventricle samples. However, the effect of hypoxia on TNC expression as well as TNC on MMPs formation in cardiomyocytes has not been known. This study aims to evaluate the effect of hypoxia on the expression of TNC, MMP2 and MMP9 as well as whether TNC influences MMPs formation in a rat cardiomyocyte cell line. H9C2 rat cardiomyocytes cell line was submitted to 6 and 24 hours of hypoxia in a 95% N2 and 5% CO2 atmosphere. Additionally, TNC was added to the same cell line under normoxic
conditions in four different concentrations, 1, 3, 5 and 10 µg/mL, for 6 and 24 hours. The mRNA expression of TNC, MMP2 as well as MMP9 were determined by RT-qPCR and normalised to ß-actin as housekeeping gene. Under normoxic conditions TNC expression was not detectable. In contrast, hypoxia significantly induced TNC expression in all time points (P<0.05, respectively). The formation of MMP2 and MMP9 were increased by hypoxia. MMP2 expression maximum was obtained after 6 hours of hypoxia (2.1 fold-change); MMP9 upregulation reached the highest levels following 24 hours hypoxia (15.8 fold-change). The expression of MMP2 and MMP9 were markedly increased by the administration of human TNC protein following 24 hours (1.5 and 2.8 fold change, respectively). This study evidenced that hypoxia and reoxygenation markedly increased TNC, MMP2 and MMP9 expression in rat cardiomyocytes. Moreover, TNC has a significant effect on MMP2 and MMP9 upregulation. These results might explain the pathological importance of TNC on MMPs following myocardial infarction and hypertension. Additionally, the present model may allow the test of novel compounds affecting TNC and MMPs expression in cardiomyocytes.

P 11 Plasma NEP concentration is not associated with acute ischemic myocardial injury


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Systemic Neprilysin (NEP), N-terminal B-type natriuretic peptide (NT-proBNP), clusterin, neutrophil gelatinase-associated lipocalin (NGAL), endothelin-1 (ET1) and osteopontin are currently thoroughly investigated as cardiac biomarkers characterizing acute myocardial ischemic injury. The aim of our study was to assess suitability of these biomarkers in acute myocardial infarction (MI) in pigs. A total of 24 pigs underwent MI induced by 90min percutaneous balloon occlusion of the mid left anterior descending coronary artery (LAD), followed by balloon deflation (reperfusion). Cardiac magnetic resonance imaging (cMRI) was performed at day 3 and after 6 weeks of MI to determine infarction size and left ventricular (LV) parameters. NEP concentrations alongside other cardiac biomarkers such as NT-proBNP, clusterin, NGAL, ET1 and osteopontin were measured by ELISA and miRNA21 and miRNA29 through rtPCR during the acute and subacute phase of MI. The course of biomarkers and correlations with cMRI parameters were investigated. cMRI showed an area at risk of 16.4% LV at day 3 after acute MI accompanying a depressed LV function with 36.1% of LV ejection fraction (LVEF). LVEF remained depressed with signs of adverse remodeling reflected by increased LV end-systolic and -diastolic volumes during the 6-week follow-up. NT-proBNP, NGAL as well as miRNA21 and miRNA29 were significantly elevated at 3 weeks and clusterin was significantly decreased at 3 and 6
weeks compared to baseline levels. ET1 showed an inverse correlation with infarction size and LVEF \(r= -0.67, p=0.033\) and \(r= -0.64, p=0.035\). Changes in plasma NEP concentrations could not be observed \(p=0.593\) and no correlations were found with infarction size or LVEF \(r= -0.10, p=0.664\) and \(r= -0.14, p=0.502\). In contrast to recent data on NEP in chronic heart failure, plasma NEP levels were not associated with acute ischemic injury in porcine model of reperfused myocardial infarction.

P 12  **Myocardial transcriptome in clinically relevant porcine model of ischemic postconditioning**


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Introduction. Ischemic postconditioning (IPostC) present sublethal ischemia/reperfusion stimuli applied after prolonged ischemic insult aiming to render the myocardium more resistant against ischemia/reperfusion injury. Although the concept of IPostC showed a promising strategy to attenuate ischemic injury in pre-clinical animal models, translation to clinical scenarios has not met success. This study proposes a comprehensive analysis of transcriptional changes elicited by IPostC in the translational porcine model of reperfused MI.

Methods. Anaesthetized pigs were randomised to groups IPostC(n=3) and myocardial infarction(MI, n=3); sham-operated animals served as controls(n=3). MI was induced by 90min percutaneous balloon occlusion of the left anterior descending artery following reperfusion (balloon deflation). IPostC was elicited by 6x30sec cycles of ischemia/reperfusion after 90min ischemia. Left ventricular (LV) function was assessed by cardiac MRI (three days follow-up) and transcriptome analysis was performed using next-generation sequencing of myocardial samples collected at three days follow-up from the infarcted and remote area in each experimental group.

Results. IPostC had no effect on LV function and infarct size; however, the myocardial oedema and microvascular obstruction were significantly attenuated as compared to the MI group. We detected relevant molecular changes in remote area of MI and IPostC groups and identified genes with the opposite regulation in MI and IPostC groups. Genes with opposite regulation were organized into functional clusters associated with regulation of extracellular matrix, coding of ribosomal subunits, vesicle transport, activation of blood cells and cardiac hypertrophy.

Conclusion. Our results indicate that the effect of IPostC is limited to confer benefits on coronary microvasculature. In addition, analysis of transcriptome indicates that substantial genomic response occurs in the remote area of the myocardium.
PET-reporter gene transfection in porcine MSCs for in vivo cell tracking after transplantation of tissue engineered heart valves

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Introduction: Transient cell transfection using a plasmid vector, such as human adeno-associated virus (AAV) does not carry the risk of introducing foreign genetic material into the genome of eukaryotic cells, therefore this method is frequently used in human gene therapies. The aim of our sub-study of the LifeValve EU project was to seed tissue engineered heart valves (TEHV) with PET-reporter gene transfected porcine mesenchymal stem cells (MSCs) that should be implanted into sheep (xenogeneic transplantation). The fate of the transiently transfected stem cells was tracked via serial in vivo positron emission tomography-computer tomography (PET-CT).

Methods: Porcine MSCs were transiently transfected with PET-reporter gene using Lipofectamine and seeded in a concentration of 7x10⁶ cells onto TEHV, which were then implanted percutaneously into the pulmonary position of sheep (n=8). mCi [18F]-FHBG PET tracer was produced for each procedure and serial PET-CT imaging of the sheep was performed after valve implantation.

Results: Implanted PET-MSC TEHV showed a clear PET signal after 3h (calculated cell number 4.95x10⁶). There was no meaningful decrease of living cells at 24h and 3 weeks after valve implantation (estimated cell number 4.67x10⁶). PET-CT images after 6 months, with a clear PET signal (estimated 3.16x10⁶ cells) on the valves are indicating a spontaneous stable transfection with PET-reporter gene.

Conclusion: This is the first report on serial non-invasive in vivo tracking of long-term survival of xenogeneic MSCs seeded onto TEHVs that were percutaneously implanted into sheep. Long-time follow-up revealed spontaneous stable transfection of the PET-reporter gene, which suggests the risk of genomic mutation induced by plasmids. The study was supported by the LifeValve EU project.

BMPRII signaling of fibrocytes is increased in STEMI and dyslipidemia and predicts poor systolic function at follow-up

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BACKGROUND: Inflammation is a hallmark feature of ST-elevation myocardial infarction (STEMI). Fibrocytes, Collagen-I+CD34+CD45+ mesenchymal progenitor cells with both leukocyte and fibroblast properties, accumulate in cardiac tissue of a murine ischemia/reperfusion model and are decreased in patients with acute coronary syndrome. Physiological expression of bone morphogenetic protein receptor II (BMPRII) is lost in advanced atherosclerotic plaques. Therefore, we studied the frequency and BMPRII expression of fibrocytes at the culprit lesion site (CLS).

METHODS: Blood samples from the CLS and femoral site were drawn in the course of primary percutaneous coronary intervention (pPCI) from STEMI patients (n=50, male=78%, mean age=61±13y). Another sample was acquired 72h after pPCI (n=21). Fibrocytes were characterized using flow cytometry. Wall motion score index (WMSI) was assessed by transthoracic echocardiography 23 [IQR 17-28] months after STEMI (n=19).

RESULTS: Fibrocytes were significantly increased two-fold at the CLS compared to femoral blood. No differences were found in BMPRII expression between CLS and femoral blood. However, in patients suffering from dyslipidemia, BMPRII on fibrocytes was increased both at the CLS and femoral site. 72h after pPCI, BMPRII was significantly upregulated. After adjusting for cardiovascular risk factors, BMPRII expression on fibrocytes at baseline positively predicted WMSI at follow-up.

CONCLUSION: Fibrocytes contribute to adverse cardiac remodelling and poor functional outcome. Increased BMPRII expression could promote enhanced fibrocyte migration.

P 15  Molecular Imaging of the Antigen Recognition Dynamics in CD8+ Cytotoxic T-Cells

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Cytolytic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for
ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. In this fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

**P 16  A potential role of osteopontin in NAFLD-induced hepatocellular carcinoma development**

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Hepatocellular carcinoma (HCC) is the cause of approximately one million deaths yearly. In addition to chronic hepatitis B and C infections, nutrition-related diseases significantly contribute to the increasing prevalence of HCC. In Obesity, osteopontin (OPN, gene Spp1) is upregulated in adipose tissue and liver and induces inflammatory and metabolic processes, which lead to insulin resistance, type 2 diabetes, fatty liver and NASH. Using a recently established mouse model, which faithfully reproduces human NAFLD-induced HCC development by treating newborn mice with streptozotocin (STZ) and feeding them a high fat diet (HFD) (STAM mice), we investigated metabolic and immunological parameters throughout the entire disease evolution. Compared to HFD-fed-only (HFD-o) mice, STAM mice showed comparable increased hepatic expression levels of metabolic genes. The expression of pro-inflammatory markers was, however, significantly higher in the STAM animals, indicating the induction of inflammation to further develop fibrosis and HCC. Between 15 and 19 weeks of age, STAM mice but not HFD-o and STZ-injected-only (STZ-o) mice developed liver tumors. In contrast to HFD, STZ alone recruited comparable levels of liver macrophages (LM) as observed in STAM mice, but the expression of M1-activation marker Cd11c and notably of Spp1 was significantly lower. Furthermore, Spp1 and other main immunological cytokines look like to be fine-tuned between the 15th and 19th week in order to elicit first a M2-like (implicated in tumor expansion and metastasis) and later a M1-like LM polarization. Our preliminary data confirm the necessity of inflammation for the establishment of HCC and point for the first time to a potential role of Opn in NAFLD-induced liver cancer.

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Mononuclear phagocytes are skewed towards a pro-inflammatory phenotype in X-linked adrenoleukodystrophy


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X-linked adrenoleukodystrophy (X-ALD) is a rare neurodegenerative disease with remarkable phenotypic variability ranging from a default spinal cord involvement (adrenomyeloneuropathy, AMN) to a fatal cerebral inflammatory demyelination (cerebral ALD, CALD). X-ALD is caused by mutations in the ATP binding cassette subfamily D (ABCD) 1 gene encoding a peroxisomal transporter crucial for the import and subsequent degradation of very long-chain fatty acids (VLCFA). Thus, ABCD1 deficiency leads to pathognomonic accumulation of VLCFAs in tissues and body fluids of X-ALD patients. Intriguingly, monocytes and macrophages are the immune cells most affected caused by the ABCD1 deficiency. Previously, whole transcriptome analysis of untreated CD14+ monocytes derived from blood of AMN patients revealed alterations in pro-inflammatory pathways. Furthermore, LPS-stimulated AMN derived monocytes showed a more pronounced pro-inflammatory cytokine expression when compared to healthy controls. In this study, we analyzed the alteration of gene expression in AMN derived macrophages upon in vitro myelin phagocytosis that normally triggers anti-inflammatory gene expression. By immunohistochemistry of post mortem brain tissue of X-ALD patients using markers directed against activated macrophages/microglial cells (CD68) as well as T and B cells (CD3 and CD20), we characterized the pattern of immune cell infiltration in active lesions. Furthermore, we determined immunohistochemically the expression of pro- (CD86) and anti-inflammatory (CD206) markers in active lesions and compared the results to active lesions of multiple sclerosis (MS) patients. We observed a decreased expression of the anti-inflammatory marker CD206 in X-ALD lesions compared to MS. Taken together and in line with our in vitro data, we conclude that X-ALD derived macrophages are skewed towards a pro-inflammatory phenotype and show a reduced ability to repolarize towards an anti-inflammatory state.

Bacteria Educated Platelets (BEPs)- Gram negative and Gram positive bacteria induce different mRNA expression profile changes in human platelets

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Background: Circulating blood platelets contain ~9500 different mRNAs, variable classes of non-coding RNAs and ~ 16 kb mitochondrial DNA. Due to the lack of DNA they are unable of de novo gene transcription. But as they contain mRNA splice and translation machinery they can produce functional proteins.

Aims: To analyze the platelet transcriptome following exposure to Gram negative or Gram positive bacteria.

Materials and methods: Citrated blood of healthy donors (n=4) was used to isolate platelets by OptiPrep density gradient centrifugation. The washed platelets were incubated with three Gram negative (E.coli K12, E.coli O18, K.pneumoniae) and two Gram positive (S.aureus NWT, S.epidermis) bacteria strains. Platelet RNA was isolated by Trizol method and sequenced on the Hiseq 2500 Illumina platform. Using flow cytometry, CD41, P-selectin and CD63 platelet surface proteins were evaluated (BD FACSCalibur).

Results: Studying intron-spanning reads (log counts per million > 3), we found 24 differential expressed genes in platelets after exposure to Gram negative bacteria, and 75 affected genes in the Gram positive treated samples (P-value < 0.05). None of these genes were overlapping between the two groups. The majority of these genes show ~2-8 log-fold change in platelets exposed to Gram negative bacteria, ~2-4 log-fold change incubated with Gram positive strains. According to the gene ontology and pathway analysis both bacteria groups affected the mRNA splicing processes, the Gram negative bacteria also had an effect on mitochondria related changes.

Conclusion: The different bacteria have strain specific effects on human platelet transcriptome. These differ strongly between Gram negative and Gram positive bacteria. Besides the influenced splicing, in certain cases also mitochondria processes were affected. Further detailed investigations are necessary towards this direction.

P 19 Somatodendritically located SERT: is there physiological relevance?

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P 20 The role of urokinase-type plasminogen activator receptor [UPAR] on T cells and the impact of UPAR T cells in human diseases
Activation and migration of T cells to sites of infection are crucial steps in orchestrating adaptive immune responses. Urokinase-type plasminogen activator receptor (uPAR, CD87) is an established surface marker of migrating monocytes and granulocytes, enabling binding to vitronectin, and extracellular matrix degradation. Vitronectin is an extracellular matrix component and located at vessel walls and the skin, especially in the dermis. By T cell receptor stimulation with CD3 and CD28, 50% of peripheral blood human T cells are able to neo-express uPAR upon in vitro activation. We employed immunohistochemistry and identified uPAR positive T cells in healthy human dermis sections obtained from elective surgery patients. We have also indications that uPAR positive T cells are present in peripheral blood. The aim of our study is the further phenotypical characterization of peripheral blood T cells, identifying their activation and differentiation state as well as interacting immune cells. To this end we will conduct polychromatic Image Stream analysis to combine flow surface staining statistics with visual inspection of singlets and duplets. Based on the findings obtained by flow, further functional assays will be performed in vitro. In addition, immunohistochemistry will be performed on skin sections from patients with systemic skin immune disorders (e.g. lupus erythematosus) and compared to skin samples from healthy donors. Supported by FWF W1205-B09 and the Cell Communication in Health and Disease PhD Program.
Nociceptive signals from peripheral fibers are processed in the spinal cord’s superficial dorsal horn [SDH] before they are relayed to the brain. Plasticity of the SDH’s microcircuits is thought to underlie pain conditions such as allodynia and hyperalgesia. In spite of the network’s important role in nociceptive processing, knowledge about its components has been limited to the characterization of neuronal subtypes through connectivity tracings and morphological and electrophysiological classifications of SDH neurons. This approach allows few conclusions about the relative temporal and spatial processing of sensory signals within the circuit. The constraints have been methodological: patch-clamp recordings are fast but are restricted to few neurons at a time. Calcium imaging increases the spatial dimension of recordings at a cost of a temporal resolution limited by calcium dynamics. Overcoming both constraints, voltage-sensitive dyes [VSD] shift their fluorescence emission spectrum upon cellular voltage changes, allowing the direct, fast optical readout of activity from populations of cells. We optimized imaging conditions for VSDs and applied them to the SDH of transversal and parasagittal preparations of rat spinal cord with attached dorsal root. For this purpose we stimulated the root at Ad and C fiber intensities and compared the spread of cellular excitation across the SDH. We master VSD’s notoriously low-signal-to-noise ratio with a custom-written image analysis algorithm, with which we detected intensity, spatial and temporal changes to the population response upon pharmacological disinhibition, hindrance of action potential propagation and stoppage of glutamatergic synaptic transmission. In further experiments, we will determine the ability of this voltage imaging system to reliably detect plastic changes in the SDH in vitro. Once its suitability is established, we will employ it to tackle pressing questions about changes in chronic pain conditions.

**P 23  Sex differences in nociceptive transmission**

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Background: A growing body of evidence suggests that glial cells play a major role in the processing of nociceptive information at the spinal level. A recent, however controversially discussed study suggested that this holds true only for male mice, whereas in female mice T-cells might play a crucial role. Long-term potentiation (LTP) at spinal C-fiber synapses is a well-established cellular model for pain amplification. However, research was so far exclusively performed on male rodents. Here, we tested whether there are sex-differences in LTP in male versus female rats. Additionally, we analysed the role of glial cells in this
mechanism in both sexes.

Methods: C-fiber-evoked field potentials were recorded in the superficial laminae of the dorsal horn in deeply anesthetised male and female rats. LTP was induced by high electrical frequency stimulation (HFS) of the sciatic nerve or by abrupt morphine-withdrawal. The glial-cell blockers minocycline as well as fluoroacetate was applied to assess the contribution of glial cells on the induction of synaptic LTP.

Results: HFS as well as morphine withdrawal significantly potentiated C-fiber-evoked field potentials in both male and female rats. Systemic application of minocycline or spinal application of fluoroacetate completely prevented HFS-induced LTP induction in rats of both sexes.

Conclusion: Thus, our data obtained so far from rats do not support the sex differences reported in mice. Planned experiments will address if species differences exist or if pain models are relevant.

P 24  Revealing the molecular basis underlying T cell antigen recognition in health and autoimmunity

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Thymic selection is essential for shaping the T-cell antigen receptor (TCR) repertoire. To ensure central tolerance developing T cells undergo with their newly recombined TCRs negative selection, in which T cells with high affinity TCRs towards self-antigens become eradicated. However, as recently demonstrated clonal deletion does not efficiently remove all self-specific T cells, which then colonize the periphery where they can cause harm if not under control by peripheral tolerance mechanisms. Interestingly, self-specific T cells are present in frequencies similar to those specific for non-self-antigens. While it is plausible that retaining a pool of TCR specificities against self-determinants promotes efficient pathogen defense, it bears the risk of causing autoimmunity. We hypothesize that autoreactive T cells, i.e. self-specific T-cells which have breached central tolerance, differ significantly from self-specific quiescent T cells with regard to TCR:antigen binding, in particular because of alterations within the immunological synapse, the transient interface between the T-cell and its antigen presenting cell (APC). To test this we will compare the synaptic TCR-pMHC binding dynamics as they occur for auto-reactive T cells, self-specific but quiescent T cells as well as pathogen-specific T cells with the use of a Förster Resonance Energy Transfer (FRET)-based molecular imaging system. To this end T-cells will be isolated from peripheral blood in an antigen-specific manner with the use of pMHC-streptamers. The reversible nature of pMHC-streptamer binding will also allow us to quantitate TCR:pMHC dissociation rates on a single cell level outside the context of the immune synapse. Correlating TCR:pMHC binding in situ and in vitro with the ensuing T-cell response will be instrumental to reveal the molecular basis underlying antigen recognition.
in health and autoimmunity. 
Supported by the CCHD PHD program

P 25  **Actions of hydrogen sulfide in the autonomic nervous system**


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Introduction: Hydrogen sulfide (H2S) is a toxic gas also produced in mammalian tissues where it can exert various functions as gasotransmitter, such as opening of smooth muscle KATP channels resulting in vasorelaxation. Recently H2S was found to be synthesized and released in sympathetic ganglia and to potentiate ganglionic transmission [1]. This project aims to elucidate the role of H2S in autonomic nervous system. Methods: Primary cultures of rat superior cervical ganglion (SCG) were used to determine release of previously incorporated [3H]noradrenaline and to measure membrane potential and ion currents via the perforated patch clamp technique. Results: In electrophysiological experiments, NaHS hyperpolarized the SCG membrane potential and reduced action potential firing probably via KATP channels. In SCG neurons, hyperpolarization of membrane potential can be caused as well by an enhancement of currents through Kv7 channels [2]. Unexpectedly, NaHS inhibited currents through Kv7 channels in a concentration-dependent manner. We studied the possible effect of NaHS on cholinergic miniature excitatory postsynaptic currents (mEPSC) in long-time cultured SCG neurons and found that NaHS increased their frequency, thus indicating an increase in the probability of acetylcholine release. Furthermore, in radiotracer release experiments, outflow triggered by either electrical fields or high k+ and sucrose concentrations were enhanced by 0.1 to 1 mM of the H2S donor NaHS in a concentration-dependent manner. Conclusion: These results show that H2S hyperpolarizes SCG neurons and reduces membrane excitability. Since KATP blockers prevented the effects of H2S, we conclude that the effect on membrane excitability was caused by an opening of KATP channels. In addition, increased the frequency of cholinergic mEPSCs. These excitatory actions most likely underlie the observed increase in ganglionic transmission.

P 26  **On the role of phosphodiesterase 5 inhibitors in sympathetic transmitter release**

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Phosphodiesterases (PDE) are enzymes responsible for the hydrolytic breakdown of the second messenger molecules cAMP and cGMP and lead to the termination of their respective intracellular signaling. PDE5 has been identified in various tissues, including platelets, vasculature and lungs. To date, several inhibitors of PDE5 are marketed and used to treat medical conditions including pulmonary arterial hypertension and erectile dysfunction. Although effects on the aforementioned tissues have been broadly studied, data on possible actions on the innervating autonomic nervous system are lacking.

In primary cultures of rat superior cervical ganglion (SCG) neurons application of the PDE5 inhibitors sildenafil and vardenafil, but not tadalafil or zaprinast, increased basal as well as electrically evoked transmitter release. These enhancing effects remained unchanged in presence of the guanylyl cyclase inhibitor ODQ. Omission of extracellular Ca2+ left the increase of basal noradrenaline release unaltered. Besides, an action of sildenafil and vardenafil on noradrenaline uptake was ruled out. In electrophysiological experiments resting membrane potential, action potential parameters and excitability of SCG neurons were unaffected upon application of sildenafil and vardenafil. Hyperosmolar stimulation with sucrose indicated a direct action of vesicular transmitter release.

Collectively, PDE5 inhibitors sildenafil and vardenafil affect sympathetic transmitter release independently of cGMP signaling, most likely via a direct effect on vesicle exocytosis.

**P 27** Epinephrine induced a regulatory M2b-like macrophage phenotype, which attenuates cord blood-derived mast cell (CBMC) IgE-mediated degranulation in a human model of allergic inflammation


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**P 28** Axl collaborates with transforming growth factor-ß signaling to express pro-invasive CXCL5 in hepatocellular carcinoma


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Background: Hepatocellular carcinoma (HCC), the most common form of liver cancer, is the third most lethal cancer worldwide. The epithelial to mesenchymal transition (EMT) plays a central role in the invasion and metastasis of HCC cells. EMT is mostly regulated by transforming growth factor (TGF)-β signaling which acts either tumor-suppressive or tumor-promoting upon HCC development. Here we address the molecular mechanisms responsible for this switch in TGF-β functions dependent on the receptor tyrosine kinase Axl.

Methods: Protein and mRNA levels regulated by Axl and TGF-β signaling components were analyzed in human HCC cells by immunoblotting and quantitative PCR. Immunoprecipitation was performed to study protein-protein interaction. Cell migration was assessed by wound healing and Transwell assays. Axl protein knockouts were generated by CRISPR/Cas9. Truncated and wild-type Axl were re-expressed by lentiviral transmission.

Results: We show that Axl is upregulated and activated in EMT-transformed HCC cells. Activation of Axl by its ligand Gas6 collaborates with canonical TGF-β signaling through phosphorylation of the Smad3 linker region at S213 via c-Jun-N-terminal kinase, leading to autocrine TGF-β signaling and activation of pro-metastatic TGF-β targets. Using a complete Axl knockout model including re-introduced wildtype or truncated receptor constructs, we analyzed the interplay of Axl and TGF-β pathways as well as Gas6-independent Axl signaling in HCC cells. Importantly, the knockout of Axl abolishes CXCL5 expression in mesenchymal HCC cells which use the TGF-β/CXCL5 axis for cancer cell invasion.

Conclusion: Taken together, we show that TGF-β and Axl signaling are molecularly linked by the aberrant phosphorylation of Smad3 and the upregulation of CXCL5 upon HCC progression. CXCL5 represents a novel TGF-β target which allows immunomodulation and early steps of HCC cell dissemination.

P 29  Understanding the heterogeneity of B-cell subsets: from dissecting tumor complexity to prognostic modeling


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Despite accumulated knowledge positioning tumor-infiltrating B cells among powerful contributors to tumor immunity, many questions remain given complexity of multifarious B-cell subsets and unique ability to assemble into functional ectopic lymphoid structures (ELSs). The presence of CD20+ B lymphocytes and/or ELSs and their association with a
better prognosis was demonstrated for both primary colorectal cancer CRC (at certain stage of disease) and metastatic CRC in the liver (our data). We hypothesize that B cells get instructed as anti-tumoral players along CRC development already at primary site and are able to transfer this knowledge to distant metastatic site. For understanding ELSs assembling mechanisms and their potential anti-tumor effects, we performed a comparative alignment of primary and matched metastatic B-cell-attributed patient-specific immunological imprints.

We developed a computerized microscopy-based algorithm, allowing quantitative assessment of various B-cell subsets across large-scale tissue specimens of primary CRC and matched metastatic CRC (n=21). To characterize the B-cell subsets we stained the tissues for CD20, AID, IgM, CD27, CD73, and CD138. We assessed the distribution and quantities of tumor-infiltrating B cells including those organized into ELSs and furthermore discriminated the IgM+/CD27+, IgM+/CD73+ and CD20+/CD27+ memory cells and CD138+/CD27high plasma cells. The developed algorithm consolidates the complexity of tumor anatomy and the immune landscape in term of B-cell subsets at primary and metastatic sites.

As outcome, the obtained patient-specific B-cell-attributed immunological imprint, described by diverse staining-derived datasets, will be used for alignment with clinicopathological parameters for building-up of complex prognostic/predictive survival models allowing to propose novel immune check points and/or targeting strategies.

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### P 30 A comparison of ROI-based MRI analysis strategies in dementia


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Evidence showed that temporal brain areas are especially affected by neuronal loss in Alzheimer’s disease (AD). Neuroimaging techniques allow these assessments in the living human brain while the FreeSurfer software is often chosen for this type of analysis. Recently, the CAT12 toolbox for SPM has been introduced offering a fast alternative approach. In this study, we assessed cortical thickness (CT) with both software solutions and compared the AD cohort to healthy controls subjects (HC). 45 healthy females (77.4±8.5) and 38 matched AD patients (77.6±7.7) were analyzed. MRI scans (1.5T, T1-MPRAGE) were taken from the OASIS database (http://www.oasis-brains.org/). All subjects were processed with FreeSurfer5.3 and CAT12. T-tests were calculated for each region (Desikan-atlas) between the HC and AD subjects using both methods. FreeSurfer as well as the CAT12 toolbox showed lower thickness values in the AD subjects. These decreases were significant (p<0.05, corr) in 18 ROIs for FreeSurfer and in 22 ROIs using CAT12. Reductions (d=mean difference HC vs AD) were most pronounced in the entorhinal cortex for both methods (FreeSurfer: t=5.2, d=0.53mm; CAT12: t=4.2, d=0.45mm). Further highly
atrophic areas were the temporal pole (FS: t=3.1, d=0.25mm; CAT12: t=3.4, d=0.29mm), the parahippocampal region (FS: t=3.8, d=0.27mm; CAT12: t=4.7, d=0.27mm) followed by the middle-, inferior- and superior temporal regions. The reductions of both methods for each ROI revealed a high correlation (R2=0.70). Both methods were able to detect atrophic brain areas in AD subjects. As expected, highest decreases were found in temporal areas with most reductions in the entorhinal cortex. ROI-wise decreases (HC vs AD) observed in both methods were highly correlated. Here we could show that CAT12 delivers a reliable, fast and easy-to-use alternative for cortical thickness measurements next to the already established and often used FreeSurfer software.

P 31 Evaluation of Escherichia coli Nissle and Salmonella typhimurium Bacterial Ghosts in Inducing Immunomodulatory Responses in Conjunctival Epithelial Cells


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Bacterial ghosts (BGs) are empty cell envelopes derived from Gram-negative bacteria. One main characteristic of BGs is that they possess all cell membrane structures which induce local immunity, while not posing any infectious threat. These characteristics give a potential to use BGs as an adjuvant delivery system. In the present study, we investigated the potential of Escherichia coli Nissle (EcN) and Salmonella typhimurium (Sty) BGs in modulation of immune responses in human conjunctival epithelial cells (HCjE). Confluent monolayers of HCjE cells were treated for 2 and 24 hours with EcN and Sty BGs at a concentration of 1x10^8 particles/ml, as well as with Interleukin-1α[alpha] as a general stimulator. 24 hours after cell stimulation the supernatants and cells were collected. Concentration of IL-6 and IL-8 were measured using ELISA. Quantification of the gene expression levels for toll-like receptor (TLR)-4 and TLR-5 was performed using qRT-PCR. HCjE cells responded to EcN BGs and Sty BGs with increased secretion of pro-inflammatory cytokines and expression of TLRs. Expression of TLR-4 and TLR-5 was significantly higher in EcN BGs stimulated cells compared to Sty BGs stimulated HCjE cells (P<0.05). EcN and Sty BGs induced enhanced secretion of IL-6 and IL-8 in HCjE cells. Our results suggest that both used BGs may modulate the secretion of IL-6 and IL-8 mainly via the activation of TLR-4 and TLR-5 in HCjE cells.

P 32 The difficulty of measuring executive functioning in patients treated for pediatric cerebellar tumors

OBJECTIVES: The current study aimed to analyze tests measuring executive functioning and behavioral aspects in patients treated for pediatric cerebellar tumors and to identify risk factors influencing their performance. Additionally, the administered measures were compared in terms of their validity to detect executive dysfunctioning.

METHODS: A group of 27 patients (8-16 years) treated for a pediatric cerebellar tumor was assessed with three different executive functioning measures: the Behavioral Rating Inventory for Executive Functioning (BRIEF), the Trail Making Test (TMT) and the Wisconsin Card Sorting Test (WCST). Furthermore, the Strength and Difficulties Questionnaire (SDQ) was administered. Subsequently, test results of the patients were contrasted against the normative samples of these tests using one sampled t-tests. The role of the risk factors (tumor histology, treatment type, age at and time since diagnosis) was analyzed via correlations. To examine whether the different instruments detected the same patients as impaired, cross-tables were used.

RESULTS: The data indicate that the investigated patients suffered from impairments in executive functioning (e.g., flexibility in thinking, concept formation, problem solving, etc). Behavior was affected in peer relationships and in dealing with emotional problems. Patients with a high-grade tumor were more impaired in executive functioning and showed more behavioral problems than patients with a low-grade tumor. It can be concluded that the different test instruments did not detect the same patients as impaired, with the behavior rating (BRIEF) showing the fewest amount of detections.

SUMMARY: This study did not only demonstrate that pediatric cerebellar tumors and associated risk factors have a substantial impact on executive functioning, but also that it is vital to assess it with differentiated diagnostic methods, especially since neurological impairments are not always visible on a behavioral level.

P 33 Axon quantification in the facial nerve

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Background: Facial nerve palsy can have various causes and has a devastating effect on patients. Next to the considerable loss of functionality of mimetic muscles patients frequently report of great psychological distress related to facial palsy. Despite comprehensive knowledge on the anatomical course of the facial nerve and its branches, little is known about the definite quantity of motor and sensory axons within the nerve.

Methods: To analyze the sensory and motor axon contribution of the facial nerve, the main nerve trunk is harvested at its emergence from the skull base in human heart-beating organ donors. Sensory and motor axons are visualized via immunofluorescence double
staining. The neurofilament (NF) antibody labels all neurons present within a nerve, whereas the antibody targeting acetylcholine transferase (ChAT) exclusively labels motor axons, allowing for the first time a detailed quantification of all nerve fibers in the facial nerve.

Results: Preliminary data on so far completed analyses will be presented at the PNS 2017. At the moment two facial nerves have been analyzed with a total axon count of 9,999 and 10,428 respectively. In both donors the motor axon contribution was found to be 24%.

Conclusion: The sensory axon contribution of more than 75% within the facial nerve allows the conclusion that rich proprioceptive feedback is of utmost importance for smooth control of mimetic muscles.

P 34  Amphetamine-induced cortisol release in patients with schizophrenia and healthy subjects: influence of sensitization and sex


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P 35  The post-transplant course of patients undergoing liver transplantation for nonalcoholic steatohepatitis versus cryptogenic cirrhosis: a retrospective case-control study

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P 36  Weight loss, reoperations and reflux – 10 years of lap. Sleeve gastrectomy. Our first 100 patients.

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Background: Laparoscopic Sleeve gastrectomy (LSG) is currently the most frequently performed procedure for obesity and its comorbidities worldwide. As a restrictive surgical method it involves the resection of a major part of the stomach, thus creating a narrow sleeve. Aspects of interest in this context are de-novo reflux and its possible effects, such as esophagitis and Barrett's esophagus, as well as adequate weight loss in a long-term follow-up. Method: This cross-sectional study of the first 100 LSG patients was conducted in a multi-center setting. The mean follow-up was between 10 and 14 years. Data on weight loss success, complications and reoperations was collected from all participating patients. Non-converted patients were also asked to complete questionnaires about their quality of life (BAROS, SF36, GIQOL, BQL). Patients also received gastroscopies (including biopsies), manometries and 24h pH-metries. Results: These first 100 patients, treated in one of the three bariatric centers mentioned above, had their Sleeve gastrectomy between 2003 and 2006. A third of them was converted to a Roux-en-Y gastric bypass within the follow-up period. Today, half of the patients who were not converted suffer from active gastritis and ulcers; Barrett's metaplasia at the gastroesophageal junction was found in 15%. The 24-h pH-metry and manometry's results were pathological for 50% of the non-converted patients. Primary Sleeve patients as well as those who were converted in the follow-up period managed an Excess Weight Loss (EWL) of 50% at 10 years or more. Data on patients' quality of life will be presented at the congress as well. Conclusion: The results of this longterm study reveal that 10 years after LSG a number of patients has had to deal with conversions and/or postoperative reflux and weight regain. This suggests that a careful selection of patients is necessary when considering LSG.

P 37 Slit2 is a novel human brown fat derived factor with thermogenic function


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Obesity is associated with the expansion of white adipose tissue (WAT) which serves as the main energy storage site for excess energy. In contrast, brown adipose tissue (BAT) is specialized in energy dissipation and fatty acid oxidation. This process is mainly mediated by uncoupling protein-1 (UCP-1) and results in heat generation (thermogenesis). Recent clinical studies using 18F-fluorodeoxyglucose positron emission tomography combined with computed tomography (18F-FDG PET/CT) have established BAT as an important metabolic organ in human adults with beneficial effects on energy expenditure, fat mass, and glucose homeostasis. However, the distinct functions of human BAT and its role as secretory organ has not been well studied yet. Preclinical studies have shown that Slit2, a member of the Slit extracellular protein family, is a factor secreted by murine brown fat cells and promotes a thermogenic phenotype including protection against obesity in mice. Using 18F-FDG PET/CT scans we show here that modest cold exposure (14-17°C) for approximately two hours results in robust BAT activation in young healthy adults. Cold-induced BAT activation was accompanied by a significant increase of circulating Slit2 concentrations. Human BAT biopsies taken before and after cold activation showed elevated Slit2 mRNA and protein expression in response to cold exposure. Next we isolated human stromal vascular cells from subcutaneous adipose tissue obtained from abdominoplasty and differentiated these cells to mature adipocytes followed by stimulation with human recombinant Slit2. Notably, Slit2 treatment increased expression of the key thermogenic mediator UCP-1 in human adipocytes. In summary, we have identified Slit2 as a novel human BAT-secreted factor that promotes a thermogenic program in white adipocytes.

P 38  Angiotensin formation in a model of skin infection
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The regulation of electrolyte balance is considered the main action of the renin-angiotensin system (RAS); Yet, crosstalk between immunological processes and the RAS via angiotensin (Ang) II and Ang-(1-7), the antagonizing main effectors, have been uncovered in vitro. We characterized local tissue RAS (Ang levels and formation rate) in a mouse model of experimental infection to define disease-dependent local RAS (dys)regulation. Two mouse strains with different healing capacity (Balb/c and C57/Bl6) were infected
locally (L. major) into one hindlimb. At maximum swelling (day 30-40), mice were sacrificed, plasma and skin of infected areas as well as the uninfected contralateral limb was harvested for subsequent mass spectrometric analysis of RAS activity.

In infected skin, tissue Ang II content was lower (~50 to -90%) than in uninfected areas from the contralateral limb. Tissue activity of angiotensin-converting enzyme, but not chymase was lower (~66% vs. 2%) in infected areas. Endogenous Ang-(1-7) was present at very low concentrations (<10pg/mL), yet activity of Ang-(1-7) forming enzymes was over 40-fold higher than Ang II forming enzymes. Both mouse strains were similar in their RAS regulation.

Unexpectedly, pro-inflammatory and pro-fibrotic Ang II was present at lower amounts in inflamed areas, yet anti-inflammatory Ang-(1-7) was only present at minute amounts. How the RAS is affected during the course of infection, will be investigated in a dedicated study.

P 39  A Salt Mine under the Skin: Subcutaneous Sodium Storage in Haemodialysis Patients


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The largest organ of the human body – the skin – seems to possess an enormous capacity of storing sodium, as recently discovered by 23Na-magnetic resonance imaging (MRI). On one hand beneficial, sodium is thought to ward off infections on local sites, on the other hand chronic accumulation of skin sodium seems to correlate with left ventricular hypertrophy, even independent of blood pressure and overhydration. Since haemodialysis patients often lack significant residual kidney function to excrete water and electrolytes, they are at high risk of accumulating sodium. Measurement of skin sodium content by 23Na-MRI is a non-routine method, therefore we sought an alternative method by measuring sodium in induced sweat. This test will help to identify patients with high tissue sodium content, where a directed sodium reduction therapy should be forcibly anticipated. This exploratory, cross-sectional evaluation of haemodialysis patients (n=8) was performed before dialysis and two days after the last dialysis (maximum sodium/water buildup). As reference, healthy volunteers (n=8) were invited. Iontophoresis with pilocarpine was performed at least two sites of the lower arms for five minutes. Sweat was collected for 30-45min, later sodium concentration was determined by a specialized blood gas analyzer. Medication and vital parameters were recorded at time of sample collection. We observed a considerable higher sweating rate in male participants, some females exhibited almost no inducible sweat production. Male haemodialysis patients had highest sodium concentration (67mM), female patients lowest (25mM) compared to healthy controls (male: 48mM, female: 46mM)

In haemodialysis patients, a considerable gender-specific tissue sodium accumulation
seems to be present. How this test can help to improve dialysis quality and sodium removal is currently under investigation.

P 40  Genetic diagnosis of fetal akinesia syndrome and associated abnormalities by means of whole exome sequencing

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Introduction: The term fetal akinesia represents disorders within a broad spectrum of diseases leading to reduced or absent fetal movements. This clinical entity is often recognized as a sequence of related deformational changes and includes features like intrauterine growth restriction, craniofacial anomalies, limb contractures, together with pregnancy complications such as polyhydramnios. In the last years, gene discovery was revolutionized by implementation of NGS technologies, whereas research mainly focused on postnatally well-defined phenotypes and less on fetal lethal disorders.

Materials and Methods: We included 15 affected fetuses in 11 families. Fetal akinesia syndrome was diagnosed prenatally by ultrasound and/or MRI. Mainly the clinical diagnosis was leading to decision of termination of pregnancy or later to perinatal death. In all cases chromosomal aberrations were excluded. Exome sequencing was performed in the index case of each family.

Results: In 4/11 families, including two consanguineous families, more than one fetus was affected and an autosomal recessive disorder was likely. We correlated exome data with known "33" disease-related genes and found pathogenic variants of CNTN1-gene and RYR1-gene, respectively in two cases. In one case exome sequencing was negative, but a DMPK-gene repeat expansion was identified. In the remaining eight cases no previously reported variant was found.

Conclusions: Fetal akinesia syndrome is a genetically heterogeneous disorder, following different types of inheritance. In a minority, a variant in a known disease-related gene was found. So far, in the majority the underlying genetic cause remained unknown, but several new candidate genes were identified based on our exome data.

P 41  Intra- and interrater reliability of fat and muscle thickness ultrasound measurements in 10 healthy subjects

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Whole body fat and muscle mass influence pharmacokinetics, functional status and mortality. The objective was to test the intra- and interrater reliability of an ultrasound method to measure fat and muscle thickness in 10 healthy subjects. The ultrasound examination comprised measurement of limb length, landmarking of measuring points, performance and evaluation of ultrasound scans. 2 measuring points on the left upper arm and 3 measuring points on the left thigh were landmarked with an erasable pen. On each measuring point a transversal and sagittal ultrasound scan was taken. 5 examiners performed the ultrasound examination in 10 healthy subjects. Each examiner repeated the ultrasound examination in each subject. 2000 fat and muscle thickness measurements were recorded.

For limb length measurement a Bland-Altman comparison between the first and second examination yielded a bias of -0.1 cm (95% CI -0.3 to 0.1 cm) with a range of agreement from -2 to 2 cm. Comparisons between each examiner pair revealed a bias ranging from -0.03 to 0.6 cm (all 95% CIs included 0) with a range of agreement from -4 to 4 cm. For fat and muscle thickness measurement a Bland-Altman comparison between the first and second examination yielded a bias of -0.01 cm (95% CI -0.03 to 0.01 cm) with a range of agreement from -0.65 to 0.62 cm. Comparisons between each examiner pair revealed a bias ranging from -0.08 to 0.10 (all 95% CIs included 0) with a range of agreement from -0.75 to 0.90 cm. The mean intrarater difference for fat and muscle thickness measurements improved from the first 5 healthy subjects to the last 5 subjects (bias (95% CI) [range of agreement] from -0.04 cm (-0.07 to -0.01 cm) [-0.71 to 0.63 cm] to 0.01 cm (-0.02 to 0.04 cm) [-0.58 to 0.60 cm], P=0.02).

The ultrasound examination has clinically acceptable intra- and interrater reliability with ranges comparable to other studies. There was a training effect in thickness measurement within examiners.

P 42  A novel mechanism of antibody-induced enhancement of flavivirus cell attachment and infection

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The major surface protein E of flaviviruses mediates cell attachment and membrane fusion after endocytic uptake. Recent evidence has indicated that the E dimers on the virus surface undergo dynamic movements leading to transient exposure of structures that would be cryptic in a rigid viral envelope like the buried fusion peptide (FP). The FP is known to become exposed and initiate fusion at the low pH of endosomes. Many molecules have been described as candidate receptors, but their specific roles in virus entry remain obscure. Flaviviruses can also infect Fcγ-receptor-positive cells through the internalization
of virus-antibody complexes, thereby bypassing the requirement for a “true” virus-specific receptor.

We investigated factors influencing cell attachment and infectivity of the flavivirus tick-borne encephalitis virus (TBEV). Specifically, we assessed whether structural dynamics together with antibody-mediated effects can allow the FP to contribute to cell attachment. We used TBEV, TBEV E-specific monoclonal antibodies (mabs) and different cell lines. Virus cell binding was quantified by qPCR and infectivity was measured by focus forming assays. The flavivirus dengue virus type 2 (DENV), recognizing DC-SIGN, and the human rhinovirus 2 (HRV2), with a known receptor, served as controls.

Binding of TBEV and DENV to permanent and primary skin cells was very low compared to HRV2. In contrast to DENV, TBEV did not bind efficiently to DC-SIGN bearing cells. Pre-incubation of TBEV with one of the E-specific mabs enhanced cell binding 5 to 6 fold and infectivity 2.5 to 3-fold. Biochemical analyses revealed that this mab dissociated the E dimer and led to the exposure of the FP, which we could show to be responsible for enhanced binding and infection.

In summary, we have identified a novel mechanism of antibody-induced enhancement of binding and infection of flaviviruses, independent of Fcγ-receptors, relying on the exposure of the otherwise cryptic FP.

P 43 Zika virus cross-neutralization by polyclonal sera after dengue virus infection

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The explosive spread of Zika virus to Pacific Islands as well as South- and Middle America underscores the potential threat of newly emerging arthropod-borne viruses. At present it is unclear which factors contributed to this emergence, potentially including mutational adaptations that changed the character of this virus. In addition, however, there is increasing evidence that pre-existing cross-reactive flavivirus (especially dengue) antibodies can enhance Zika virus replication and thus might play a role in pathogenesis. Recent structural and monoclonal antibody studies have identified epitopes involved in infection enhancement as well as cross-neutralization between Zika and dengue viruses and provided new insights into their antigenic structures. Information on cross-neutralization of Zika virus by polyclonal antibodies induced by other flavivirus infections and/or vaccinations is currently lacking. We have therefore conducted a systematic analysis of Zika virus neutralization by serum samples from dengue virus-infected people to obtain new and quantitative data on the extent and individual variation of cross-neutralization between the two viruses. We analyzed a panel of 31 serum samples from travelers returning to Austria from South America and Asia that were diagnosed with dengue. For measuring virus neutralizing antibodies, we have established high-throughput microneutralization tests (NTs) with Zika and dengue viruses. Four of the 31 dengue serum
samples (13%) showed considerable Zika virus-neutralizing activity (50% neutralization titers > 100), but the titers were still significantly lower than the homologous dengue NT values. These data indicate that Zika-dengue cross-neutralizing antibodies represent a rare subset in only a few dengue virus-infected individuals. Currently, we are investigating the reciprocal situation, i.e. whether and to what extent polyclonal sera obtained from Zika virus-infected patients can neutralize dengue virus.

P 44 Flavivirus E protein stem interactions in virus entry

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Introduction: Flaviviruses enter cells by receptor-mediated endocytosis. After virus uptake, the viral envelope protein E mediates fusion of the viral and the endosomal membrane, triggered by the slightly acidic pH in endosomes. The current fusion model is based on atomic structures of truncated forms of the E protein in their dimeric pre- and trimeric post-fusion conformation. These structures lack the two transmembrane-domains and the so-called “stem”-region. The stem connects the ectodomain and the membrane anchor and is hypothesized to be essential for fusion by “zippering” along the trimer core during the conformational changes of E necessary for fusion.

Objectives: Since stem interactions are essential for fusion and hypothesized to provide part of the energy required for this process, we wanted to gain information on these interactions as well as their role in the fusion process by a mutagenesis approach using tick-borne encephalitis virus (TBEV), a major human pathogenic flavivirus.

Materials and Methods: We introduced modifications (point mutations, deletions) into the stem of recombinant E proteins as well as an infectious clone of TBEV and analyzed their effect on E protein trimerization, trimer stability and infectivity.

Results and Conclusion: We identified important interaction sites between the stem and the trimer core involved in the stabilization of the post-fusion conformation. In addition, replacing conserved residues in the stem resulted in a strong reduction in the production of infectious particles. Passaging experiments allowed the identification of resuscitating mutations which led to a recovery of the viability of mutant viral particles to wildtype levels. Currently, we investigate whether the observed phenotypes are caused by defects in virus entry, assembly or both processes.

P 45 Showing Strength: Mechanical Forces in T-Cell Antigen Recognition

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The recognition of antigenic peptide/MHC complexes (pMHC) on antigen presenting cells (APC) by T-cells is mediated by the T-cell receptor (TCR) and leads to the formation of an immunological synapse. This process is remarkably specific, sensitive and efficient but up to this date the underlying mechanisms are only poorly understood. There is mounting evidence that mechanical forces acting on the TCR are indeed instrumental in TCR-ligand discrimination and TCR-mediated signaling. To investigate this in sufficient detail, we will attach force sensors to pMHCs, which will be embedded either on a well-defined functionalized planar lipid bilayer system or on the surface of live APCs. Fluorescent dyes on both ends of the sensor will serve as donor and acceptor for Förster resonance energy transfer (FRET). We expect high FRET values when no tension is applied and the sensor is relaxed. However, cell-imposed forces on the TCR should stretch the sensor and reduce FRET due to larger distances between the FRET dye pair. To correlate measured FRET to corresponding forces we will calibrate sensors with optical tweezers. We intend to perform FRET measurements in bulk to map forces within the immunological synapse and also to perform single molecule experiments to assess the true molecular force dynamics. To determine the role of TCR-imposed forces in T-cell triggering and ligand discrimination we will correlate them with the stimulatory potency pMHCs and simultaneously imaged downstream signaling. In summary, we expect to visualize TCR-imposed forces most directly, establish them as a crucial factor in T-cell antigen recognition. We expect that many of the lessons learned will be applicable to studying other cell-cell interactions as they might be relevant in the activation of other immune cells, cancer progression, infection biology and neurosciences.

P 46  Quantification of the TBEV neutralising antibody response to antibody subsets in human and mouse sera after depletion


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Tick-borne encephalitis virus (TBEV) is an important human-pathogenic flavivirus endemic in large parts of Europe, Central and Eastern Asia. Long-lasting protection after natural infection or vaccination with TBEV is mediated by neutralising antibodies, recognising the viral envelope protein E. Recently, recombinant virus particles carrying a luciferase reporter gene provide a new possibility for sensitively measuring antibody-mediated neutralisation in polyclonal sera.

In order to characterise the neutralising antibody responses against TBEV in human and mouse serum samples and to quantify the contribution of defined antibody subsets to overall neutralisation, we wanted to establish a novel neutralisation test (NT) using single-round infectious TBE reporter virus particles (RVP).

We designed and optimised a neutralisation test with TBE RVPs in which infection of cells
was measured as a function of luciferase activity. For further quantification of antibody subpopulations with different fine specificities and their contribution to overall neutralisation, different test sera were depleted with recombinant antigens representing viral surface protein domains and domain combinations.

The novel reporter virus neutralisation test proved to be a robust, sensitive and fast assay, allowing us to analyse human and murine neutralising antibody responses. By using the reporter NT before and after depletion with different recombinant protein domains of E, we could define the contribution of distinct antibody subsets to the overall neutralisation and thereby distinguish differences in the immunodominance patterns of individual test sera.

P 47 The two faces of Hepatitis A virus: Quasi enveloped versus non enveloped
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Recently, a lipid enveloped [LE] form of the traditionally non lipid enveloped [NLE] Hepatitis A virus [HAV] was described in human serum and cell culture derived HAV stocks. This discovery challenges the understanding of HAV reduction in virus clearance studies of plasma products, which were performed under the premise of a NLE nature of this virus. Here, the presence of LE particles in HAV stocks used for reduction studies was verified by isopycnic gradient centrifugation. Preparations of wildtype [wt] HAV from human plasma and cell culture lysates of two cytopathic HAV variants, HM175/18f and HM175/24a, contained either LE particles (HM175/24a, wt HAV) or LE and NLE particles (HM175/18f). The LE and NLE HAV fractions were characterized for relative infectivity and then subjected to solvent detergent [S/D], heat and low pH treatment to investigate their respective physico-chemically stability. After S/D treatment, all three HAV samples had lost their LE HAV fractions and showed an intermediate [IM] density HAV fraction upon isopycnic centrifugation analysis, whereas HAV HM175/24a, but not wt HAV, showed in addition a high-density NLE HAV fraction. The specific infectivity for HM175/24a LE, IM and NLE fractions, respectively, was equivalent, whereas only LE and NLE fractions of HM175/18f had a comparable infectivity titer. Heat treatment at 58°C for 590 minutes inactivated all three HM175/24a phenotypes to below the limit of detection at the end of the incubation period. However, heat treatment was not sufficient to inactivate HM175/18f LE and NLE virions, suggesting that HM175/18f is the more heat resistant variant. In comparison, low pH (pH 2, 3 or 4.7) treatments of HM175/24a and HM175/18f NLE particles had no significant effect on viral inactivation. In conclusion, the findings suggest that the LE or NLE HAV phenotype is less important than the choice of HAV variant.

P 48 Selective free fatty acid-Pru p 3 interaction enhances its IgE binding activity
Identification and characterization of HDAC1 and HDAC2 interaction networks in Th17 cells

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The differentiation and function of CD4+ T helper (Th) subsets has to be tightly regulated, since their dysregulation is linked with immune-mediated diseases. Th cell differentiation is accompanied by reversible changes in histone acetylation, mediated by the opposing activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs), however many non-histone targets are emerging, indicating that HAT/HDACs act beyond the regulation of chromatin.

Results of my laboratory demonstrate an essential role for HDAC1 but not for the very closely related HDAC2 protein in regulating Th17 cells and the development of autoimmune diseases. Since HDAC1 and HDAC2 are part of larger multiprotein complexes, it is tempting to speculate that the crucial role of HDAC1 in Th17 cells is mediated by factors that interact with HDAC1 but not with HDAC2. The aim of my PhD thesis project is to test this hypothesis. I will utilize novel mouse models in combination with mass spectrometry approaches to generate a HDAC1 and HDAC2 interaction network map and to define HDAC1-specific interaction partners. I will study the role of selected HDAC1-specific interaction partners in Th17 cells to functionally link them with HDAC1 and Th17 cell function. My experimental approach will unravel molecular details of how HDAC1 regulates Th17 cells and the development of T cell-mediated (auto)immune responses.

Analysis of novel therapeutic approaches and potential diagnostic markers in prostate cancer

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Prostate carcinoma is one of the most commonly diagnosed types of cancer and the second most common cause of cancer mortality among men in the USA and in Europe. The major causes of the disease remain unknown, although hormonal status and lifestyle are known risk factors. Prostate specific antigen has emerged as a useful tumor marker in oncology. However, a relative lack of both specificity and sensitivity is leading to both over diagnosis and cases not being detected early enough. Therefore, we aimed to define and explore new therapeutic approaches and novel biomarkers for diagnosis and treatment of prostate cancer. The Chinese medicinal herb Panax quinquefolius has been tested for its tumor selectivity and cytotoxic efficacy. P. quinquefolius saponins (PQS) have been shown to have anti-tumor effects in vivo and in vitro. DU145 human prostate cancer cells were treated with PQS. Cell viability assays and FACS based apoptosis assays were performed after treatment. The inhibitory effects on the proliferation of DU145 indicated that PQS inhibits the growth of prostate cancer cells. A combination of microarray assays and western blotting was used to identify prostate specific gene expression patterns and to explore novel biomarkers for potential clinical use in prostate cancer diagnostics. TMEM79 was found to be highly enriched in normal prostate cells but is expressed only at low level in prostate cancer and therefore has the potential to become a novel biomarker for early diagnosis. In conclusion, PQS promotes apoptosis and inhibits the proliferation of prostate cancer cells suggesting that PQS might be an effective herbal remedy for treating prostate cancer. Microarray assays and western provide an advantageous strategy to identify specific markers of prostate cancer. Our results demonstrated that TMEM79 could be a novel candidate biomarker for prostate cancer diagnosis. Supported by the BMWF/BMG (GZ402.000/0006II/6b/2012) and the BMWFW (GZ402.000/0009-WF/V/b/2016).

**P 51  mTORC1 controls proliferation and survival of macrophages by regulating polyamine synthesis**


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The mammalian target of rapamycin (mTOR) has a central role in the effector functions of innate immune cells. Polyamines (PAs) are essential for normal cell growth and development. However, elevated levels are associated with various pathologies, especially cancer. In our previously described mouse model, we constitutively activate the mTOR complex 1 (mTORC1) specifically in myeloid cells by deleting its negative regulator TSC2 via the Cre/LoxP system (TSC2ΔM). We observe an expansion of strongly proliferative M2-like macrophages in various tissues, which also show highly increased levels of reactive oxygen species (ROS). A shot-gun metabolomic analysis of the lung indicated that the PA synthesis
pathway is affected in TSC2ΔM mice. In fact, we found significantly increased PA levels in bone marrow-derived macrophages (BMDM) of TSC2ΔM mice, which could be reduced by treatment with the mTOR inhibitors rapamycin and Torin1. This demonstrates that activation of mTORC1 promotes PA synthesis. Molecularly, we identified the antizyme inhibitor 1 (AZIN1), which is important for maintaining intracellular PA levels, as target of mTORC1. Further analysis revealed additional mTORC1 dependent enzymes. Functional evaluation showed that the highly increased proliferative capacity and elevated viability of TSC2ΔM macrophages is decreased by treatment with PA inhibitors and analogues (DFMO, Sardomozide and DENSpm). These data demonstrate that TSC2 and mTORC1 control the synthesis of PAs to regulate proliferation and survival of macrophages. As PAs as well as ROS are known to promote tumor growth, we performed co-culture experiments with a tumor cell line, and found an increased tendency of tumor cells to grow in the presence of TSC2ΔM macrophages. In order to study this phenomenon in vivo, we are performing a colitis associated cancer model. This will shed light into the role of mTORC1 and PAs in macrophages for tumor formation.

P 52  Viruses comprise an extensive pool of mobile genetic elements in eukaryote cell cultures and human clinical samples

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Viruses shape a diversity of ecosystems by modulating their microbial, eukaryotic, or plant host metabolism. The complexity of virus-host interaction networks is progressively fathomed by novel metagenomic approaches. By using a novel metagenomic method, we explored the virome in mammalian cell cultures and clinical samples to identify an extensive pool of mobile genetic elements in all of these ecosystems. Despite aseptic treatment, cell cultures harbored extensive and diverse phage populations with a high abundance of as yet unknown and uncharacterized viruses (viral dark matter). Unknown phages also predominated in the oropharynx and urine of healthy individuals and patients infected with cytomegalovirus despite demonstration of active cytomegalovirus replication. The novelty of viral sequences correlated primarily with the individual evaluated, whereas relative abundance of encoded protein functions was associated with the ecologic niches probed. Together, these observations demonstrate the extensive presence of viral dark matter in human and artificial ecosystems. — Thannesberger, J., Hellinger, H.-J., Klymiuk, I., Kastner, M.-T., Rieder, F. J. J., Schneider, M., Fister, S., Lion, T., Kosulin, K., Laengle, J., Bergmann, M., Rattei, T., Steininger, C. Viruses comprise an extensive pool of mobile genetic elements in eukaryote cell cultures and human clinical samples. FASEB J. 31, 000–000 (2017). www.fasebj.org
**P 53  The role of mTOR signaling in macrophages for iron homeostasis**


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Macrophages are essential for maintaining iron homeostasis and erythropoiesis. In spleen and liver, macrophages phagocytose senescent erythrocytes and recycle iron via transferrin to support erythropoiesis in the bone marrow (BM). However, the molecular pathways that regulate iron homeostasis in macrophages are ill-defined. Therefore, we assessed a potential role of mTORC1 for iron recycling in macrophages using the Cre/LoxP mouse model, in which macrophage-specific mTORC1 hyperactivation is induced by deletion of its negative regulator TSC2 (TSC2ΔM mice).

Freshly isolated bone marrow from TSC2ΔM mice was pale suggesting a defect in erythropoiesis. In line, reduced numbers of both immature and mature erythrocytes as well as significant lower numbers of erythroblastic islands in BM from TSC2ΔM mice indicated a failure in steady state erythropoiesis. In contrast, we observed an enhanced stress erythropoiesis in the spleen of TSC2ΔM mice, which balanced systemic erythrocyte levels. Inhibition of mTORC1 with everolimus completely restored erythropoiesis in the BM of TSC2ΔM mice. Moreover, a highly reduced iron content in the BM and spleen and strongly diminished hepcidin expression in the liver of the TSC2ΔM mice were indicative of alteration of iron metabolism as a cause of the erythropoiesis defect. Erythrocytes from TSC2ΔM mice had a significantly prolonged half-life, were more resistant to osmotic pressure and contained less haemoglobin compared to TSC2 control mice pointing to microcytic, hypochromic iron deficiency anaemia. Interestingly, the amount of transferrin bound iron in serum was highly reduced in TSC2ΔM mice, although total iron in serum was not altered. These results suggested that the erythropoiesis defect is caused by a dysfunctional regulation of transferrin iron by macrophage in the BM.

However, the mTORC1-dependent molecular mechanisms that control cellular iron metabolism in the macrophages of BM are being further investigated.

**P 54  The role of HDAC1/2 in CD4+ lineage integrity**

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P 55  Comparative assessment of antiviral immune responses in human Langerhans cells and keratinocytes


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Patients with atopic dermatitis are prone to cutaneous virus infections which can result in life threatening conditions. A better understanding of human skin immune defense mechanisms against viruses is needed for the development of more effective approaches to prevent and treat epitheliotropic virus infections. Together with keratinocytes (KCs), epidermal Langerhans cells (LCs) may also contribute to the initial innate immune response by capture invading viruses at the site of infection. To investigate the contribution of particular viral sensing receptors, we have established an ex vivo barrier-disrupted infection skin model. Using this model, we studied the effects of topically applied synthetic viral pattern recognition receptor (PRR) agonists on LCs and KCs.

We found that both, LCs and KCs were able to take up rhodamine-labeled poly(I:C) in situ and at the single cell level. Stimulation with poly(I:C) strongly induced the production of the proinflammatory cytokines IL-6, TNF-alpha and IL-8 in KC cultures. In contrast, these cytokines were not detectable in supernatants of LC cultures indicating that different downstream signaling-pathway might be activated in LC. Interestingly, the PRR MDA5 but not TLR3 and PKR was significantly downregulated in LCs in barrier-disrupted skin after treatment with poly(I:C). Furthermore, a differential activation of MAVS, IRF3 and NFκB (p65) was found in LCs and KCs upon treatment with poly(I:C). Our data suggest that MDA5 but not TLR3 and PKR may play a key role in the innate immune response of LCs to viral infection. Understanding the signaling events of LCs to viruses might promote development of attractive therapeutic strategies.

P 56  Macrophages in Acute Respiratory Distress Syndrome


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Acute respiratory distress syndrome (ARDS) remains a challenge on the intensive care unit (ICU) both in terms of the pathophysiologic understanding of the disease, as well as the therapeutic approach. In light of the high degree of ARDS heterogeneity, studies yield more reliable results, if ARDS subgroups are analyzed independently. Thus, we focus a) on ARDS associated with suspicion or clinical diagnosis of ventilator-associated pneumonia (VAP), since this is the most frequent risk factor for ARDS, and b) on ARDS in neurotrauma patients without an infectious component. We aim to investigate the types of macrophages present in the bronchoalveolar lavage (BAL) fluid in four distinct groups of patients: lung-healthy patients (1), patients with mechanical ventilation and an event-free ICU stay (2), ICU patients diagnosed with ARDS without an infectious cause (3) and ICU patients with ARDS and VAP (4). We aim to determine their contribution to the pathogenesis of ARDS and VAP, as well as patterns of gene expression characteristic to each condition. Furthermore, we investigate correlations between macrophage profile and patient outcome (e.g. mortality). We perform a BAL in all patients belonging to the above-mentioned four cohorts. Furthermore, lung-healthy preoperative patients provide a sample of induced sputum one day before surgery. Cellular subsets in the BAL fluid are analyzed by multicolor flow cytometry. The transcription profile of fluorescence-activated cell (FACS)-sorted alveolar macrophages is determined after mRNA isolation and sequencing. All BALF and sputum samples are tested for the presence of bacteria by culture or PCR. By characterizing the expression patterns in ARDS patients depending on the underlying pathology (infectious vs. non-infectious) and by discriminating these changes from those induced by mechanical ventilation, this study will provide a basis for identifying individual risk factors and susceptibility to lung disease on the ICU.

P 57  Dissecting the human host response toward borrelia


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Borrelia is one of four genera within the phylum Spirochaetes. The genus Borrelia holds the agents of relapsing fever and Lyme borreliosis [LB]. In Europe, the genospecies B. afzelii, B. bavariensis, B. burgdorferi sensu stricto [s.s.], B. garinii and B. spielmanii are known as pathogens of LB. In the United States B. burgdorferi s.s. is almost the only agent of LB, while patients in Europe are mostly infected by B. afzelii and B. garinii. The bacterium is maintained in nature through an enzootic cycle between ticks of genus Ixodes, different mammalian hosts and certain bird species. Humans can act as accidental hosts when these spirochetes are transmitted by the bite of an infected tick. Disseminated infections comprise disorders of the nervous system, joints, heart, eyes and other organs. The aim of our study is to detect characteristics in the interaction of Borrelia with various host cells. Hence, we will grow pathogenic and non-pathogenic Borrelia strains to late logarithmic phase. Subsequently, we will use the bacteria for co-cultivation with human cells known as
first contact points, i.e. endothelial cells, different types of innate immune macrophages and dendritic cells. We will analyse how the host cells bind and uptake the bacteria and how they handle and process them. We will use advanced microscopy, flow cytometry and image stream. To obtain comprehensive insight into the host response we will perform transcriptome profiling. Finally, we will analyse how the first line innate immune cells translate the recognition of Borrelia to adaptive immunity: T cell proliferation and differentiation will be analysed by expression profiling of receptors, signalling molecules and cytokines. These studies should provide insight why Borrelia are able to frequently escape the immune system and survive for long periods in hosts. Moreover, we hope to identify novel biomarkers for differentiation of acute versus chronic infection.

P 58  Immunodominance of human CD4 T cell responses induced by yellow fever vaccination


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The live attenuated yellow fever virus (YFV) vaccine, YF-17D, stimulates all arms of the immune system. Neutralizing antibodies directed against the major envelope protein (E) are an established correlate of protection. The production of such antibodies by E-specific B requires direct interaction with CD4 T cells which recognize MHC II-restricted epitopes derived from the three structural proteins E, prM/M (membrane) and C (capsid) that are internalized as part of the virion. The mechanisms that lead to epitope dominance are still largely unknown but in addition to peptide-MHC affinity, the 3D structure of virus proteins may be critical for epitope selection.

In this study, we determined human CD4 T cell specificities to YFV structural proteins in a large panel of 76 YF-vaccinated individuals. The epitope data obtained in IL-2 ELISPOT assays were analyzed in relation to the three-dimensional structures of C and E proteins, as well as to in silico predictions of peptide-MHC II affinity and were compared to those elicited with the structurally similar tick-borne encephalitis virus (TBEV).

The epitope hierarchies generated in response to YF-17D showed a high degree of homology with those of the distantly related TBEV which shares only 40% identical amino acids in the E protein. Thus, our data suggest a strong impact of conserved structural protein features on the immunodominance of flavivirus CD4 T cell responses.

P 59  Tracking T cells in human skin: Turnover and phenotype of tissue-resident memory T cells
Most of our knowledge about the recirculation and distribution of cutaneous tissue-resident T cells (Trm) is based on observations from murine studies, while the fate of Trm in human skin is still poorly understood. Therefore we followed patients who underwent myeloablative therapy, total body irradiation and subsequent allogenic hematopoietic stem cell transplantation (HSCT) in order to track the distribution and phenotype of cutaneous T cells over time.

51 biopsies and blood samples from 12 patients taken at four time points before and after conditioning regimens/HSCT were analyzed by immunofluorescence and flow cytometry. T cells were characterized using T cell homing molecules and markers defining cutaneous Trm. Tissue stainings were automatically quantified using TissueQuest software.

In concordance with data of peripheral blood, the overall number of CD4+ and CD8+ T cells in the skin declined upon HSCT as compared to baseline (pre-conditioning). In contrast to studies in mice, CD69+ and CD103+ Trm presented a mixed CD4+/CD8+ phenotype and clustered in the upper dermis. Interestingly, numbers of cutaneous CD69+ T cells remained relatively stable throughout all time points, arguing for the presence of a pool of radiation-resistant cutaneous Trm. The majority of dermal T cells were CCR7-CD62L-, a marker profile expressed by non-recirculating T cells. Small populations of central (CCR7+CD62L+) and migratory memory T cells (CCR7+CD62L-) were present in the dermis before conditioning therapy but vastly declined in subsequent skin biopsies early after HSCT (days 0–21) and resurfaced in biopsies taken at days 95–105 after HSCT.

These data present new insights into the resilience of skin TRM and contribute to our understanding of T cells recirculating peripheral tissues. In addition, they raise the question of the importance of host-derived T cells in the initiation and propagation of graft-versus-host disease, a common and potentially fatal complication of HSCT.

P 60  Differently composed IgE-Bet v 1 complexes modulate specific T cell activation and induce the phosphorylation of ERK 1/2 upon its binding to CD23

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Most ticks of the family Ixodidae produce a material called cement that supports their anchoring to the host skin while feeding. Due to its adhesive properties in humid conditions, the material is interesting as bionic template for new tissue adhesives. In the current project we analyse the structure and composition of the cement of two tick species. Amblyomma hebraeum and Dermacentor marginatus were cultivated in vitro on silicone membranes in specific tick feeding units. Cement was harvested and analysed by histology, electron microscopy, element analysis (EDX) and biochemistry. For histochemistry paraffin sections were performed and stained with Biebrich Scarlet (BBS), Periodic acid–Schiff (PAS), Martius Scarlet Blue (MSB) and Alcian blue. Protein content and characterization is assessed by gel electrophoresis (SDS-PAGE) and amino acid analysis after total acid hydrolysis.

Whole cement deposits of A. hebraeum primarily appears at the lower side of the artificial membrane, D. marginatus also build small cement cones at the upper surface. Histochemistry on cement gave spherical and whorl-like patterns with BBS for basic proteins and PAS reaction for carbohydrate. Additionally, MSB suggest different densities within these substructures. Alcian blue for mucopolysaccharides only stained at the surface of cement cones. EDX revealed carbon, oxygen, nitrogen, phosphorus and sulphur as main elements in cement.

The data show that even though the material appears homogenous macroscopically, it has an internal compartmentation, probably resulting from the secretory process. Histochemistry indicates that cement is based on proteins and contains carbohydrate. Biochemical analyses will bring more detailed information on the composition of cement such as the amino acids, proteins and the nature of the carbohydrate. This work is funded by the FWF grant # P 28962.

P 62 NKG2D: friend or foe in NK cell mediated tumor surveillance?


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NKG2D is an activating receptor expressed in mice and men on NK, NKT, [gamma][delta]T and CD8[up+] T cells. It has been implicated in autoimmune disease, infection and cancer. Transformed cells express ligands for NKG2D making tumor cells visible particularly for NK cell mediated tumor surveillance. In line NKG2D[up-/] mice are tumor prone and develop more aggressive tumors in a prostate cancer model. Escape mechanism for NKG2D-mediated cytotoxicity have been described including ligand shedding or cytokine-induced downregulation of NKG2D itself or its ligands. Recent reports added a new twist showing that persistent receptor-ligand interaction downregulates the NKG2D receptor and impairs NK cytotoxic functionality. Importantly it was found, that tumor associated myeloid cells also express NKG2D ligands that contribute to the desensitization of NK cells accompanied by deceased tumor surveillance. NK cells are of particular importance for surveillance of hematopoietic cancers. It is unclear if and how NK-cell activation or NKG2D-dependent desensitization interferes with tumor surveillance as both, stromal cells and cancer cells express NKG2D ligands. To study this complex NK cell mediated relations we employed a mouse model specifically deleting the NKG2D locus (KLRK1) in NK cells under the control of the Nkdp46 promotor (hereafter termed as NKG2DΔNK mice). We find that NK cells lacking the NKG2D receptor are viable and display enhanced expression of effector molecules after cytokine stimulation. This relates to an increased ex-vivo killing capacity. In line NKG2DΔNK mice have a significant survival advantage and delayed disease onset in a model of slowly evolving B cell leukemia. This underscores the complexity of NKG2D receptor mediated effects on tumor surveillance and shows that lack of NKG2D on NK cells can be beneficial in hematopoietic cancer surveillance.

1Guerra N. et al. 2008 Immunity 2 Deng W. et al. 2015 Science

P 63  The role of STAT1 and STAT3 in polarization of tumor-associated macrophages


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STAT1 and STAT3 belong to the same family of transcription factors, yet display opposing functions in tumorigenesis. While STAT1 is considered to be a tumor suppressor, STAT3 promotes tumor cell proliferation and survival. Moreover, STAT3 suppresses anti-tumor immune activities. Consistently, formation of azoxymethane/dextran sulfate-induced colorectal cancers (CRC) was strongly reduced in mice lacking STAT3 in myeloid cells/macrophages, partly due to a dominant M1 polarization of STAT3-deficient macrophages. We investigate putative opposing effects of STAT1 and STAT3 signaling in myeloid cells on tumor formation. We use in vitro macrophage cultures to identify tumor –
stroma interactions modulated by STAT1 or STAT3. As a cellular source, we isolate bone marrow cells from mice with conditional deletions of STAT1 or STAT3 in the myeloid compartment and differentiate them into mature macrophages. These macrophages are stimulated with TLR ligands to assess STAT1- and 3-dependent functions in macrophage polarization. We demonstrate a differential response of STAT1- and STAT3-deficient macrophages to stimuli received, reflected by differences in activity and polarization profiles. Our data suggest that certain TLR ligands, present within the intestinal microbiome, might influence macrophage activity in a STAT1/3-dependent manner and modulate pharmacological effects of STAT3 inhibitors in CRC therapy.

P 64  The role of the protocadherin CDHR5 in colorectal cancer
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Protocadherins constitute the largest subgroup of the cadherin protein superfamily and are frequently downregulated in human cancers suggesting a negative role in oncogenesis. The protocadherin CDHR5 is a transmembrane protein that is located in the microvillar brush border of enterocytes, cholangiocytes and kidney epithelial cells. CDHR5 crosslinks microvilli and has been implicated in regulation of beta-Catenin activity. We are interested in CDHR5 functions in colorectal cancer. We found that CDHR5 expression is downregulated in altered crypt foci, adenomas, carcinomas and colorectal liver metastasis. We further demonstrate a tumor-suppressive role of CDHR5 in colorectal cancer using transplantation experiments of cell lines with gain or loss of CDHR5 function. We generated CDHR5 knock-out mice to further investigate CDHR5 functions in autochthonous colorectal tumors. Knock-outs were viable and did not show an overt intestinal phenotype but displayed shortening of microvillus length. Formation of colorectal cancer, induced with the chemical Azoxymethane/Dextran sulfate protocol, was not affected in CDHR5 knock-out mice but the number of aggressive carcinomas invading the muscularis mucosa was substantially increased. These data suggest that CDHR5 is a metastasis suppressor gene in colorectal cancer. We are currently using intestinal organoid cultures, cotransfection experiments and RNASeq of RNA, isolated from intestinal epithelial cells, to unravel molecular function of CDHR5 in colon cancer metastasis.

P 65  Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance.
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Cancer is one of the leading causes of death in the industrialized world. Every third diagnosed cancer is a skin cancer. Imiquimod (IMQ) is an immune modifying compound used as a 5% cream formulation (Aldara) to treat warts and basal cell carcinomas (BCC). The mechanism of action of IMQ relies on the activation of Toll like receptor 7/8 (TLR 7/8) expressing immune cells, prominently a subtype of dendritic cells called plasmacytoid dendritic cells (pDC). pDCs are Type I interferon producing innate immune cells. We have recently shown that if activated they can be converted into tumor killing cells. The tumor killing ability of pDCs relies on the production of lytic molecules like Granzyme B (Gzmb). The production of these tumor killing molecules in pDCs as well as other proinflammatory molecules like tumor necrosis factor alpha (TNF-α) are controlled by a defined subset of transcription factors like interferon regulator factor 7 (IRF 7). Another family of immune regulators is the AP-1 family whose role in pDCs and IMQ mediated tumor clearance is poorly understood. In order to investigate the role of c-Jun in pDC development and function, we are employing mice harbouring floxed c-Jun alleles to delete c-Jun in all bone marrow (BM)-derived cells with the poly I:C inducible Mx-Cre transgenic line. Our results indicate that c-Jun is dispensable for the development and maturation of pDCs. Furthermore, we could show that c-jun is an important factor for the production of Interleukin-6 (IL-6) and Interferon beta (IFN-β) in IMQ stimulated pDCs.

P 66  The role of EGFR in c-fos-dependent osteosarcoma formation


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P 67  Cytoskeletal effects on drug-induced endovesiculation of erythrocytes


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Many biophysical and biochemical studies on lipid bilayer interactions with the cytoskeleton continue to be done on erythrocytes. For physiological reasons they are capable of undergoing rapid cell shape changes within seconds from 'normal' discocytes to e.g. elongated, even cigar-shaped cells to enable passage through narrow capillaries. Thus,
red blood cells can be also used as a model system to improve understanding of both, exo- and endovesiculation. The unequal lipid composition of the inner and outer leaflet of the membrane can cause preferential drug partitioning. Permeable cationic amphiphiles like chlorpromazine mainly intercalate into the inner leaflet, causing endovesiculation, while anionic amphiphiles, like 3-(dodecyldimethylammonio)-1-propanesulphonate insert themselves into the outer membrane leaflet, causing exovesiculation.

Using our novel flow cytometry-based assay, we explored conditions and effectors of chlorpromazine-induced endovesiculation. Vanadate, an agent promoting extrusion of thorn-like projections from the cell surface, and metabolic depletion both strongly inhibited endovesicle formation. In contrast, protein kinase C-dependent phosphorylation of protein p4.1R and destabilization of spectrin tetramers by urea increased endovesiculation. This indicates that a deregulated cytoskeleton and/or reduced interaction between membrane and cytoskeleton enhance this process. Co-stimulation with inhibitors and activators partly compensated their effects.

Moreover, misshaped erythrocytes of chorea acanthocytosis (ChAc) patients revealed a reduced capacity to drug-induced endovesiculation. ChAc erythrocytes respond to endovesiculation effectors similar as erythrocytes from healthy donors, albeit at a reduced level. These data corroborate the notion of alterations in the cytoskeletal organization and dynamics of erythrocytes from ChAc patients.

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P 68 Role of JAK2 in the initiation and progression of KRAS-mutated NSCLC

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P 69 A novel PAK1/Notch-1 axis contributes to intestinal inflammation and cancer

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p21 activated kinase (PAK1) belongs to a family of serine-threonine kinase and contributes to various oncogenic pathways like MAPK and Wnt-ß catenin. Our lab recently linked activation of PAK1 to IBD as well as colitis-associated cancer. PAK1 is also overexpressed in IL-10 KO mice which develop enterocolitis and intestinal neoplasia. We tested the effects of PAK1 deletion on intestinal inflammation and carcinogenesis in IL-10 KO mice. Methods: IL-10 KO mice were crossed with Pak1 KO mice to generate double-knockout mice (DKO).
Inflammation was triggered with piroxicam (200ppm mixed to chow for 14d). After 12 wks mice were euthanized, intestines were prepared and length was measured. Paraffin embedded Swiss rolls were stained with H&E and PAS. IHC was performed with antibodies against Ki67, ERK1/2, activated Notch1, γH2AX, p65/NFκB, lysozyme and LGR5. Results: DKO mice displayed higher disease activity [DAI: 2.448±0.5 vs 1.095±0.34], higher grades of inflammation [3.08±0.22 vs 1.25±0.5] and more dysplastic lesions [6.5±1.8 vs 0.25±0.25] compared to IL-10 KO mice. Despite higher degree of inflammation DKO mice had longer colons compared to IL-10 KO [10.83cm±0.57 vs 8.50cm±0.46]. IHC revealed increased Ki67; MAPK pathway activation, as shown by increased pERK1/2 staining and an increase in γH2AX positive nuclei, indicating more DNA double-strand breaks. A reduced differentiation of goblet cells and Paneth cells was marked by PAS- and Lysozyme staining, together with an expansion of the stem cell compartment (shown by increased activated Notch1 and LGR5 staining) in the colonic crypts of DKOs. Interestingly NFκB was found to be reduced in DKO mice. Conclusion: Our data suggest a novel interaction of PAK1 with the Notch pathway in regulating crypt cell stemness, proliferation and differentiation. Lack of mature goblet cells and mucus can exacerbate colitis in IL-10 KO mice. Hyperproliferation may promote dysplastic lesions independent of NFκB.

P 70  Micro-structural features of Paget’s disease of bone at cortical and trabecular sites


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Paget’s disease of bone (PDB) is a non-inflammatory, metabolic, skeletal disorder characterized by excessive bone resorption that is followed by increased osteoblastic activity. Although PDB is the second most common metabolic bone disease, there is only limited information about the microarchitecture of affected bones. Therefore, the aim of
this study was to determine cortical and trabecular bone properties in clinically relevant locations by µCT. 10 femora and 5 tibiae affected by Paget’s disease taken from the Pathological-Anatomical Collection, Natural History Museum Vienna, were compared to 13 femora and 5 tibiae of non-affected body donors provided by the Division of Anatomy, Medical University of Vienna. We performed analyses of the cortical and trabecular bone microarchitecture with an X-ray based µCT scanner (Viscom X-8060-II). Voltage, current, and scan parameters were adjusted to balance adequate contrast. We have selected the central region of the shafts of femur and tibia as cortical bone sites, and the proximal metaphyses for analyzing the trabecular microarchitecture of each bone. Moreover, semi-quantitative gradings of trabecular and cortical architectural parameters of the femoral head and the femoral midshaft (10 affected versus 13 non-affected) were included. Computed tomography images showed marked cortical porosity, and trabecularization of cortical structures. Moreover, severe disorganization of trabecular structures, trabecular defects, and thickening of (remaining) trabeculae were detected. Preliminary analyses of the femoral neck showed higher trabecular BV/TV (107%, p<0.01) and trabecular thickness (56%, p<0.001), but lower connectivity density (-42%, p<0.05) in Paget’s disease compared to control bones. There is a major and consistent structural alteration of PDB at cortical and trabecular sites. Our findings are relevant for the differential diagnosis of PDB and for the pathogenesis of associated complications.

**P 71  Effects of UVA1 on the Repair of UVB-Induced DNA Damage in Normal Human Melanocytes**


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It is generally accepted that chronic sun exposure is a risk factor for the development of non-melanoma skin cancer. In contrast, the exact correlation between melanoma and sunlight is still a controversially debated issue. Natural sunlight contains various ratios of UVA and UVB, depending on daytime, season, altitude and latitude. Nevertheless, most investigators focused on the effects of single solar wavebands and neglected possible interactions. Therefore we investigated the impact of UVA1 on UVB-induced apoptosis and the repair of UVB-induced DNA damage in normal human melanocytes of three different Caucasian donors. Cells were simultaneously exposed to physiologic doses of UVA1 (340-400nm) and UVB (390-320). On the next day the apoptotic rate was determined using a cell death detection ELISA and Annexin/Propidium Iodide staining followed by FACS analysis. UVA1 did not influence UVB-induced apoptosis and UVA1 alone did not induce detectable amounts of apoptosis at all. Accordingly, UVA1 did not alter UVB-induced activation of caspase 9. Likewise, UVA1 alone did not induce caspases 9. South Western Slot Blot analysis using antibodies against cyclobutane pyrimidine dimers (CPD) revealed that UVA1
slightly enhanced the amount of UVB-induced CPD 6 hours after irradiation whereas this CPD elevation was not seen directly after exposure. This indicates that in human melanocytes UVA1 leads to an impaired repair of UVB-induced CPD. Taken together, since UVA1 on the one hand causes decreased repair of UVB-induced CPD while on the other hand it does not affect UVB-mediated apoptosis, UVA1 might contribute to melanomagenesis.

### P 72 Characteristics of Mesenchymal Transition of Melanoma

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Melanoma is a malignant tumor of melanocytes and is responsible for 75% of skin cancer deaths. The overall survival of patients with metastatic melanoma has only in the last few years shown modest improvements due to pharmaceutical intervention. However, once the tumor cells switch from a non-invasive to an invasive phenotype, they undergo mesenchymal transition and spread to other parts of the body. One marker which is downregulated and – amongst others – responsible for the invasive potential of melanoma cells is E-Cadherin (CDH1). The expression levels of E-Cadherin can predict the probability of metastasis to sentinel lymph nodes, but the intracellular regulation of this marker is poorly characterized.

By correlating the mRNA expression levels of a set of 22 melanoma cell lines, we found a significant correlation between E-Cadherin and the regulation of JNK target genes.

We hypothesize that JNK signaling determines the differentiation status of melanoma: a curtailed JNK pathways fosters mesenchymal transition and renders melanoma cells less sensitive to treatment. We will analyze the molecular basis for the loss of E-Cadherin induced by loss of JNK signaling in melanoma. Furthermore, we will develop strategies to re-express E-Cadherin and to revert the mesenchymal phenotype. Finally, we want to translate our findings from mouse models to human melanoma patients.

This approach aims to identify new therapeutic targets to reduce the metastatic potential of melanoma and to increase sensitivity to treatment.

### P 73 The natural fungal metabolite Beauvericin exerts anticancer activity in vivo


In recent studies, antitumor properties of Beauvericin [BEA], a fungal metabolite, were shown in vitro using various cell culture models. In order to further assess the therapeutic potential of this compound we evaluated its anticancer activity in vivo in mouse models. Colon carcinoma cells (CT-26) were subcutaneously injected into BALB/c mice and human cervix carcinoma cells (KB-3-1) into CB-17/SCID mice. Once tumors were palpable, treatment with 5mg/kg bw/day BEA i.p. or, for the control group, with solvent alone was started and maintained over two cycles of five to four days. Tumor size and animal weight were measured during the whole study. After sacrificing the mice, histological sections of tumor specimens were prepared and stained with Ki-67, H/E and TUNEL [TdT-mediated dUTP nick end labeling]. Concentrations of BEA were determined in tumors, organs, serum, urine and feces by HPLC-MS/MS. Tumor volumes and weights of treated mice were 52.8% and 60% lower in the allograft model, in the xenograft model a reduction of 31.3% and 31.2%, respectively, was observed as compared to the control groups. No severe adverse effects of BEA on the treated animals were detected as animal weight and behavior were unaltered. In both models, no significant differences were detected in tumor sections from treated and untreated mice in terms of percentages of proliferating and mitotic cells. In treated CT-26-, but not KB-3-1-derived tumors, more dead cells were counted in H/E stained sections. However, in both the allograft and the xenograft model, we measured a significant, 2x or 1.5x increase of necrotic regions in whole tumor sections in the treated groups which was in line with more TUNEL-positive, i.e., apoptotic cells in treated tumors. Furthermore, a moderate enrichment of BEA in the tumors compared to serum levels was detected. Our results demonstrate in vivo anti-tumor activity of BEA indicating its potential value as novel chemotherapeutic agent.

**CXCL5 and Neutrophils increase lymphatic metastasis in cutaneous melanoma**

CXCR2 ligands are prominently expressed in various types of cancer and were found to influence angiogenesis, tumor growth and metastasis. Previous analysis of human and mouse melanoma chemokine profiles showed that high expression of the CXCR2 ligand CXCL5 is in accordance with a worse disease progression.

To investigate the role of CXCL5 in a syngeneic melanoma model in more detail, we intradermally transplanted murine CXCL5 overexpressing and control B16F1 and HCmel12 melanoma cells into C57BL/6J wt mice. Chemokine profiling of CXCL5 overexpressing tumors versus controls showed that changing the expression of this particular chemokine upregulates CXCL2, CXCR2 and CXCR4 but does not affect the expression pattern of other well-known pro-tumorigenic chemokines. Besides that, a positive correlation of CXCL5 with other CXCR2 ligands (CXCL1,2,3,6,7 and 8) was found in human melanoma samples in various publicly available databases.

CXCL5 overexpression resulted in strong neutrophil infiltration and showed an increased frequency of lymph node metastasis in B16F1-CXCL5 and HCmel12-CXCL5 melanomas compared to controls. Additionally, CXCL5-overexpressing melanomas showed a reduced extent of lung metastasis, which could be verified in a model of experimental lung metastasis. Analysis of intra- and peritumoral lymph and blood vessel densities revealed no differences between CXCL5 and control tumors. Therefore, CXCL5 and its recruited neutrophils seem to influence the metastatic behaviour directly. Flow cytometric characterization of these tumor associated neutrophils (TANs) showed a distinct phenotype with high PD-L1, CCR5 and CXCR4 expression compared to neutrophils from bone marrow, blood and lungs. PD-L1 expression on neutrophils is an important regulator for tumor immunity and we could show that peritumoral Poly(I:C) treatment suppressed T-cell mediated antitumoral effects. The exact mechanism how TANs modify the metastatic route still remains to be elucidated.

P 75  Intestine-specific calcium-sensing receptor signalling

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The calcium-sensing receptor (CaSR) is a ubiquitous class C G protein-coupled receptor that primarily mediates calcium homeostasis via regulating parathyroid hormone release.
In non-calciotropic tissues it is involved in various processes including neuronal development, fluid secretion, cell proliferation and differentiation and thus plays a key role in tumourigenesis. Such pleiotropy is owed to the numerous signalling pathways that the CaSR regulates, resulting from coupling to a variety of G-protein subtypes. Moreover, the CaSR displays biased signalling which is influenced by the ligand type, duration of stimulus, receptor localization, pH, ionic strength and the presence of interacting proteins. In the intestines, the CaSR regulates fluid transport and loss of CaSR expression is strongly linked to colon cancer progression. Furthermore, intestine-specific CaSR knockout mice have impaired epithelial barrier function, increased levels of inflammatory markers and higher susceptibility to chemically-induced colitis. However, its role in the small intestines remains enigmatic.

We aim to investigate the function and downstream signaling of the CaSR using mouse small intestinal organoids in the presence of intestine-specific ligands, such as aromatic amino acids and polyamines. In addition, we will test the effects of positive and negative allosteric modulators on the choice of downstream signalling. Forskolin-induced organoid swelling will be used as an assay for luminal fluid uptake. Intracellular Ca2+ oscillations will be measured by fluorescence microscopy using the calcium-sensitive dye Fura-2. In addition, organoid proliferation will be assessed using the MTT viability assay. The findings of this study will improve our understanding of the CaSR signalling and its role in small intestinal physiology. The allosteric modulators may have promising therapeutic benefits as adjuvants for intestinal cancer treatment.

**P 76** The ETS factor inhibitor YK-4-279 induces mitotic arrest followed by cell death in human melanoma cell models

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The small-molecule ETS factor inhibitor YK-4-279 designed for treatment of Ewing Sarcoma (ES) showed promising results in first preclinical studies. ES is characterized by the fusion protein EWS/Fli1 driving malignant transformation. Melanoma, though not driven by a specific ETS translocation, often overexpresses ETS factors. We analyzed cell viability upon YK-4-279 treatment in human ES and melanoma cell models. A rapid cytotoxic effect was revealed in ES while melanoma cells showed a profound increase in mitotic cells. Underlying mechanisms were analyzed by Western blot, flow cytometry, immunofluorescence and mRNA expression microarrays. Moreover, the mechanism of mitotic arrest induction by YK-4-279 as well as two Aurora kinase inhibitors, VX-680 and Alisertib, was compared.
Application of YK-4-279 forced human melanoma cells into a mitotic cell cycle arrest already after 24h followed by cell death. Cell cycle arrest was more pronounced in BRAF wt as compared to BRAFV600E cells. In contrast, aurora kinase inhibition did not exhibit cytotoxic activity on melanoma cells. Both Aurora kinase inhibitors led to cell cycle arrest and increase in cell size, presumably dependent on p53/p21 activation with p53/p21 unresponsiveness leading to an increase in DNA content of >8n. The mitotic arrest induced by YK-4-279 was accompanied by accumulation of cyclin B1, Aurora kinase A and increased phosphorylation of retinoblastoma and histone 3 on serine 10. Furthermore, incomplete chromosome alignment in the metaphase plate and deregulated spindle pole formation was detected. Gene expression analysis upon YK-4-279 treatment showed upregulation of several mitotic checkpoint molecules. Summing up, YK-4-279 induces mitotic arrest and cell death in human melanoma cells. Next, we aim to establish whether an ETS factor plays an important role in mitotic fidelity or whether YK-4-279-mediated mitotic arrest represents an off-target effect.

P 77 Development of an affinity purified antibody for the detection of metastatic melanoma


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Melanoma is the most aggressive type of skin cancer and one of the most frequent tumours in young adults. Even though it only accounts for 4% of all cases of skin cancer, melanoma is responsible for 79% of all skin cancer related deaths. Identification of primary tumours prone to develop metastasis is of paramount importance for further patient stratification. However, till today, no markers exist that are routinely used to predict melanoma progression.

To ameliorate this problem, we generated antiserum directed against metastatic melanoma tissue lysate and applied a novel approach to purify the obtained serum via consecutive affinity chromatography steps. The established antibody, termed MHA-3, showed high reactivity against metastatic melanoma cell lines both in vitro and in vivo. We also tested MHA-3 on over 250 melanoma patient samples and compared staining with the melanoma marker S100b. Interestingly, MHA-3 was able to differentiate between primary tumour samples from metastatic and non-metastatic melanoma, whereas S100b was not. Analysis of antigen bound by MHA-3 by immunoprecipitation revealed 18 distinct proteins. Importantly, only the combined expression profile of all identified antigens could reveal a significant survival difference in melanoma patients.

In conclusion, we developed a polyclonal antibody, which is able to detect metastatic
melanoma on paraffin embedded sections. Hence, we propose that this antibody will represent a valuable additional tool for precise melanoma diagnosis.

**P 78  Investigation of novel cell death mechanisms induced by anticancer thiosemicarbazones**

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Triapine, an a-N-heterocyclic thiosemicarbazone (TSC), has been long known for its anticancer activity. Consequently, Triapine was already investigated in multiple clinical phase I and II studies. However, these studies revealed that Triapine is only effective in hematologic malignancies. Underlying reasons may be inappropriate drug delivery into the solid tumor nodules, fast excretion or intrinsic/acquired drug resistance. To improve this situation, novel terminally dimethylated TSCs, such as Dp44mT and Me2NNMe2 are being studied with increasing interest due to their highly improved (nanomolar) anticancer activity compared to terminally unsubstituted compounds such as Triapine. Therefore, the aim of this study was to investigate the differences in the modes of action of Triapine and Me2NNMe2. Various cell death assays indicated only a minor induction of apoptosis with both drugs. In contrast, appearance of vacuoles in the endoplasmic reticulum as well as mitochondrial dilation pointed to the induction of an alternate cell death pathway, namely paraptosis, especially with those TSCs showing activity in the nanomolar concentration range. In accordance, several paraptosis inhibitors such as a MEK1/2 inhibitor and N-acetylcysteine were found to protect cells from Me2NNMe2 treatment. In conclusion, this is the first report on paraptosis induction by promising anticancer TSCs. This is not only of interest for the further (pre)clinical development of nanomolar TSCs for cancer treatment but will also help to better understand the molecular mechanisms of paraptosis, an only recently discovered form of cell death.

**P 79  CaSR therapeutics for colorectal cancer**

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The calcium sensing receptor (CaSR) is a transmembrane receptor protein that belongs to the G protein-coupled receptor (GPCR) family. It primarily controls Ca++ homeostasis, but it also regulates many other cellular mechanisms such as proliferation, differentiation and apoptosis. Those pleiotropic functions could be explained by the fact that the CaSR can activate different downstream signals, in a cell-type specific manner. This receptor seems to act as a tumor suppressor in colorectal cancer and in neuroblastoma, where it is down regulated. Moreover, pre-cancerous colorectal lesions seem to gradually lose CaSR expression, gaining tumorigenic features. Recent in vitro studies revealed that the CaSR is able to inhibit proliferation and to induce apoptosis of colon cancer cells.

We hypothesize that the calcium sensing receptor represents a promising molecular target for colorectal cancer therapy. Our aims are: i) to find means to restore CaSR expression in colorectal cancer cells where the CaSR is silenced; ii) to test whether the expressed CaSR affects tumor growth in in mouse xenografts; iii) to study also the impact of this receptor on the treatment of colorectal tumors with known chemotherapeutics.

First, we will screen different substances such as CaSR allosteric modulators (e.g. calcimimetics), epigenetic drugs (e.g. sodium butyrate) for their ability to restore CaSR expression in colorectal cancer cell lines and in human tumor spheroids. We will use subcutaneous xenografts to test the anti-tumorigenic potential of the CaSR and its impact on chemotherapeutic treatments. Those results will be confirmed in orthotopic grafts, where it will be possible to test the effect of CaSR expression on inhibiting metastasis. In conclusion, we will able to demonstrate whether the CaSR has an anti-tumorigenic effect in colorectal cancer. Moreover, we will test which compounds are able to re-establish CaSR expression and be employed as therapeutic agents.

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**P 80  SR-BI as a Key Player in the Metastatic Process of Human Melanoma**


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The incidence of melanoma in Austria is still increasing and although novel treatments are available, patients with metastatic melanoma still have poor survival rates. We found that human metastatic melanoma cells showed elevated high density lipoprotein (HDL) receptor expression when compared to non metastatic cells. Therefore, the aim of the recent study was to evaluate the contribution of the HDL receptor, also termed SR-BI, to the metastatic process in human melanoma. We investigated i) expression profiles of melanoma patients...
for clinical relevance and ii) performed siRNA mediated knockdown studies to identify the cellular role of SR-BI. Clinical data of two independent cohorts showed that patients with higher mRNA levels of SR-BI had a worse survival than patients with lower levels. In our in vitro studies we found by whole genome analysis that SR-BI is necessary for metastatic melanoma cells to perform glycolysis and hypoxia signaling. Furthermore, we found that cells lacking SR-BI down-regulated the MET receptor, HIF1a and VEGFA on the protein level. Functional assays revealed that depletion of SR-BI leads to a reduced migratory capability as well as reduced exosome secretion. Here we have shown that SR-BI facilitates a glycolytic phenotype, important for metastatic melanoma. Hence SR-BI will represent a valuable target for future therapeutic therapy.

P 81  CRISPR/Cas9-mediated generation of accurate cellular models for clinically relevant combinations of genetic lesions to investigate epigenetic and transcriptomic effects of mutated CEBPA in Acute Myeloid Leukemia


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The gene encoding the transcription factor CCAAT-enhancer-binding protein (CEBPA) is mutated in 9% of Acute Myeloid Leukemia (AML) cases. These mutations include N-terminal frameshift mutations, resulting in expression of a smaller variant of C/EBPa, termed p30. Mutations in the C-terminal basic leucine zipper domain (bZip) disrupt the DNA-binding ability of C/EBPa. CEBPA-mutated AML genomes frequently have concurrent mutations in NPM1, FLT3, GATA2, RUNX1, ASXL1, TET2 and DNMT3A. While individual combinations of mutations can strongly influence the outcome of AML, the mechanisms for this are still unknown.

We hypothesise that the N-terminally truncated p30 variant of C/EBPa differs from the longer C/EBPa p42 in its potential to interact with components of the chromatin modifying machinery, leading to global alterations in the epigenome. Resulting changes in gene expression might thus depend on combinatorial effects of CEBPA mutations together with additional genetic lesions that are present in the same leukemic clone.

We have analysed global genomic and transcriptomic changes upon Cebpa knockdown in Cebpa[p30/p30] cells by ChIP-Seq and RNA-Seq, with a focus on enhancer and super-enhancer regions. We are using the CRISPR-Cas9 technology to establish novel murine and human cell models harbouring combinations of genetic lesions that associate with mutant CEBPA in AML patients. To elucidate molecular mechanisms underlying changes dependent on mutant CEBPA together with other relevant AML mutations, we will profile the genomic and transcriptomic landscapes of the established cell lines to identify common and differentially regulated pathways.

Understanding and deciphering global epigenetic and transcriptomic changes that depend on CEBPA mutations in a physiologically relevant mutational context will enhance our
understanding of gene cooperativity in the induction and progression of AML and could provide starting points for the development of novel treatments.

P 82  Modification of radiation-induced oral mucositis by heparin – preclinical studies

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Severe oral mucositis is a frequent and often dose-limiting early side effect of radio(chemo)therapy for head and neck tumors. Although it has a major impact on the patient’s quality of life and tumor control probability, no biology-based prophylactic or mitigative approach has been generally introduced into clinical practice. Unfractionated or low-molecular-weight heparin (UFH, LMWH) both modulate multiple biological processes, such as proliferation, inflammation, infection, immune response and others. The purpose of the present, preclinical study was to investigate the effect of systemic administration of UFH or LMWH, on radiation-induced oral mucositis. The study was performed in the well-established animal model of the lower tongue mucosa in the inbred C3H/Neu mouse strain. Mice were irradiated using either single dose or fractionated irradiation protocols with 5x3 Gy/week, given over one or two weeks. All fractionation protocols were concluded by local top-up irradiation using graded doses to generate complete dose-effect curves. Daily doses of UFH or LMWH (40 or 200 I.U./mouse, respectively) were applied subcutaneously over varying time intervals. Mucosal ulceration was analyzed as clinically relevant endpoint. For mechanistic studies, groups of 5 mice were sacrificed daily, the tongues excised and subjected to histological/IHC analyzes. Preliminary data clearly show that systemic application of LMWH significantly increased isoeffective doses for the induction of mucosal ulceration in fractionated irradiation protocols. Moreover, a tentative reduction of ulcer duration and prolonged latency was observed. In conclusion, these data provide the first evidence of a protective and/or mitigative effect of heparins for radiation-induced oral mucositis. Further studies are ongoing in order to optimize the drug administration protocols and to characterize the underlying mechanisms of the muco-protective effect.

P 83  The role of the relapse associated gene CALCRL in therapy resistance of acute myeloid leukemia

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Acute myeloid leukemia (AML) is a hematologic malignancy characterised by the clonal expansion of myeloid blasts in the bone marrow and peripheral blood. Following standard chemotherapy based on the two agents cytarabine (araC) and daunorubicin (DNR), complete remission (CR) is achieved in approximately 50-80% of adult cases. Nevertheless, only 20-30% of patients experience long-term disease-free survival, due to a high incidence of relapse with therapy resistant disease. Relapse is thought to stem from the outgrowth of mostly quiescent, chemotherapy resistant leukemic stem cells (LSCs) and can be viewed as a central problem in the treatment of AML. Through microarray data analysis, our group identified the CALCRL (calcitonin receptor-like receptor) gene as a potential contributor to chemoresistance and relapse. CALCRL is a G-protein coupled receptor (GPCR) and was shown to be upregulated in relapsed AML compared to diagnosis and is associated with poor overall survival. Together with one of three receptor activity modifying proteins (RAMP1-3) it forms a cell surface receptor specific for either adrenomedullin (ADM, heterodimer with RAMP2 or 3) or calcitonin gene-related peptide (CGRP, heterodimer with RAMP1). Using functional assays, we demonstrated that incubation with either of the CALCRL ligands enabled receptor positive human myeloid cell lines, but not those without the receptor, to tolerate higher doses of araC and DNR. Overexpression of CALCRL in different cell lines was achieved through retroviral infection and the effects on chemotherapeutic resistance were analysed using viability assays. In conclusion, our data suggest CALCRL to be a putatively crucial player in the development of chemotherapy resistance in AML.

P 84  Relevance of apical pore tyrosine residues in substrate gating of human P-glycoprotein.


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The X-ray structure of a eukaryotic half transporter from Cyanidioschizon merolae (CmABCB1), which is a homolog of human ABCB1 (Kodan et al. 2014), suggested an apical tyrosine residue in the apex of the central cavity (Y358) as being part of the extracellular gate. We mutated analogous tyrosine residues in both the N- and C-terminal halves of the human full transporter ABCB1 (Y310 and Y953) to alanine and characterized these mutants functionally. Our group previously showed that rhodamine 123 (rh123), as well as other paradigmatic substrates, are able to bind to the transporter in two modes, which are
related to each other by 180° rotational symmetry. Introduction of positively charged arginine residues in helices 2 (Q132R) and 8 (Q773R) deselects one of these two rh123 binding modes. The Y310A and Y953A mutations were combined with these binding-mode selector residues to yield 4 double mutants. These mutants were characterized in efflux and uptake experiments. Interestingly, two of the mutants lost active transporter characteristics, while the other two retained it. This shows that depending on the binding mode, one or the other tyrosine residue is involved in gating. Therefore not only ATP and substrate binding, but also gating follows a dual principle. A kinetic model for transport will be presented. Supported by the Austrian Science Fund within the scope of “Spezialforschungsbereich” SFB35 (project part 3506 to HHS, 3509 to PC and 3524 to TS). YDC was partially funded by Austrian Science Fund project 23319 to TS.

P 85  Analyses of key regulators of the Hippo signalling pathway in first trimester cytotrophoblast

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P 86  The role of STAT3ß in acute myeloid leukemia

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STAT3, the main mediator of IL-6 signaling, plays a central role in proliferation, differentiation and oncogenic transformation. It is known to be expressed in two alternatively spliced isoforms, STAT3a and truncated STAT3ß, which is lacking the C-terminal transactivation domain. STAT3ß was formerly postulated as the dominant negative form of STAT3, however it has been shown that it has various STAT3a-independent regulatory functions. Furthermore, STAT3ß recently gained attention as a powerful anti-tumorigenic molecule, so far shown in patients with esophageal squamous cell carcinoma, as well as in breast, skin and colon cancer models. The aim of this study is to gain better understanding of STAT3ß and its specific function in acute myeloid leukemia. We hypothesize that STAT3ß acts as an anti-tumorigenic molecule in AML by shaping the cellular response of myeloid blasts in regard of differentiation and cell mobilization, thus impairing leukemogenesis. We further propose that STAT3ß serves as a favorable
prognostic marker in AML patients. This study is using patient data as well as a Stat3ß-transgenic and knock-out mouse models and cell culture-based methods to investigate the role of STAT3ß. In our first approach we combine a Pten-dependent AML mouse model with inducible Stat3ß-transgenic mice. We observed that Stat3ß significantly delayed disease progression in Pten-deficient mice and impaired hematopoietic stem cell mobilization from the bone marrow to the spleen. We aim to further examine this STAT3ß-specific function and its underlying mechanisms using a similar approach with Stat3ß knock-out mice and in a MLL-AF9-dependent AML transplantation model. Moreover, analyses of samples derived from AML patients for Stat3ß/Stat3a mRNA expression levels revealed a correlation between predicted prognosis and Stat3ß/Stat3a expression ratio. We therefore aim to further unravel the contribution of STAT3ß to myeloid differentiation and cell mobilization in AML.

P 87  NUP98-DDX10-rearranged acute myeloid leukemia expresses high levels of Cdk6 and is hypersensitive to Palbociclib treatment

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Chromosomal rearrangements involving the Nucleoporin 98 (NUP98) gene are recurrently found in patients suffering from acute myeloid leukemia (AML) and are often associated with poor prognosis. The NUP98-DDX10 translocation is among the most frequent rearrangements of the NUP98 gene in AML, in which the N-terminal part of the NUP98 gene is fused to the C-terminal portion of the RNA helicase DDX10, resulting in the expression of an oncogenic fusion protein. In contrast to other NUP98 fusions that have been postulated to act as aberrant transcription factors, the NUP98-DDX10 fusion does not harbor any DNA-binding or chromatin-interaction domain. Effective therapy of patients is currently impossible, as the molecular mechanism underlying NUP98-DDX10-dependent leukemogenesis is still unresolved.

To study NUP98-DDX10-driven leukemogenesis we employed tetracycline (tet)-mediated control of oncogene expression in vivo to establish the first mouse model of NUP98-DDX10. Mice transplanted with NUP98-DDX10-transformed hematopoietic progenitor cells developed a transplantable, AML-like disease with full penetrance. Phenotypic analysis of moribund mice exhibited severe splenomegaly and > 80% oncogene-expressing myeloid blasts in bone marrow and spleen. RNA-seq analysis upon acute tet-mediated oncogene repression during leukemogenesis identified a set of NUP98-DDX10-regulated genes that are potentially involved in disease development and maintenance. Within this core of regulated target genes, we found that Cdk6 was highly expressed in leukemic blasts, but rapidly down-regulated upon oncogene shutdown. In consequence, NUP98-DDX10 transformed cells, but not MLL-rearranged or RUNX1-rearranged AML cells were...
hypersensitive to treatment with the CDK4/6-inhibitor Palbociclib. Therefore, inhibition of CDK6 could represent a promising approach to treat patients suffering from NUP98-DDX10-driven AML.

P 88  Prevention of oxidative DNA damage in the liver of obese mice by gallic acid


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P 89  CD222 in human body liquids: origin and diagnostic value

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The mannose 6-phosphate/insulin like growth factor II receptor (CD222, M6P/IGFIIIR) is a multifunctional receptor, mostly localized intracellularly, less on the cell surface of all types of mammalian cells. It is involved in the processes like the transport of acid hydrolases containing mannose 6-phosphate moieties in its structures into lysosomes, or in binding and internalization of extracellular ligands like IGFII or plasminogen. This is related to functions of CD222 in regulation of cell proliferation, migration and apoptosis.

Recently, it has been shown that soluble CD222 (sCD222) can be proteolytically released from the surface of human endothelial cells by the tumour necrosis factor-[alpha]-converting enzyme (TACE). It was reported that the level of sCD222 in rat serum increased on liver damage.

In this study, we investigated the serum and urine of same cohort of oncological patients for presence of CD222 and its form by ELISA and Western blot approaches. Similarly to rat serum, we found sCD222 in patients’ sera. Despite that, in urine we found CD222 of different origin. Finally, we questioned its diagnostic value in both human body liquids.

P 90  Synthetic Rescue Interactions to Correct Defective DNA Damage Repair

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The DNA damage response (DDR) has evolved to maintain the integrity of the human genome from endogenous and exogenous sources of damage. Several highly conserved DNA repair pathways deal with specific types of DNA lesions and their importance is highlighted by the occurrence of heritable diseases from patients carrying germline mutations in genes within these pathways. Most of these diseases have no available treatments and they are characterized by a wide range of pathologies, including immunodeficiencies, neurodegeneration and developmental defects. While the molecular mechanisms underlying the mode of action of these specialised pathways are broadly characterised, the regulatory complexity of the DDR remains to be fully understood. In this project, we aim to systematically identify genes and gene products, whose loss (in the background of a disease relevant mutation) gives rise to synthetic viability. Our approach consists of genome-wide Clustered-Regularly-Interspaced-Short-Palindromic-Repeats (CRISPR)-Cas9 knock-out (KO) screens, in loss of function mutant cell lines for DNA repair genes associated with diseases, broadly covering all DNA repair pathways. Additionally, phenotypic reporter assays will be used to identify candidate genes, whose loss not only rescues cell viability, but also promotes the clearance of DNA damage. A bioinformatics approach will be used to map the identified rescue interactions into a network of the DDR. Moreover, functional experiments, with different cellular and relevant in vivo models, will elucidate the molecular mechanisms underlying selected synthetic rescue interactions and provide clues about their clinical relevance. This integrated view of the DDR has the unique potential to expand our knowledge on the repair of DNA damage and intervenient players, but also significantly improve the way we treat related diseases.

**P 91 INFLAMMATION RELATED TUMOR/FIBROBLAST INTERACTION MARKERS IN CRC**


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Inflammation has been identified as an important factor in development and progression of various types of cancer, including colorectal cancer [CRC]. To determine the role of inflammation and activated fibroblasts we analyzed six human CRC tissue specimens and paired normal adjacent mucosa samples by liquid chromatography coupled with tandem mass spectrometry [LC-MS/MS]. Several inflammation associated proteins were
differentially expressed compared to normal colonic mucosa, including SPARC and THBS2 which were recently identified as proteins of a fibroblast inflammation signature. Analysis of the CRC consensus molecular subtype dataset, which includes both publicly available RNA expression datasets and CRC data from the TCGA database, revealed both an upregulation of SPARC and THBS2 in CRC as well as an association with the mesenchymal subtype. Consistently, immunohistochemistry staining demonstrated upregulation of SPARC and THBS2 in the tumor stroma compared to normal adjacent mucosa and co-localization with the mesenchymal marker αSMA. In vitro 3D co-culture experiments with human colon derived fibroblasts and CRC cell lines resulted in enhanced SPARC and THBS2 protein levels when both cell types were cultivated in direct physical contact, compared to the culturing of fibroblasts or cancer cells alone. This study was a pilot study demonstrating the feasibility of detecting tumor-specific signatures by LC-MS/MS and RNA expression datasets. We identified an inflammation signature in CRC tissue and the data emphasized the contribution of activated fibroblasts in these events.

P 92 The role of CDK6 in transcription

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Cyclin-dependent kinase 6 (CDK6) allows, together with the cyclin-dependent kinase 4 (CDK4), the progression of cells through the cell cycle by binding to D type cyclins. Alterations of components of these CDK4- and CDK6-cyclin complexes are frequent in cancer. As such, enhanced levels of CDK6 expression have been reported in hematopoietic malignancies. The central role of CDK4/6 for tumour development is underscored by the fact that the US FDA selected inhibitors of the kinase activity of CDK4/6 as "breakthrough of the year 2013". Recent findings have shown a novel role for CDK6, but not for CDK4, in regulating transcription in a kinase dependent and independent manner. For example, CDK6 has been identified as a transcriptional regulator of the angiogenesis promoting factor VEGF-A, the cell cycle inhibitor p16INK4a as well as the hematopoietic stem cell dormancy by down-regulation of EGR1. To gain further insights how CDK6 interferes with transcription we generated Bcr/Ablp185 cell lines expressing different mutants of CDK6. One of the mutants appeared of particular interest as it lacked kinase activity as it failed to bind D type cyclins. This is accompanied by a distinct transcriptional profile analyzed by Microarray. Interestingly we failed to observe changes in cell growth in vitro. The reduced protein expression level of the mutated CDK6 - in the presence of comparable mRNA levels compared to wildtype CDK6 - were detectable in p185+ cell lines by Western Blotting. Treatment with the proteasome inhibitor Bortezomib showed a stabilizing effect on mutated CDK6. These results assume instability of the mutated CDK6 protein due to proteasomal degradation. The aim of this study is to define novel regions in CDK6, which
can be targeted to inhibit the tumour promoting function of CDK6 in hematopoietic malignancies.

**P 93** Epithelial growth factor receptor tyrosine kinase inhibitor enhances statin induced cell death in non-small cell lung cancer cell lines by activation of necroptosis

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Non-small cell lung cancers (NSCLC) with primary epidermal growth factor receptor (EGFR) mutations are treated with EGFR-tyrosine kinase inhibitors (TKI). However, resistances may arise against EGFR-TKIs, via T790M mutations, upregulation of MET oncogene or primary k-RAS mutations. Inhibitors of the HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase (statins) can overcome these resistances in vitro. Statins are usually prescribed to lower the cholesterol level. In addition, they have been shown to exhibit anti-tumor effects including growth inhibition, induction of apoptosis and prevention of metastasis. We could show that the combined treatment of erlotinib and pitavastatin or fluvastatin enhances cell death in NSCLC cell lines with primary resistance to TKIs. These effects are highly dependant on the HMG-CoA reductase pathway as the addition of mevalonate can fully abrogate these effects.

Assessment of cell viability in NSCLC cell lines treated with pitavastatin and fluvastatin via flow cytometry using annexin V and propidium iodide (PI) double staining resulted in prominent appearance of apoptotic cell populations. However, large populations were also positive for PI, which requires a leaky cell membrane to enter the cell. This indicates that necrotic processes are taking place perforating the cell membrane secondary or parallel to apoptosis, as apoptotic processes alone would result in cells disintegrating into apoptotic bodies labelled by annexin V only. Inhibitors such as zVAD-fmk or necrostatin 1 can block apoptosis or necroptosis, respectively. Effects of these inhibitors were assayed on NSCLC cell lines A549, Calu6 and H1993, which respond to erlotinib-statin co-treatment. Co-administration of each inhibitor or both will tell whether necroptotic signalling is underlying the observed distribution of dead cell populations which does not fit apoptosis alone.

In future, this data may help to employ more effective targeted treatments for NSCLC patients.

**P 94** Time-resolved molecular characterization of Ibrutinib effect on CLL reveals widespread downregulation of proliferation pathways

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Chromosomal translocations in cancer can result in the production of oncogenic fusion proteins (FPs). In leukemia, a particularly high number of fusion oncogenes has been identified. FPs involving the Nucleoporin 98 (NUP98) gene are found in ~2% of acute myeloid leukemia (AML) patients. The NUP98 multi-partner translocation family features >20 different FPs, all harbouring an N-terminal NUP98 fragment fused to distinct C-terminal partners. Previous studies showed that different NUP98-FPs cause similar AML phenotypes in humans and mouse models. We postulate that NUP98-FPs share molecular mechanisms that depend on conserved protein-protein interactions to modulate important leukemogenic pathways. Thus, we aim to identify critical effector proteins through mass spectrometry (MS)-based profiling of the interactomes of 5 representative, yet distinct NUP98-FPs.

Inducible and tagged variants of 5 selected NUP98-FPs (NUP98-HOXA9, -JARID1A, -DDX10, -NSD1 and -PSIP1) were stably expressed in human AML cells and purified protein complexes were characterized by MS. Data were analysed using SearchGUI and PeptideShaker to identify the interactomes of NUP98-FPs. Exogenous NUP98 and known NUP98-binding partners, such as RAE1 and RAN, were highly abundant in all datasets. Upon stringent filtering, NUP98-FP interactomes revealed a network of 308 proteins, of which 83 were present in [>=]4 interactomes. Functional annotation of the 83 conserved NUP98-FP-interactors revealed a significant enrichment for proteins involved in transcription, while no components of the nuclear pore complex (NPC) were found, indicating that NUP98-FPs have active roles in transcriptional control but do not co-localise with the NPC. Altogether, this study provides the first comprehensive protein interactome of 5 NUP98-FPs.

Together with the future functional investigation of common NUP98-FP interactors, this study will significantly enhance our understanding of the molecular mechanisms of NUP98-FP driven AML.
DNA methylation is an epigenetic process, by which methyl groups are added to the C5 position of Cytosines on DNA. Methylation modifies the function of DNA by altering gene expression and is essential for normal gene regulation and development. Hypomethylation of gene promoters is usually associated with transcriptionally active DNA, while hypermethylation causes gene repression. Aberrant DNA methylation patterns are widely observed in a variety of tumors. This includes hypermethylations of CpG-Islands in promoters of tumor suppressor genes leading to epigenetic silencing.

Prostate cancer is one of the most common cancers worldwide and the second leading cause of cancer related mortality in men. Elevated levels of prostate specific antigen (PSA) are detected in serum of prostate cancer patients, which is currently used to screen for the presence of prostate cancer in elderly men. However, the use of PSA as biomarker for prostate cancer has several limitations. PSA itself is not a prostate cancer specific, but prostate specific biomarker, which often results in the detection of false positives. This and other limitations make novel biomarkers with high specificity for prostate cancer a necessary and attractive target for research.

By doing genome-wide DNA methylation analysis of primary prostate cancer and adjacent normal tissue, we identified several genes that are hypermethylated and therefore epigenetically silenced in prostate cancers. These genes were successfully validated in an independent cohort. On top of this finding, one of those potential biomarkers might have an important biological role for prostate cancer progression. Overexpression of said gene in prostate cancer cell lines results in a distinct reduction of the invasive potential.

Further experiments will include invasion assays with organotypic spheroid models and more prostate cancer cell lines, in order to validate these results.

P 97 Exploring the role of charged amino acid residues in the transmembrane regions of the bile salt export pump (BSEP, ABCB11) for substrate transport

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The bile salt export pump (BSEP/ABCB11) transports bile salts from hepatocytes into bile canaliculi. Its malfunction is associated with severe liver diseases. My work will initially focus on the role of 4 charged residues, which are present in the membrane spanning portion of the transporter. Two of these are negatively charged D215 and E381, which lie close to the leaflet water interface. These residues are also present in another member of the ABCB subfamily, P-glycoprotein (P-gp). On the other hand two pseudosymmetric arginine residues are unique to BSEP (R223, R1033). These arginine residues are located close to the leaflet interface. Interestingly, P-gp has a preference for neutral and positively charged substrates, while BSEP transports negatively charged conjugated bile acids. Therefore, we hypothesize that positively charged arginine residues in the substrate translocation path may be involved in determining charge preference. While R223 is located at a C-alpha distance of 10.68 Å from the negatively charged residue D215 and 14.89 Å from E381 and likely forms charge interactions with them, R1033 might be involved in the interaction with BSEP-substrates. We thus are in the process of replacing this residue by lysine, glutamate and histidine. The latter will possibly allow to influence the charge state by changing pH in the medium. These experiments will address the role of charged residues for substrate transport.

**P 98 Understanding the domain interplay in the Bile Salt Export Pump**

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The bile salt export pump (BSEP/ABCB11) transports bile salts from hepatocytes into bile canaliculi. Its malfunction is associated with severe liver disease. One reason for functional impairment of BSEP is the systemic administration of drugs, which as a side effect inhibit the transporter. Therefore, drug candidates are routinely screened for potential interactions with this transporter. Hence understanding the functional biology of this
transporter is of key importance. In this study we engineered BSEP in order to dissect inter-domain communication paths. Interestingly, the transporter mutant M584E/E1244Q, in which the non-canonical nucleotide binding site 1 (NBS1) was made hydrolysis competent, while at the same time NBS2 was rendered hydrolysis incompetent, retained 15% residual transport activity. This mutant is devoid of active transport characteristics, but adopts properties of an ATP-gated facilitator. This result is unprecedented in ABC proteins with one non-canonical NBS and aids in understanding the domain interplay in asymmetric ABC-transporters.

P 99  **Statistical properties of tests for finding a treatment effect using goal attainment scaling**

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Goal Attainment Scaling (GAS) is a measurement instrument to evaluate the effect of an intervention on the basis of individual, patient-specific goals. The effect of a treatment on a particular goal is mapped in a pre-specified way to a common ordinal scale. The advantages of this measurement approach are the utilization of patient-centered outcomes and the possibility to combine the information from patients in heterogeneous populations. The latter is of particular interest in rare disease research, because it allows for as large as possible samples. Here we focus on the statistical aspects of using GAS data for the comparison of two treatment groups in a randomized clinical trial. We discuss the scope of possible null hypotheses for the between-group comparison and review methods to aggregate the data on multiple goals within each patient. The statistical properties of hypothesis tests for the between-group comparison are studied for a data generating model that is based on the assumption of underlying latent multivariate normal responses, which are assumed to be connected with the unknown treatment effect on some common underlying physiological process. The actual ordinal observations are obtained by discretizing these continuous outcomes via thresholds. Key parameters of the model, such as the number of goals per patient, their correlation and the choice of thresholds are under some control of the trialist, and we derive guidance on the choice of these parameters when aiming for good discrimination between treatment groups. Different analysis methods are studied corresponding to how to weight either the individual goals of each patient or their aggregated scores to achieve high power when testing if there is a significant treatment effect in a randomized clinical trial. A real data set of a clinical trial concerning children with cerebral palsy is analysed for illustration.

P 100 Deep Convolutional Neural Networks for Parathyroid Gland Classification in HE-Stained Images
Parathyroid (PT) glands are very small endocrine glands which are located in the neck region of mammals. They have a key role in regulating the amount of calcium in the blood. Analyzing their structure from consecutive histological (HE-stained) images could be highly beneficial for assessing the impact of diseases or mutated molecules on the development and function of the organ. However, this analysis has become very challenging for biologists due to significant variability in shape, size, location, texture and staining of the images.

In general, segmentation and classification are fundamental to quantitative analysis of tissues in histological images. Common method in clinical practice is manual delineation of structures which is performed by human experts, but this is time consuming and suffers from intra- and inter-observer variability. Therefore, computerized methods have been proposed for tissue segmentation in microscopic images. Among those methods, machine learning-based segmentation approaches were found to be superior over standard image processing methods.

The aim of this study is to develop a method based on deep convolutional neural networks (CNN) to segment and classify PT glands in light microscopic images of consecutive histological sections obtained from the neck regions of 10 mice. Images are provided with ground truth masks from two biologists.

The implemented algorithm for quantitative analysis of PT gland structure shall supersede the time consuming manual segmentation with competitive precision performance.

**P 101 Standardization for L1-penalized regression**

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Since its introduction in 1996, L1-penalized regression [LASSO] has become a very popular method to combine optimal prediction performance and variable selection. It is especially useful in situations when there are many candidate predictors, such as risk factor studies or analysis of genetic and SNP data. The estimation process involves the absolute norm of
the regression coefficients, hence it is essential to bring variables to a common scale. Otherwise, variables which exhibit small coefficients will be favored unduly. However, what exactly constitutes this common scale? Discussions about how to properly standardize variables have a long history in statistics. We will investigate some of the proposed methods in the setting of LASSO estimation and assess their impact on conclusions drawn from such an analysis.

We are mainly concerned about categorical predictors, which are very common in medical applications. We will demonstrate how standardization affects selection probabilities and prediction performance when the pool of candidate predictors includes balanced and unbalanced variables. Furthermore, we question if categorical and continuous variables are treated similarly by standardization. There are indications that differences in variability affect the LASSO estimates even after simple standardization in certain situations. As variability of biomarkers and laboratory measurements often differ by orders of magnitude, this may potentially influence the conclusions.

We will present simple considerations, complemented by a simulation study for more complex scenarios. Our focus will be on low-dimensional problems with more observations than variables. However, we will also explore the high-dimensional case, where the LASSO is a common method to analyze e.g. SNP data.

Real data will be used to demonstrate our recommendations for practical applications.

P 102 Optimized Adaptive Enrichment Designs


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P 103 In vitro evaluation of a novel apelin-receptor targeted contrast agent for molecular ultrasound imaging of tumor angiogenesis


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Introduction: The apelin-receptor (APJ) and its endogenous ligand, apelin, not only induce neoangiogenesis but also promote the maturation of newly formed tumor vessels. This APJ-driven process appears to make tumors more susceptible for anti-cancer therapies. Considering this fact, APJ expression of tumor vessels may predict treatment response. Molecular ultrasound imaging enables quantification of a target receptor expressed by vascular endothelial cells using targeted microbubbles as a contrast agent. To date, in vitro
evaluation of a suitable target precedes molecular ultrasound imaging studies. Our aim in the current preliminary in vitro study was to evaluate the binding specificity of a novel APJ-targeted contrast agent for molecular ultrasound imaging.

Materials and Methods: APJ expression of HUVECs was confirmed with immunofluorescent microscopy after staining of HUVECs with a biotinylated anti-APJ antibody and a streptavidin-conjugated fluorescent dye. Target-ready microbubbles were either conjugated with the biotinylated anti-APJ antibody or a biotinylated isotype-matched control antibody. HUVECs were incubated with APJ-targeted microbubbles or control microbubbles for 5 min, then HUVECs were washed with phosphate buffered saline. The binding of each type of microbubbles per cell was visually assessed under an inverted brightfield microscope by counting microbubbles manually.

Results: Mean number of microbubbles bound to HUVECs was 5.57 per cell for APJ-targeted and 1.78 for control microbubbles, respectively. APJ-targeted microbubbles showed a significantly higher binding specificity to HUVECs than control microbubbles \( (p=0.021) \). Binding of microbubbles corresponded to findings of immunofluorescent microscopy.

Conclusion: APJ expression of HUVECs targeted by a novel contrast agent for molecular ultrasound imaging may serve as a potential imaging biomarker in tumor angiogenesis.

P 104 High Resolution Mouse Retinal Imaging by Visible Light Polarization Sensitive Optical Coherence Tomography


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Since its first demonstration in 1991, optical coherence tomography [OCT] has proven itself to be a useful tool in ophthalmic imaging, offering a non-invasive technique well suited to longitudinal studies in both humans and animal models. In recent years, many functional extensions have been made to basic OCT, which is a 3D imaging technique based on interferometry. One of these extensions is polarization sensitive OCT [PS-OCT], which identifies birefringent and depolarizing tissues – both of which are found in the retina. Another development in OCT is visible light OCT [vis-OCT] which, in addition to providing a better axial resolution due to a lower central wavelength, can also provide spectroscopic data of a sample due to its large bandwidth when the full range is used. As the eye is designed to see visible light, it is particularly interesting to observe how the different wavelengths within this range behave. In this work, we present the design of the first combined visible light polarization sensitive OCT [vis-PS-OCT] system. A high power supercontinuum white light laser was chosen as the light source, with the desired wavelength selected by a filter box. The system has a central wavelength of 560 nm with an overall spectral range of 280 nm. The axial resolution was measured to be 1.07 [μm] in air, which corresponds to a resolution of 0.76 [μm] in tissue, assuming the group index of the
retina to be 1.4. Using this system, preliminary images of healthy mouse models were acquired and post-processing algorithms were tested, proving that this system offers the possibility to look at high resolution spectroscopic image data and PS-OCT image data simultaneously. Such a system could therefore be suitable for a longitudinal study of mouse models of ophthalmic diseases.

P 105 Ocular fundus pulsation changes as a response to increased intraocular pressure in the posterior rat eye

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Optical coherence tomography [OCT] can be used to detect subtle displacements and deformations in ex-vivo specimen as well as in-vivo. In this work we demonstrate the use of functional OCT to measure pulse induced motion between the retinal and the choriocapillaris complex along with the visualization of ocular blood flow by the use of OCT angiography [OCTA]. The effect of increased intraocular pressure [IOP] on the ocular fundus pulsation and the retinal perfusion was investigated in the posterior rat eye.

A custom-made OCT system for small animal retinal imaging was utilized to image the posterior eye of Sprague-Dawley rats. To investigate the impact of increased IOP on the ocular fundus pulsation and the chorioretinal perfusion, the IOP level of 5 rats was increased experimentally. The anterior chamber of the right rat eye was cannulated and the IOP was increased from 15 to 105 mmHg.

To determine the fundus pulsations, the relative motion between the retinal and choriocapillaris region was calculated between consecutive B-scans. Thus, the average relative velocity between the two regions was calculated and an analysis of the fundus pulsations was performed. In addition, the different vascular plexuses in the posterior eye were visualized by OCTA. An increase of the pulsatile motion was observed until 65 mmHg before it lowered at higher IOP levels. A local increase of pulsatile motion was observed at higher IOP levels around large choroidal vessels. Retinal perfusion showed a slight decrease above 55 mmHg and was substantially decreased above 75 mmHg.

In this work we proposed a method to determine subtle tissue deformations in-vivo as a response to an elevated IOP, which is known to play a key-role in glaucoma. Hence, functional OCT may be a promising tool in preclinical research to study the biomechanical properties of the eye and to reveal insights into the pathogenesis of ophthalmic diseases.

P 106 Ex vivo brain tissue imaging using a high resolution visible light spectral domain optical coherence microscopy system
Optical imaging techniques play an important role in neuroscience research. In particular, optical coherence microscopy (OCM) may be a promising technique for many in vivo and ex vivo studies. The principle of OCM is similar to ultrasound imaging, however using light instead of sound. The major advantages, compared to other optical imaging modalities are that three-dimensional data can be acquired in real time and for imaging no staining or slicing of the tissue is needed. Alzheimer’s disease (AD) is a severe neurodegenerative brain disease and among the most common forms of dementia worldwide. A lot of research is still needed to improve the early diagnosis, which is very challenging but clinically highly demanded. AD is characterized by the degeneration of neurons in the brain and preceded by the formation of tangles and deposition of beta-amyloid proteins. Investigating the brain by using visible light OCM may prove a promising candidate for finding new biomarkers for AD. In this work a spectral domain optical coherence microscope was developed to investigate brain tissue ex vivo. A supercontinuum light source was used to operate the system in the visible wavelength range. An ultrahigh axial resolution of 0.88 µm in tissue was achieved by the use of an extremely broad bandwidth. The system was built on the basis of a Michelson interferometer and a homemade spectrometer. A microscope objective was used to focus the light onto the tissue, which results in a 2 µm transversal resolution. Unstained fragments of formalin-fixed, post-mortem healthy and Alzheimer diseased brain tissues from human subjects were investigated. As a result white and gray matter could be well distinguished based on their different scattering properties. Furthermore a first effort was made to differentiate healthy and AD affected brain tissue by the occurrence of senile amyloid beta plaque structures. Spectroscopic analysis of the images was performed to enhance the image contrast.

**P 107 Scattering characterization in optical coherence tomography using a few-mode fiber detection**


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Optical coherence tomography (OCT) is a non-invasive imaging technique that is well-established in medicine. It uses the intensity of the backscattered light when a sample is illuminated to construct an image of its morphology. Over the years, OCT has become an important imaging technique in fields like ophthalmology, dermatology and neurology, to
diagnose different pathologies of the human body. Scattering and other optical properties of tissues vary depending on the tissue composition and morphology. Features like Alzheimer’s disease plaques or cancerous tumors have different scattering properties than their surrounding tissue when they are illuminated with a light beam. In conventional fiber-based OCT systems, only the light directly backscattered from the sample is measured. We propose a novel detection method for OCT systems based on a few-mode fiber for collecting the backscattered light. These fibers transmit the light by several transversal modes depending on the angle of the incident beam, reconstructing the final image encoded at different depths for each mode. Since with this method we can distinguish light scattered at different angles, few-mode fiber OCT may provide extra information of the optical properties of the tissue sample. In order to test the method, ex vivo brain tissue samples were imaged for detecting scattering regions and characterizing the morphology of the tissue. A relation between the angle of backscattered light and the distribution of the modal intensity of the different images has been demonstrated.

**P 108 Evaluation of Decoupling Ring Effects for Designing a Flexible Transceiver Array for 7 T Cardiac MRI**


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Transmission line resonators (TLRs) have been applied to develop mechanically flexible arrays for high field MRI combining the advantages of parallel imaging with the possibility of form-fitting. The aim of our work is to develop a flexible transceiver array based on TLRs for cardiac MRI at 7 T. One of the major technical challenges is the mutual decoupling between individual elements. Efficient decoupling has been demonstrated using coil annexes with the limitation of interfering with the coil’s geometry. In this work, we evaluate the performance of a decoupling ring-based technique, including decoupling efficiency and effects on the transmit field of the TLR, using electromagnetic simulations (EMS) and MR measurements at 7 T. Each element is composed of two conductors (84 mm outer diameter, 2 mm width) deposited on both sides of a 150 µm thick polyimide substrate. In array configuration, decoupling rings of nearest neighbors are deposited on opposite sides of the substrate for overlapping. Decoupling optimization was done by analytically calculating the shared magnetic flux as function of the decoupling ring overlap distance. Initially, the performance of the decoupling rings for a single element TLR, 4-element and 12-element array of TLRs was evaluated in 3D EMS and circuit co-simulation. For the single
element, the transmit efficiency \( (B_1+/\sqrt{P}) \) with and without decoupling ring was simulated and validated by MR measurements. To test decoupling efficiency, S-parameters were simulated in a 4-element array. With the results from the single TLR and the 4-element array, the final 12-element array was designed and S-parameters were simulated using 3D EMS. Efficient decoupling by decoupling rings was demonstrated using 3D EM simulation. MR experiments have successfully demonstrated that the presence of the decoupling ring does not degrade the transmit performance of the TLR. Also, image quality is not affected by the presence of the decoupling ring.

**P 109 High-resolution line-field OCT using digital aberration correction**

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**P 110 An automatic approach to calculate the image-derived input function for combined PET/MRI brain studies**


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Fully-quantitative PET brain studies require the knowledge of a subject-specific input function (IF), which describes the concentration of non-metabolized radiotracer in arterial plasma as a function of time. Traditionally, the IF is acquired invasively by arterial cannulation. Here we present an automatic approach in calculating a non-invasive image-derived IF (ID-IF) from 18-FDG PET/MRI data of the brain.

Five subjects underwent dynamic FDG test-retest PET/MR examinations in a fully-integrated PET/MRI (Siemens, Biograph mMR). The protocol included a time-of-flight MR angiography (TOF-MRA) sequence and sparsely sampled MR-navigators. Arterial blood samples were collected as the gold standard (A-IF). The calculation of the ID-IF consists of three automatic steps: (1) segmentation of the carotid artery from co-registered TOF-MRA images, (2) motion correction of the dynamic PET frames using the vector fields obtained from MR-navigators, and (3) a point spread function (PSF) dependent partial volume correction using a single region modified Müller-Gartner method. The ratio of the area-under-the-curve (AUC) of A-IF to the AUC of ID-IF (RAUC = AUC A-IF/AUC ID-IF) was used as a similarity measure.

The extracted ID-IF overestimates the A-IF faintly, given the average RAUC of 0.98 ± 0.09. This may be related to the heuristic assumption of the PSF of the PET component of the
PET/MR.
In conclusion, based on the obtained results, the IDIF calculated by the proposed method serves as a viable alternative to the invasive arterial input function.

**P 111 Assessment of attenuation correction for myocardial PET imaging using combined PET/MRI**


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**P 112 Data-driven respiratory motion detection and compensation of myocardial viability examinations using NH3 and FDG.**


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**P 113 Reduced dose rate dependence at high dose rate regimes for sulfur-based polymer gel dosimeter**

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**P 114 Investigating the influence of multiple knee angles on metabolic parameters of different exercising human calf muscle groups using dynamic multivoxel 31P-MRS**

Purpose: Oxygen (O16) ions are a promising alternative to carbon ion beam therapy due to their enhanced linear energy transfer, which is expected to yield a higher relative biological effectiveness and a reduced oxygen enhancement ratio. In order to allow realistic investigations on O16 ion beams using Monte Carlo (MC) simulations, a benchmark against experimental data is mandatory.

Methods: Several physics lists in Geant4 were benchmarked using the GATE environment. Five nuclear models and two electromagnetic options were validated against measured integrated depth dose (IDD) distributions and charge changing cross sections from literature.

Results: Simulated beam ranges (R80%) deviated by less than 0.6 mm to the measurements for all physics lists and energies. For all physics lists, the relative dose differences up to the Bragg peak were found to be less than 4% compared to measurements. Beyond the Bragg peak, in the so-called fragmentation tail, differences increased notably, by up to one order of magnitude. However, the absolute dose difference in the fragmentation tail was comparable to the absolute difference before the Bragg peak. Overall, deviations to the measurement were less than 2% of the maximum dose for all models, disregarding the dose fall off region due to the steep dose gradient.

Partial charge changing cross sections simulated with the BIC, BERT and QBBC models deviated up to 51% from the measurements, INCLXX up to 43% and the QMD model up to 20%.

Conclusion: IDDs simulated with Gate/Geant4 deviated less than 2% of the maximum dose to the experimental results for all physics lists. Measured charge changing cross sections could best be reproduced using the QMD model, whereas the BIC model showed considerable discrepancies. Therefore, Gate/Geant4 can be considered a valid dose calculation tool for O16 ion beams and we suggest the QMD model for accurate prediction of fragmentation.
P 116 Quantitative MR Microscopy of Human Menisci Using Mono and Bi-exponential T2* mapping with a Variable Echo Time Sequence


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P 117 Neurofeedback interventions within resource-oriented treatment of inpatients with alcohol use disorder

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Alcohol use disorder is one of the most prevalent mental health disorders and leading causes of sickness and death globally. As quantitative electroencephalographic (qEEG) and low resolution brain electromagnetic tomography (LORETA) mapping studies of detoxified alcohol-addicted patients show, there is an increase in absolute and relative beta power and a decrease in alpha and delta/theta power.

The aim of this study is the examination of the effects within a group of inpatients with alcohol use disorder getting treatment as usual (TAU) at the Anton Proksch Institut in Vienna in comparison to a group of inpatients getting add-on therapy with neurofeedback following the Scott-Kaiser modification of the Peniston protocol. The neurofeedback group (NFB) will receive in total 30 sessions of neurofeedback training combining sensorimotor rhythm (SMR) training (SMR augmentation with theta suppression) with the Peniston protocol (alpha-theta training).

As specific patterns of qEEG abnormality were found in alcohol-addicted patients, qEEG patterns before and after interventions will be analysed within both treatment groups. With an effect size of $r = .30$, in total N=56 participants will be randomized to TAU group (n=28) and NFB group (n=28).

Due to a combination of a psychophysiological and neuropsychological approach, all participants will receive a baseline measurement (T1) of the heart rate variability (HRV) and a qEEG measurement prior to the application of interventions. Additionally, participants have to fill out a set of psychological and clinical questionnaires recording domains of executive functions, inhibitory control, emotional abilities and coping strategies amongst others.
At the second point of measurement (T2), which follows after interventions in both groups, participants will again be measured in terms of a HRV, qEEG and clinical measurements.

P 118 The impact of binaural-beats on QEEG-metrics of alcohol addicted patients


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The main research interest is to investigate whether or not the prolonged exposure to binaural-beats evoking stimuli [BB] can influence the activity of the CNS in alcohol-addicted patients, as quantified by the QEEG-metrics absolute and relative power within the delta, theta, alpha and beta frequency bands. BB refer to an auditory perception which occurs when two sounds of nearly similar frequency are simultaneously presented, one to each ear. BB can be perceived at low frequencies characteristic of the EEG spectrum and seem to entrain the brain activity. Furthermore, it should be also examined if and how potential changes of QEEG-parameters have an impact on therapy outcome and relevant, self-reported psychometrics related to stress coping strategies, fluctuating feelings and enduring affect states, personal competences to deal with problems and critical situations of daily life, thoughts about alcohol and drinking behavior, impulsive behavior, transient and enduring anxiety levels, severity of depression and, last but not least, life satisfaction.

P 119 Effects of heart rate variability-biofeedback (HRV-BF) on alcohol-addicted inpatients

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Heart rate variability (HRV) is an index of beat-to-beat changes in heart rate and generally acknowledged as a psychophysiological marker for physical and mental health. According to Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), chronic and severe alcohol consumption is diagnosed as alcohol abuse disorder (AUD) and leads to an autonomic nervous systems’ (ANS) imbalance. This dysregulation of sympathetic and parasympathetic activities results in reduced HRV, which is linked to an increase in craving and risk for relapse in addiction treatment. By using a matched-pair design, this interventional pre-post study explores the potential effects of HRV-biofeedback (HRV-BF) training on various HRV-parameters, feelings of craving, impulsivity, self-efficacy and alcohol-related comorbidities such as depression and anxiety in alcohol-addicted
inpatients’ therapy. All participants (n = 60) are inpatients at the Anton Proksch Institute (API) and will be either allocated to an experimental (n = 30) or control (n = 30) group on a randomized basis. While the former faction undergoes treatment as usual plus 15 sessions of HRV-BF, the latter only receives standard inpatient therapy. Furthermore, the complex interrelation between the autonomic nervous system (ANS) and the central nervous system (CNS) will be investigated for the first time. For that purpose, a quantification of treatment-associated brain activity changes by means of quantitative electroencephalogram (qEEG) will be performed before and after HRV-BF interventions. This enables a neurometric comparative analysis of brain activity and EEG signal patterns between both experimental conditions. In addition to a detailed introduction of this non-invasive and novel method in addiction therapy, this presentation provides first results, an evaluation of the effectiveness of HRV-BF and its applicability among alcohol addicted inpatients.

P 120 ADHD & HCV prevalence in a standardized investigation in Austrian prisons in opioid maintained subjects & imprisoned adolescents


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Background: The burden of psychiatric and somatic comorbidities is higher in people who inject drugs, especially in prison. Symptoms of attention deficit-hyperactivity disorder (ADHD) are correlated to delinquent behavior and ADHD is increased in persons who suffer from substance-use disorders. Furthermore, inmates often continue or start using illicit substances when imprisoned, which elevates the risk of Hepatitis C (HCV) infection.

Research Question: Investigation on prevalence of ADHD symptoms as well as HCV infectious status, delinquency, opioid maintenance therapy (OMT) and other psychiatric comorbidities in two target groups in Austrian prisons. Methods: Standardized psychiatric assessment was administered on either inmates enrolled in opioid maintenance treatment or adolescents/young adults in youth detention centers. Applied instruments: Structured psychiatric questionnaires and assessment of medical status, substance use and delinquency were conducted. HVC testing including genotyping was performed. Results: Total sample of 153; results in 112 adult inmates undergoing OMT show that 50% report ADHD symptoms in their childhood, whereas 17% of adult inmates in OMT score for both current and childhood ADHD. In 41 young adults/minors 45% score for ADHD in their childhood and 13% report current ADHD symptomatic. HCV investigation show a prevalence of HCV infection in the OMT sample with 56% positive results. In contrast, HCV prevalence in the adolescent/young adult sample showed negative results. Prevalences of other psychiatric comorbidities were high, especially antisocial personality disorder (56%) and current episodes of major depression (21%). Conclusion: The implementation of a consistent diagnostic and consecutive treatment delivery for ADHD could prevent continued crime related behavior. Standardized treatment of HCV has the potential to
reduce transmission in a target risk group. However, a structured aftercare program requires implementation.

**P 121** Applied Behaviour Analysis [ABA] in Austria – First Results of the Binational EU-Project “Autism Competence Exchange”

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In the framework of the binational EU-project “Autism Competence Exchange” of two non-profit autism societies in Vienna and Bratislava 18 professionals (psychologists, pedagogues and medical doctors) were being trained in the methodology of ABA in a specially developed 1⅓-year training course. The training course was implemented together with the „Institute for Child Development” in Gdansk and characterized by an immediate implementation of the taught method under supervision. The project was accompanied by a scientific study to evaluate the training course as well as the effectiveness of the taught ABA-method. In contrast to many international studies, that have demonstrated the efficacy of intensive ABA-treatment under laboratory conditions, this pilot study focused on the effectiveness of low-intensity ABA-treatment in everyday care.

The experimental group consisted of 15 children receiving approximately 5 hours of ABA per week over a period of 14 months. Outcome parameters (intelligence, language, adaptive behaviour, autism-specific symptoms, problem behaviour and level of parental stress) were assessed at three time points (baseline, 7-month follow-up and 14-month follow-up) by psychological tests, standardized interviews and questionnaires.

Pre-Post-Comparisons after 14 months showed significant improvements regarding autistic core symptoms. Outcome measures of intelligence and adaptive behaviour showed progress in terms of developmental age and large inter-individual differences. Parental stress related to child characteristics significantly decreased from baseline to 14-month follow-up assessment. Evaluation questionnaires showed a high level of satisfaction of the ABA-therapists and the parents with the therapeutic method and the progress of the children.

The results suggest that low-intensity ABA-treatment in everyday care can lead to positive effects and developmental progress.

**P 122** Molecular insights into subtype selective modulation of gamma-aminobutyric acid type A receptors

GABAA receptors constitute important inhibitory neurotransmitter receptors of the central nervous system, as well as important elements in other tissues including the peripheral nervous system and diverse non-neuronal cells. They are ligand-gated ion channels composed of five subunits and modulated by multiple drugs. Different combinations drawn from the nineteen GABAA receptor subunits constitute a large variety of different receptor subtypes with distinct pharmacological profiles, as well as many known and putative allosteric binding sites. For the recently described allosteric site at the extracellular \( \alpha^{+}/\beta^{-} \) interfaces, ligands of the pyrazoloquinolinone class with functional subtype selectivity have been identified and proven useful tools for the further investigation of GABAA receptor function. In this study, their interactions with different receptor subtypes are characterized in recombinant GABAA receptors expressed in Xenopus laevis oocytes, utilizing two-electrode voltage clamp electrophysiology. Moreover, computational homology modelling and docking are employed to generate structural hypotheses as basis for mutational analysis. Here, we present a set of \( \beta^{1} \) preferring pyrazoloquinolinones with nanomolar potencies. Molecular mechanisms that govern unselective and subtype selective modulation mediated by the \( \alpha^{+}/\beta^{-} \) site are also addressed. We also characterized some natural compounds with unknown binding sites. Here, we report the antagonizing effect of falcarindiol on toxic natural compounds with potential benefits for the treatment of water hemlock plant poisoning. Continued exploration of ligand interactions with novel and unknown binding sites of the GABAA receptor can provide more insights to the subtype-selective modulation of the receptors, not only for potential therapeutic benefits but also for establishing useful pharmacological tools that will shed more light on these complex receptors.

**P 123 The intracellular gating network of the human dopamine transporter**

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**P 124 Evaluation of receptor interactions during Reelin-signaling**

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The extracellular matrix molecule Reelin plays a central role in migration and positioning of neurons in the cortex, hippocampus and cerebellum during embryogenesis. The canonical Reelin signaling pathway starts with binding of Reelin to apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR), which triggers Src-family kinase induced tyrosine phosphorylation of the intracellular adaptor protein disabled-1 (Dab1). Clusterin (apolipoprotein J) is also a ligand for ApoER2 and VLDLR which mimics Reelin signaling. Clusterin can bind to amyloid beta (Aβ) oligomers and inhibits the aggregation of Aβ and therefore prevents further formation of senile plaques in the Alzheimer disease (AD) brain. Genome wide association studies (GWAS) identified clu as a risk gene for AD.

Binding of Reelin is thought to result in receptor oligomerization and subsequent clustering of Dab1. Until now the size of clusters and type of oligomerization (homo- or hetero-oligomerization) of the receptors is unknown. Understanding cell signaling mechanisms requires analysis of protein interactions at the subcellular level which still remains a challenge without disruption of the cell. We applied advanced microscopic methods to evaluate receptor interactions resolved in space and time.

Homodimerization state of ApoER2/VLDLR upon Reelin/Clusterin binding was analyzed using steady-state and time-resolved anisotropy (homo-FRET, Förster resonance energy transfer between identical fluorophores), whereas heterodimerization was analyzed using FLIM (Fluorescence Lifetime Imaging Microscopy) hetero-FRET. These FRET and FLIM studies demonstrate that hetero- and homodimerization of ApoER2 and VLDLR is present even in the absence of Reelin. These results were confirmed using biochemical methods such as Co-IP experiments.

Slight differences on the homo-, heterodimerization state of ApoER2 and VLDLR upon ligand binding were detected.

**P 125 ICAM-1 binding human rhinoviruses enter HeLa cells via multiple pathways**

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Of the more than 150 human rhinovirus (HRV) serotypes, 91 utilize intercellular adhesion molecule-1 (ICAM-1) for cell entry. These belong either to the phylogenetic species A or B. We recently demonstrated that HRV-B14 and HRV-A89, despite binding this same receptor, are routed into distinct endosomal compartments for release of their RNA into the cytosol. To gain insight into the underlying mechanism we now comparatively investigated the port of entry, temperature dependence of uncoating and intracellular routing of HRV-B3, HRV-B14, HRV-A16 and HRV-A89 in HeLa cells. The effect of various drugs blocking distinct
stages on the individual pathways was determined via comparing the number of infected cells in a TissueFaxes instrument. We found that HRV-B14 and HRV-A89 enter via clathrin-, dynamin-, and cholesterol-dependent pathways as well as by macropinocytosis. Drugs interfering with actin function similarly blocked entry of all four viruses indicating their dependence on a dynamic actin network. However, uniquely HRV-A89 was able to produce progeny when internalized at 20°C followed by neutralizing the endosomal pH and heating to 34°C. Blocking dynein-dependent endosomal transport prevented uncoating of HRV-A16 and HRV-A89 but not of HRV-B3 and B14, indicative for routing of HRV-A16 and HRV-A89 into the endocytic recycling compartment for uncoating.

**P 126 Targeting STAT5 Oligomerisation in Hematopoietic Cancer**


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Targeted inhibition of hematopoietic malignances is predominantly achieved through small molecular weight tyrosine kinases (TK) inhibitors (TKI). TKI treatment is often jeopardized by development of resistance and severe side-effects. TK are frequently mutated in cancer associated with their hyperactivation. One of their critical substrates is downstream activation of the STAT5 family. STAT5 was shown to be critical for disease initiation and progression in multiple forms of hematopoietic cancer. Therefore, strategies are required to target STAT5A/B gene products. STAT5 oligomers generated via interaction of STAT5 N-terminal dimerization, contribute to neoplastic cell growth and survival. STAT5 promotes direct oncogene mRNA up-regulation in hematopoietic cells. Mice expressing a STAT5 variant lacking the N-terminus provided evidence of its importance to drive neoplasm. Moreover, STAT5 variants engineered to form only dimers failed to induce hematopoietic neoplasm. Here, we specifically target the N-terminus of STAT5 to disrupt STAT oligomers. Aim of the study: Establish a screening system that allows for search of STAT5 inhibitors to disrupt STAT5 oligomers to find a new scaffold of drugs that target the STAT5 N-domain dimer interface.

We developed a unique screening system with engineered hematopoietic cell lines that are driven for growth and survival through the STAT5 N-domain dimerization. This allows for high-throughput and high content compound library screen. Specifically, by fusion of the N-terminus of STAT5A/B to an active TK domain we will identify molecules disrupting STAT5 oligomers. Constructs with respective controls displayed that the STAT5-TK fusions convert cytokine-dependent cells into cytokine independent cells. Once potential lead compounds are characterized, combinatorial effects of selected STAT5 inhibitors with FDA approved drugs will be evaluated. Our long term aim is a better understanding of STAT5 N-domain function in cancer progression.
The transcription factor FOXO3 differentially regulates the expression of proinflammatory genes in rheumatoid fibroblast-like synoviocytes


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Objective: The transcription factor (TF) FOXO3 is known to integrate information from multiple upstream signals (e.g. cytokines, oxidative stress, growth factors) in order to maintain tissue homeostasis during stress. Interestingly, although an association between a FOXO3 genotype (SNP) and the severity of rheumatoid arthritis (RA) was recently reported, the role of this TF in rheumatoid fibroblast-like synoviocytes (FLS) has not yet been investigated.

Methods: With approval by the ethics committee synovial tissues from patients fulfilling the ACR/EULAR classification criteria for RA were obtained as discarded specimens following synovectomy. RA-FLS were isolated according to standard procedures. FOXO3 phosphorylation was determined by western blots. MK2206 was used to inhibit AKT. Nuclear-cytoplasmatic shuttling of FOXO3 was visualized and quantified by confocal immunofluorescence microscopy. FLS were transfected with siRNA pools in order to investigate the role of FOXO3 in the TNF-induced inflammatory response. The expression of cytokines, chemokines and proteases, that are all known to be involved in RA pathogenesis, was assessed by ELISA, qPCR and western blots.

Results: TNF, the apex of the proinflammatory network in RA, promoted the phosphorylation and nuclear export of FOXO3 in FLS. FOXO3 phosphorylation by TNF was inhibited by the AKT-inhibitor MK2206, demonstrating that TNF induces AKT phosphorylation to subsequently control FOXO3 activity in FLS. Interestingly, while IL6 and IL8 expression was not affected by FOXO3 knockdown, a significant reduction in MMP3 expression was observed. In contrast, FOXO3 knockdown by siRNA promoted the TNF-induced expression of BAFF, TNFSF10 and CXCL11, suggesting that FOXO3 is a negative regulator of these genes.

Conclusions: Our data reveal differential regulation of arthritis-associated genes by FOXO3 in FLS and thus support the idea that FOXO3 plays a crucial role in rheumatoid synovitis.

Cortical segmentation of the human brain using distributions of serotonergic key proteins quantified by PET in vivo

P 129 Role of JAK2 in the initiation and progression of KRAS-mutated NSCLC

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P 130 EGFR controls epidermal homeostasis and skin inflammation

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The epidermal growth factor receptor (EGFR) orchestrates epidermal differentiation and is an important regulator of epidermal function, homeostasis and overexpression is involved in several human tumor types. Consequently, cancer patients are treated with EGFR inhibitors (EGFRIs) and frequently develop acneiform skin toxicities, which correlate with patient's anti-tumoral response. Previous work of our group showed that the early inflammatory infiltrate of the skin rash induced by EGFRIs is accompanied by the expression of chemokines (CCL2, CCL5, CCL27, and CXCL14) in epidermal keratinocytes. Furthermore, EGFRIs-treated keratinocytes show a reduced production of antimicrobial peptides and skin barrier proteins leading to a reduced cytotoxic activity against Staphylococcus aureus. Mice lacking epidermal EGFR (EGFRΔEP) partially phenocopy patients treated with EGFRIs, displaying a chemokine-driven skin inflammation, hair follicle degeneration, decreased host defense, and compromised skin barrier function. Skin toxicities were not ameliorated in a Rag2−, MyD88−, TNFR1/2−, CCL2-deficient, hairless (hr/hr) background or in mice depleted of epidermal Langerhans cells, macrophages or mast cells, respectively. Therefore the exact mechanisms at the basis of the skin inflammation still remain elusive. We hypothesize that a combination of deranged epidermal chemokine expression, hair follicle and barrier defects is responsible for the observed phenotypes. This project should clarify the molecular mechanisms behind EGFRI-induced cutaneous toxicities and further identify drugable targets responsible for the pathogenesis of the skin rash. Ultimately, the results of this study should be directly translated into clinics leading to the development of novel supportive care therapies to avoid cutaneous toxicities of EGFR inhibitor treatment.
A "multi-omic" investigation of the effects of long wavelength ultraviolet light on primary human keratinocytes

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Ultraviolet A light is the dominant environmental oxidative stressor for the skin and a major factor for skin aging. In dermal fibroblasts UVA oxidizes phospholipids which in turn induce Nrf2 activation and autophagy as stress responses. The role of keratinocytes (NHEK), in the initial events of UVA - photoaging is not well understood. We studied which lipid mediators UVA would generate in KC and how they are correlated to UVA induced changes in mRNA, microRNA and protein expression.

The oxidized phospholipidome of cultured NHEK immediately or after 24h stress recovery showed, using HPLC-MS/MS, that 141 distinct lipid species were significantly induced immediately after UV exposure. 120 UVA generated lipid species had declined to their baseline values after 24h.

In parallel, gene ontology and pathway analysis of global mRNA expression 7h after the stress showed that the 81 mRNAs induced both by UVA and in vitro oxidized lipids which partially mimic the UVA response could be attributed to the action of upstream regulators Nrf2, the UPR, and PPAR signalling.

Among the upregulated genes we found mitochondrial phospholipases, peroxiredoxins and other enzymes capable of metabolizing isoprostanoid-PL and other lipid mediators recently correlated to (skin) aging. Together our data suggest that UV-induced phospholipids initiate a transcriptional response that not only induces synthesis of antioxidant stress response genes but also enzymes to specifically metabolize and detoxify bioactive oxidized membrane lipids.

Oligomerization of human dopamine transporter (hDAT)

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Pharmacological Rescue of Dopamine Transporter (DAT) Mutants Responsible for Infantile Parkinsonism-Dystonia

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The presynaptic dopamine transporter (DAT), is responsible for sequestering released dopamine from the synaptic cleft. DAT is a target of therapeutically relevant and illicit recreational drugs. Point mutations within the coding sequence of human membrane proteins can result in their retention in the endoplasmic reticulum (ER) and thus give rise to clinically relevant phenotypes. Folding-defective mutants of the human dopamine transporter (hDAT) cause a syndrome of deficiency infantile parkinsonism-dystonia (IPD). We hence created 13 mutations responsible for IPD by site-directed mutagenesis and examined their cellular localization and functional activity. Confocal microscopy experiments indicated that all 13 mutated DATs were retained in intracellular compartments, namely in the endoplasmic reticulum (ER) since they co-localized with an ER-resident chaperone calnexin. In terms of their functional activity, none of the mutants showed any appreciable dopamine uptake compared to the wild type DAT. Interestingly, 3 of the mutants could be functionally rescued, i.e. they responded to treatment by pharmacological chaperones, noribogaine (a non-competitive DAT inhibitor) and pifithrin-µ (an inhibitor of the heat shock protein HSP70). We verified these findings by Western blotting: cells which had been pretreated with noribogaine or pifithrin-µ, produced a larger amounts of mature core-glycosylated bands, compared to untreated control. The combination of noribogaine and pifithrin-µ produced the largest increase in the mature core-glycosylated form of hDAT. Pretreatment of cells with noribogaine and pifithrin-µ is predicted to reduce the association of hDAT mutants with calnexin and HSP70-1A. We hence verified by performing immunoprecipitation experiments, that pretreatment of cells with both these compounds reduced the association of hDAT mutants with calnexin and HSP70-1A.

Paneth-related cells promote immune escape of colorectal cancer


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P 135 Biochemical characterization of PYROXD1 and its involvement in the redox-regulation of the tRNA splicing

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Splicing is an essential step for the maturation of transfer RNAs (tRNA) in all Eukarya and entails two enzymatic steps: endonucleolytic cleavage of a precursor tRNA and ligation of tRNA exon halves. Although the enzymes responsible for these reactions have been identified, their regulation remains enigmatic. Using a phylogenetic screen, we identified PNO1, a protein predicted to be involved in redox biology, as a potential regulator of tRNA ligation in human cells. Interestingly, we observed that the tRNA ligase complex is inhibited upon redox stress. Thus, we propose PNO1 as a novel factor required for redox-regulation of tRNA splicing.

Using in vitro assays, we demonstrated that PNO1 harbors redox activity. Expression of a catalytically dead PNO1 mutant in HeLa cells did not rescue the absence of the wild-type protein, indicating that the oxidoreductase activity of PNO1 is essential for the function of the tRNA ligase complex in vivo. Furthermore, we observed that PNO1 might itself be a target of redox regulation, since both its activity and oligomeric state depend on cellular redox markers. Searching for endogenous substrates of PNO1, we noticed that PNO1 resembles a 25-years elusive mammalian cytosolic ubiquinone-10 reductase. This hypothesis is being tested by measuring ubiquinone-10 levels in cells depleted of PNO1. In summary, we identified PNO1 as a redox-active protein whose activity is essential for the splicing of tRNAs in mammals. We aim to identify the redox switch in the tRNA ligase complex and link the reaction product of PNO1 with the redox regulation of tRNA splicing.

P 136 Conformational changes associated with substrate transport in LeuT

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Biological membranes play a critical role in membrane-protein structure, stability and function. It has long been recognized that lipid molecules directly interact with membrane proteins with high affinity. Earlier work has highlighted that phospholipids and sterols modulate the structure and function of ion channels, and a flurry of recent studies have demonstrated that bound lipids can affect folding, impart stability and modulate the function and physiological role of membrane proteins. Neurotransmitter sodium symporters (NSS) are sodium-coupled symporters that drive the uptake of neurotransmitters from neural synapses and are key targets for antidepressants and psychostimulants. Extensive structural data have been collected in recent years for several members of the NSS family which opened the way to structure-based studies for a mechanistic understanding of substrate transport. LeuT, a bacterial orthologue, has been broadly adopted as a prototype in these studies. We will investigate whether the function of LeuT is influenced by the lipid environment in the LeuT Proteoliposomes of defined lipid compositions. The functional assessment in the above Proteoliposomes will be addressed based on its folding and the substrate transport activity. We will further control the directional orientation of LeuT into Proteoliposomes using His-tag which has been previously done in two separate studies on different transporters. In order to address dynamic nature of the substrate transport in LeuT, we are going to use the Lanthanide based resonance energy transfer (LRET) technique. This technique is an alternative to address the movement of helices, with great resolution. This has been employed successfully in our lab to the LeuT.

**P 137 Identification of keratin K23 as a component of the cytoskeleton in cornifying epidermal keratinocytes**

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The formation of the skin barrier to the environment depends on the terminal differentiation of epidermal keratinocytes and their conversion into dead corneocytes that are filled with keratins and build to the outermost layer of the body. Keratin intermediate filament proteins are important biomarkers of epithelial cell types and differentiation stages. Most of the 54 human keratins have been characterized but the expression patterns and functions of some keratins have remained elusive. Here, we investigated whether epidermal keratinocytes express K23, a keratin which had previously been detected in simple epithelia under stress conditions. We found that normal human epidermal keratinocytes proliferating in vitro contained only small amounts of K23 mRNA.
whereas the induced differentiation of keratinocytes led to a more than hundred-fold upregulation of K23 expression, as determined by quantitative RT-PCR. Using a newly raised anti-K23 antibody, K23 protein was detected, by Western blot analysis, in the cytoskeletal protein fraction of confluent keratinocyte cultures and, by immunohistochemistry, in the granular layer of human epidermis. Comparative genomics showed that K23 is conserved in mammals with the notable exception of dolphins and whales, in which the terminal differentiation program of keratinocytes has degenerated during the evolutionary transition to a fully aquatic lifestyle. Our results define K23 as a marker of keratinocyte terminal differentiation and suggest that this keratin contributes to the maturation of the cytoskeleton during skin barrier formation.

P 138 A mutation of A1-adenosine receptor (A1R-G279S) associated Parkinson's disease*

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P 139 Epigenome-Wide RNAi Screen Identifies an Alpha to Beta Cell Transdifferentiation Factor

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Pancreatic beta cells, the sole source of insulin in the human body, closely interact with their neighbouring cells in the islets of Langerhans, namely the glucagon-secreting alpha cells, to maintain normoglycaemia. Loss of these beta cells, as observed in Type I Diabetes (T1D), results in deregulated glucose homeostasis and severe hyperglycaemia. This underscores the need for a novel insulin cell source in T1D patients. The close developmental link between alpha- and beta-cells, evident via their common Ngn3+ progenitor, make alpha-cells a promising candidate. Hence, we are attempting to induce beta cell characteristics in alpha cells, in the hopes of replenishing beta cell mass. We conducted a chromatin-focused short hairpin RNA screen on the murine alpha cell line, aTC1, in search of proteins repressing beta cell markers. The viral library targeted over 300 potentially druggable chromatin factors. The cells were then screened for changes in their transcription profile, with a particular focus on increased insulin (Ins2) expression. Target hits were validated via rescue experiments and immunofluorescence. The strongest hit from the screen was a protein involved in post-transcriptional modifications. RNAseq
results show a general upregulation in beta cell markers, including Iapp, Gck, Pax4 and Ins2, upon knockdown in aTC1 cells. Knockout experiments reveal the essentiality of the gene for alpha cell survival and proliferation. Its function appears to be conserved in human islets, in which its knockdown induces a significant upregulation of Pax4 transcription. Affinity proteomics and mutagenesis experiments further elucidate the mechanism of this Pax4 repression. Overall, we have identified a promising candidate whose loss triggers insulin expression in alpha cells. These experiments could yield valuable information regarding transcriptional regulation in the endocrine pancreas, and potentially a new insulin cell source.

P 140 LPA-induced phosphatidylserine exposure in erythrocytes is mediated by TMEM16F and modulated by EGCG and fluoxetine

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It becomes increasingly accepted knowledge that erythrocytes are active players in blood clotting and thrombus formation. Lysophosphatidic acid (LPA), a lipid mediator secreted upon platelet activation, triggers a signaling cascade in erythrocytes that results in cellular calcium uptake and high levels of phosphatidylserine (PS) exposure at the cell surface within minutes. This process generates activated membrane sites for binding of factors of the blood clotting cascade.

Upon studying PS exposure at the cell surface of erythrocytes from fresh blood we found that it is strictly dependent on calcium influx. This contrasts other reports that, however, had employed non-physiological reagents like phorbol myristate acetate (PMA) or calcium ionophore instead of LPA. TMEM16F, one of ten members of its family (also known as anoctamins), was implied as a likely candidate molecule responsible for membrane scrambling in erythrocytes. In fact, we can show by immuno fluorescence and FACS analyses TMEM16F to be present in the erythrocyte membrane. Moreover, known effectors of TMEM16F clearly modulated LPA-induced calcium influx plus PS exposure (>80% PS+ cells) in erythrocytes. Interestingly, the scramblase inhibitors tannic acid and epigallocatechin gallate (EGCG, the most abundant catechin / polyphenol of green tea) elicited an hormetric response, i.e. TMEM16F scramblase activation at sub-micromolar versus inhibition at micromolar doses. This hormetric effect was further enhanced by micromolar doses of fluoxetine (Prozac™), a known activator of TMEM16F.

Together, this suggests an increased thrombotic risk might be considered as a potential side effect of the anti-depressant fluoxetine that warrants further study.
**P 141 Pantothenate Kinase 2 activity in red blood cells of patients with pantothenate-associated neurodegeneration**

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Pantothenate kinases (PanK) catalyses the first and rate limiting step in coenzyme A (CoA) biosynthesis. Mutations in pantothenate kinase 2 (PanK2) are associated with pantothenate-associated neurodegeneration (PKAN; incidence 1-3 per million). The occurrence of misshaped erythrocytes (acanthocytes) observed in some patients had suggested a role of PanK2 in erythrocytes.

After establishing a radiological enzyme activity assay, we indeed could confirm PanK2 enzyme activity in erythrocytes, Western blot results correlated well with activity. Next, erythrocytes of 18 PKAN patients with different mutations were studied and compared to wild type and heterozygous (usually parents) control donors. Samples homozygous for G521R, the most frequent mutation linked to PKAN, were inactive for enzyme activity and PanK2 protein absent from Western blots. Most likely this mutant protein is instable and degraded. Conversely, the second most common mutant, T528M, was found to be present and partly active. Compound heterozygotes (G5221R/T528M) also exhibited residual PanK2 (activity). Surprisingly, both amount of PanK2 on Western blots and level of activity were comparable between patients and some heterozygous but healthy control donors. Overall, 8/18 patients exhibited active PanK2 while 5/14 heterozygous control donors showed reduced enzyme activity. Of note, PanK2 is an enzyme of the intermembrane space of mitochondria, the latter being absent in erythrocytes. Despite this, further inducer and inhibitor experiments established that the entire 5-step pathway of CoA biosynthesis is active in mature erythrocytes and can be feedback inhibited by CoA.

Although erythrocyte PanK2 activity apparently cannot be used as a predictive marker for the severity of neurodegeneration or its onset in PKAN, it may still be a valuable means to approach the molecular-pathological situation in individual patients.

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**P 142 The N-terminus specifies the switch between transport modes of the human serotonin transporter.**

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The serotonin transporter (SERT) and other monoamine transporters operate in either a forward transport mode, where the transporter undergoes a full transport cycle, or an exchange mode, where the transporter seesaws through half-cycles. Amphetamines trigger the exchange-mode leading to substrate efflux. This efflux was proposed to rely on the N-terminus, which was suggested to adopt different conformations in the inward-, outward-facing and amphetamine-bound states. This prediction was verified by tryptic digestion of SERT-expressing membranes: in the absence of Na+, the N-terminus was rapidly digested. Amphetamine conferred protection against cleavage suggesting a relay between the conformational states of the hydrophobic core and the N-terminus. We searched for a candidate segment, which supported the conformational switch, by serial truncation removing 22 (ΔN22), 32 (ΔN32) or 42 (ΔN42) N-terminal residues. This did not affect surface expression, inhibitor binding and substrate influx. However, amphetamine-induced efflux by SERT-ΔN32 or SERT-ΔN42 (but not by SERT-ΔN22) was markedly diminished. We examined the individual steps in the transport cycle by recording transporter-associated currents: The recovery rate of capacitive peak - but not of steady-state - currents was significantly lower for SERT-ΔN32 than that of wild-type SERT and SERT-ΔN22. Thus, the exchange mode of SERT-ΔN32 was selectively impaired. Our observations show that the N-terminus affords the switch between transport modes. The findings are consistent with a model, where the N-terminus acts as a lever to support amphetamine-induced efflux by SERT.

**P 143 Enantio-selective effects of cathinone derivatives at the serotonin transporter**


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In nature, cathinones are the psychoactive components in the Khat plant. The leaves of Khat were used by people in the Arab peninsula and eastern Africa as psychostimulants for centuries. Structurally, cathinone and its derivatives share similarities with amphetamines. The difference is that cathinones have an extra ketone group which is absent in the amphetamines. In the illicit drug market, both S- and R-isoforms of cathinone derivatives are available. In this study, we investigated the effects of enantiomers of three different cathinone derivatives, namely, methcathinone, methylmethcathinone and trifluormethylmethcathinone on the serotonin transporter (SERT). Our results suggested that the S-isoforms were more potent in inhibiting uptake, inducing efflux and eliciting currents when compared to the R-isoforms. Moreover, we found that the S-isoforms have higher on-rates of binding than the R-isoforms, while their off-rates differed non-
significantly. In summary, the S-isoforms of the cathinone derivatives were more potent than the R-isoforms, most likely due to their higher on-rates to SERT.

**P 144 Angiotensin-II boosts neutrophil extracellular trap formation in a AT1R and NADPH oxidase-dependent manner**

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Background: Arterial hypertension is a major risk factor for coronary artery disease (CAD). By formation of neutrophil extracellular traps (NETs), neutrophils can release their nuclear content into the extracellular space, fighting pathogens. NETosis has recently been implicated in CAD. In preliminary studies of CAD patients, we observed a positive correlation between blood pressure and NETosis ex vivo, implicating that blood pressure modulates NETosis. Angiotensin–II (Ang–II) is an important mediator of blood pressure via its potent vasoconstrictive properties, but also exerts pro-inflammatory functions via the angiotensin type 1 receptor (AT1R). AT1R is expressed on neutrophils. We thus hypothesized that Ang–II might influence NETosis.

Methods: Ex vivo NETosis of isolated neutrophils upon stimulation with ionomycin was measured using Sytox® Green Nucleic Acid Stain, a dye exclusively staining extracellular DNA released from cells with disrupted membranes, a hallmark feature of NETosis. Extent of NETosis was computed as percentage of positive Triton control.

Results: In line with previous literature, ionomycin induced NETosis in a dose-dependent manner. After pre-treatment with Ang–II, NETosis was enhanced to 80–90% of positive control, irrespective of ionomycin concentration. The AT1R antagonist losartan abolished the effect of Ang–II on NETosis, suggesting an AT1R-dependent pathway. Since Ang–II induces intracellular ROS formation in neutrophils via activation of NADPH oxidase, we pre-treated neutrophils with the NADPH oxidase inhibitor diphenyleneiodonium (DPI). DPI antagonized the effect of Ang–II on NETosis.

Conclusion: Our results implicate that via Ang–II, arterial hypertension increases the propensity of neutrophils to undergo NETosis by increasing intracellular ROS production, which in turn makes neutrophils more susceptible to a second hit. This provides new insight in how effective blood pressure lowering might lead to more favorable outcome.

**P 145 Deciphering Artemether-induced signaling pathways in alpha to beta cell transdifferentiation**

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Type 1 diabetes is characterized by the loss of pancreatic beta cell mass. One promising therapeutical approach is the transdifferentiation of alpha cells into insulin-producing beta-like cells. Downregulation of the alpha master transcription factor Arx is sufficient to trigger this conversion. Our group recently reported that the drug Artemether is able to repress Arx by inducing its translocation from the nucleus, resulting in alpha cell transdifferentiation. Artemether is a small molecule that stabilizes gephyrin, leading to increase in GABAA receptor signaling. GABA treatment can induce beta-like cell formation by downregulating Arx expression in alpha cells as well as alpha cell neogenesis upon reactivation of endocrine developmental processes. Several aspects of the mechanism by which Artemether-induced GABA signaling results in Arx translocation are still unknown. In order to elucidate this complex signaling, genome-wide and targeted CRISPR screens will be employed using mouse pancreatic cell lines. At the same time, human pancreatic islets will be treated with Artemether to study the heterogeneity and complexity of the transcriptome changes in the different cell populations during transdifferentiation. This will be done in a robust approach using single-cell sequencing by Drop-seq. A genome-wide loss-of-function CRISPR screen will enable us to identify the essential genes in Artemether-induced transdifferentiation in a systematic, robust and unbiased approach. In an independent focused genetic screen, genes involved in alpha and beta cell identity and GABA signaling will be targeted in a gRNA library which will be used with the novel CROPseq technology combining CRISPR genome editing with single cell transcriptome readouts. These genetic screens will comprehensively address the mechanism of action of GABA and Artemether in alpha cells, and identify novel targets and synergistic conditions for the generation of novel functional beta cells.

**P 146 Measuring Protein Affinities using FRET**

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Every set of interacting molecules exists in an equilibrium of a bound, and an unbound fraction. This equilibrium is determined by the affinity, which describes the relation of association and dissociation of the interaction partners. In molecular biology, where most processes are guided by finely tuned molecular interactions, most common methods only give qualitative information on an interaction. Therefore, determining the affinity can give much insight into the complex processes within a living organism.

Methods to determine affinities have been around for a long time, but they often need isolated reactants and clean conditions, which are rarely the natural environment of biomolecules, and the isolation of proteins from their usually very crowded environment can vastly change their behaviour.

To overcome this, we are using FRET (Fluorescence/Förster Resonance Energy Transfer), a method that is often used to measure the interaction of molecules within their respective
environment. Here, a radiationless energy transfer transmits energy from one fluorophore to another within close proximity, which can be measured by several effects this has on the two fluorophores. By using this method to determine the extent of interaction and titrating varying concentrations of the interaction partners, the law of mass action can be used to solve for the affinity.

We explore different methods of measurement and ways to determine the affinity. The method is applied to both, a controlled in-vitro system as well as the complex environment of a living cell.

**P 147 Visualizing endogenous proteins and their interactions in live cells using CRISPR/Cas9-based genome editing**

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Numerous binding partners of NF-κB and the IKK-complex have been identified; however, little is known about the complex interaction network of these proteins and how it is dynamically regulated in living cells. Most of the molecular interactions that we know by now, have been identified by rather artificial screening approaches such as yeast two-hybrid screens and have been verified by various techniques in vitro like co-immunoprecipitation. Protein affinities have been determined in most cases by biochemical analyses in vitro, under non-physiological conditions, which differ enormously from the microenvironments of living cells. However, key effector molecules, such as NF-κB or IκB kinases, are often able to bind to a variety of interaction partners and to form complexes characterized by dynamic dissociation and re-association reactions. Significant changes in this dynamic interaction network might provide a regulatory basis for the control of cellular functions and minor changes of affinities due to protein-modifications (e.g. phosphorylation or ubiquitination) may lead to shifts in the composition of protein-complexes. Based on these consideration, the main goal of this study is to elucidate the dynamic interaction network of signaling molecules of the NF-κB pathway at their physiological expression levels and in live cells, which has not been possible so far in sufficient detail. Using the CRISPR/Cas9 technology of genome editing, we want to tag endogenous proteins with fluorescent markers in vivo. Cell lines with endogenously tagged signalling molecules will be analysed by time lapse microscopy at different conditions and states of activation to assess and investigate the interaction dynamics. We expect, that our results will contribute to a better understanding of molecular interaction dynamics and especially to a more precise knowledge of the NF-κB signaling pathway.

**P 148 The AP-1-BATF and -BATF3 module is essential for growth and survival of anaplastic large cell lymphoma**
Anaplastic large cell lymphoma (ALCL) is an aggressive T-cell lymphoma with early onset. It is characterized by the fusion of the ALK kinase with the nucleophosmin protein which can be found in half of the patients and leads to dimerization and constitutive kinase activation. Transcription factor AP-1 is constitutively activated and drives growth and survival in both ALK+ and ALK- ALCL. Here we demonstrate high-level expression of BATF and BATF3 in ALCL, irrespective of the ALK-status. Both BATFs bind to classical AP-1 motifs and interact with AP-1 factors deregulated in ALCL. Together with IRF4, they co-occupy AP-1-IRF composite elements (AICE), differentiating ALCL from non-ALCL. Gene-specific inactivation of BATFs by CRISPR/Cas9 results in ALCL growth retardation and/or cell death in vitro and in vivo. In particular, deletion of BATF or BATF3 in ALK+ ALCL cell lines led to significant growth retardation. This was also reflected in murine engraftment experiments where absence of BATF led to significantly smaller tumours. Knocking out BATF3 led to an compensatory upregulation of BATF indicating partially overlapping function in ALCL. When we knocked-down both BATF and BATF3 using siRNA approaches, cell growth was stopped suggesting BATF addiction of ALCL cells. Cell cycle analysis revealed that BATF3 knock-out results in G1 arrest and transwell assays showed reduced migratory potential of BATF3 knock-out cells. Our data highlight the crucial role of AP-1 / BATFs for ALCL biology and provide the basis for an ALK-independent ALCL pathogenic concept with clinical relevance.

**P 149 Effects of T cells-derived cytokines on neutrophils in IgE-mediated allergy**

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**P 150 Characterization of IL-9-producing T cells generated from healthy human skin explant cultures**
P 151 IgE auto-reactivity in the auto-immune condition bullous pemphigoid

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Bullous pemphigoid (BP) is an auto-immune blistering disease that has consistently been associated with IgG autoantibodies and complement activation. Two keratinocyte-produced antigens are known to be the target of IgG auto-antibodies in this disease, i.e. BP230 (BPAg1) and BP180 (BPAg2). The NC16A portion of the BP180 molecule is thought to have the greatest antigenicity. The frequent appearance of urticarial plaques in a large number of these patients points to a pathogenic role of IgE, but the exact mechanisms of such an occurrence are only poorly understood. In this study, we have addressed this question and have detected, via ELISA, significantly higher levels of NC16A- and BP230-specific IgE in BP sera than in those of healthy individuals. Using overlapping peptides of BP180 as targets, IgG and IgE were found to share the same dominant epitopes. IgE was also detected in perilesional skin of 21 out of 32 (66 %) BP patients. As opposed to reports from other investigators, this IgE was not found at the dermal-epidermal junction, but rather on the surface of mast cells and eosinophils. We have evidence that, on both cell types, the high-affinity receptor for IgE (FceRI) is the primary molecule involved in this interaction. Our further finding of BP180 co-localizing with IgE+ eosinophils suggests that this antigen (or fragments thereof), released from basal keratinocytes, may cross-link eosinophil- and perhaps also mast cell-bound BP180-specific IgE, culminating in at least some of the symptoms seen in BP. In fact, BP180-IgE complexes induce signal transduction in FceRI-expressing rat basophils. We therefore propose a new pathway of BP pathogenesis, not alternative but parallel to an IgG track, dependent on functional BP180-IgE immune complexes present in BP skin.

P 152 Interaction of natural and mutant fish parvalbumins and fish-derived food matrix with bronchial epithelial cells

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Introduction: Inhalation of aerosolized fish allergens and fish matrix components is often associated with severe IgE-mediated reactions in sensitized individuals. The calcium-binding proteins parvalbumins are major fish allergens. The role of epithelial cells in allergic reactions to fish is not well understood. We explored interactions of the human bronchial epithelial cell line 16HBE14o- with natural fish parvalbumins in presence or absence of fish-derived food matrix. To explore the role of calcium binding to parvalbumins in their interaction with the cells, we included in our study a mutant carp parvalbumin in which two functional calcium-binding sites were mutated.

Methods: We used the natural parvalbumins Gad m 1 and Cyp c 1 purified from cod and carp, respectively, and the mutant Cyp c 1 expressed in E. coli. A <3kDa fraction of fish extract was used as a fish matrix. Polarized 16HBE14o- cells were treated apically with parvalbumins with or without the respective fish-derived food matrix. Fluorescently labelled parvalbumins were detected by confocal microscopy. Gene expression of IL-6, IL-8, CCL2 and TGF-β1 was explored by qPCR and the concentration of the cytokines in the basolateral cell culture medium was measured by the Luminex assay.

Results: Apical exposure of the cells to parvalbumins resulted in their internalization. Mutant Cyp c 1 decreased IL-6, IL-8 and CCL2 release to basolateral medium by 40-60%, whereas natural parvalbumins did not. Carp matrix increased basolateral release of IL-6 and IL-8, in contrast to cod matrix which had no influence.

Summary: We observed internalization of fish parvalbumins by bronchial epithelial cells. Mutant carp parvalbumin induced a different pattern of cytokine release compared to the natural allergens which indicates a possible role of calcium binding to parvalbumins in their interaction with the epithelial cells.

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P 153 Histamine receptor 1 antagonists inhibit proliferation and survival of canine neoplastic mast cells

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Advanced canine mastocytomas are characterized by uncontrolled growth of neoplastic mast cells (MC), mediator-related symptoms, and poor prognosis. Drugs inhibiting the histamine receptor 1 (HR1) are frequently used to treat mediator-related symptoms in these patients. Some of the HR1 antagonists, such as loratadine and terfenadine, inhibit growth of neoplastic MC. In this study, we asked whether other HR1 antagonists, commonly used in canine patients, exert effects on viability of MC and on the release of histamine. We used two canine MC lines, C2 and NI-1, and investigated six HR1 antagonists: loratadine, desloratadine, rupatadine, cyproheptadine, diphenhydramine, and dimetindene. In addition, we analyzed targeted drugs that may affect IgE-dependent histamine release in NI-1 cells: KIT inhibitors toceranib, masitinib and PKC412, the JAK1 inhibitor oclacitinib and the BTK inhibitor ibrutinib. HR1 antagonists were found to decrease proliferation of neoplastic MC, with following IC50 values (µM): loratadine (5-10), rupatadine (10-20), desloratadine (10-50), and cyproheptadine (10-35). Reduced proliferation was associated with apoptosis-induction, with following ED50 values (µM): loratadine (15-25), rupatadine (25-45), desloratadine (25-50), and cyproheptadine (35-50). Diphenhydramine and dimetindene showed no significant effects on growth and viability. At higher concentrations (50 µM), loratadine, desloratadine, and rupatadine were found to inhibit the histamine release in NI-1 cells. Masitinib, toceranib, and oclacitinib did not exert inhibitory effects on histamine release. In contrast, PKC412 and ibrutinib suppressed histamine release in NI-1 cells (10 µM each). Together, our data show that several HR1 antagonists exert anti-proliferative and apoptosis-inducing effects in canine neoplastic MC. At higher concentrations, some HR1 blockers also counteract histamine release. The clinical relevance of this observation remains to be determined.

P 154 Recombinant E. coli Nissle 1917 expressing allergen chimer suppresses allergic poly-sensitization without permanent colonization when applied intranasally


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Poly-sensitization is becoming an increasing health issue in Western countries, since poly-sensitized individuals are difficult to treat by conventional therapeutic measures. Recently, probiotic bacteria such as non-pathogenic Escherichia coli Nissle 1917 are increasingly used to treat allergies and other diseases. It is therefore of interest to test if recombinant
probiotics expressing specific allergens may represent the proper tool for prevention of poly-sensitization. We previously showed that colonization with recombinant lactic acid bacteria expressing the allergen Bet v 1 successfully prevents Bet v 1 specific allergic responses. Now in this study we demonstrate the effect of mucosal application of recombinant E. coli Nissle expressing an allergic chimer (rEN-chi-m) of birch and grass pollen allergens in adult mice. Mice were pre-treated either orally or intranasally with rEN-chi-m before allergic poly-sensitization. Particularly after intranasal treatment with rEN-chi-m the mice showed a significant reduction of lung inflammation (eosinophils, IL-5, IL-13 in BAL) along with reduction in allergen specific IgE and Th2 cytokines in spleen and lung cell cultures. In contrast, allergen specific IgA in lungs and gut and serum IgG2a were significantly increased in these mice. Using in vivo imaging techniques we further demonstrated that intranasally applied E. coli Nissle were detected in nose, lungs and gut but no longer than 2 days after application, indicating that the bacteria are not colonizing at mucosal surfaces. In confocal imaging system we proved that the bacteria are taken up by epithelial cells and dendritic cells and both cells seems to be responsible for initiation of regulatory immune responses. In conclusion we demonstrate that intranasal application of rEN expressing allergen chimers can be a safe and effective strategy to prevent allergic poly-sensitization.

**P 155 Assessing basophil activation pathways via flow cytometry in the context of food allergy**


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Food allergy occurs at a prevalence of up to 5% in children. Some food allergies have a high likelihood of tolerance development up to the age of five whereas others do not. Tolerance development usually precedes loss of specific IgE or skin prick test reactivity. A considerable number of children are asymptomatic sensitized. Consequently, these methods are not appropriate to monitor tolerance development or define sensitized, non-allergic individuals. Basophil Activation Test (BAT), using CD63 as a readout parameter, has been described to be superior in assessing tolerance in peanut-sensitized, tolerant individuals as compared to other tests. In addition to CD63 assessment, changes of phosphorylation of signaling pathways may precede tolerance development or reflect clinical reactivity in sensitized individuals. Basophils from food-sensitized children sampled before an Oral Food Challenge (OFC) were evaluated for CD63 expression as well as for phosphorylation of MAPK, and ALK upon allergen-specific FcεRI crosslinking using flow cytometry. Basophil Activation Test showed a sensitivity of 89.3% and a specificity of 88.9% for nuts,
milk and egg. Six percent of the patients were so called non-responder and excluded from analysis. Kinetics of phosphorylation pathways demonstrate that the shift in the phosphorylation varies depending on the sensitization status and clinical response. Sensitivity and specificity of the BAT suggest that it may be helpful to provide a more accurate risk assessment before an OFC in equivocal patients. Phosphorylation of ERK1/2 and p38 MAPK and AKT takes place upon allergen specific activation and might depend on the sensitization profile and clinical history. Understanding the effect of the phosphorylation of these key intracellular proteins that contribute to elicit allergic symptoms may allow a precise delineation of events taking place during desensitization and tolerance development.

**P 156 INFLUENCE OF CONFORMATIONAL AND LINEAR IgE EPITOPEs ON ARA H 2-SPECIFIC IgE-BINdING**


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Ara h 2 is the most important peanut allergen. Little is known about the role of conformational versus linear IgE epitopes of this molecule. We aimed to define the patient-specific contribution of conformational and linear epitopes to the IgE-binding of Ara h 2, the most potent peanut allergen, for the use in vaccine design. An Ara h 2 mutant, mtAra h 2, lacking surface-exposed loops that contain most linear IgE epitopes, and the wild-type protein (wt) were expressed in the baculovirus insect cell system. Aliquots of purified wt, mt and natural (n) Ara h 2 were reduced with dithiothreitol and alkylated with iodoacetamide. Physicochemical characteristics were determined by mass spectrometry, N-terminal sequencing and CD spectroscopy. IgE-binding was tested by ELISA using sera of ten peanut allergic patients. Mt, wtAra h 2 and nAra h 2 displayed correct sizes, N-termini and the characteristic alpha-helical structure. Reduction and alkylation of the proteins was confirmed. In direct and inhibition ELISAs, allergic patients' sera revealed up to 70% (p<0.05) reduced IgE-binding to the mutant compared to nAra h 2 and a 40% lower IgE-binding compared to wtAra h 2 (p>0.05). Reduced wtAra h 2 revealed patient-specific decreases in IgE-binding compared to native wtAra h 2 (p<0.01) and nAra h 2 (p<0.001). Relative amounts of IgE-binding to reduced wtAra h 2 (mostly linear IgE-binding epitopes) and the native mtAra h 2 (mostly conformational IgE-binding epitopes) showed a high extent of patient-dependent variability. The reduced and alkylated mutant showed almost no IgE-binding at all. These results indicate that both conformational and linear IgE-binding
Epitopes are important for Ara h 2 specific IgE-binding. Relative contributions of linear and conformational epitopes to Ara h 2 IgE-binding are patient-specific.

**P 157 Altered Peptide Ligands derived from the major mugwort pollen allergen Art v 1, reduce Th2 cytokines upon in vitro re-stimulation of humanized allergen-specific T cells.**


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Allergic sensitization and late phase reactions have been linked to altered CD4+ T cell function. CD4+ T cell activation requires T cell receptor-dependent recognition of immunogenic peptides bound and presented by MHC class II molecules. Previous reports have shown that T cell function can be modulated by altering the sequence of the peptides recognized by them. We sought to investigate the contribution of TCR signal strength to the differentiation of allergen-specific CD4+ T cells and use a recently developed Art v 1 TCR/HLA-DR1 double transgenic humanized murine system to define possible altered peptide ligands [APL] of the major mugwort pollen allergen, Art v 123-36. We here used HLA-DR1+ K562 cells to establish a fast, flow cytometry-based competitive binding assay for the characterization of the relative binding capabilities of 25 different APL.

In the competitive assays six peptides with increased (IC50 competitor/wt ratio>1.50), nine with similar (ratio 0.50-1.50), and ten with decreased (ratio< 0.5) binding capabilities were identified. Functional evaluation in T cell proliferation and cytokine secretion assays identified 3 partial agonists (APL7, 9 and 11) inducing reductions of up to 60% in the levels of all or some of the Th2 cytokines IL-4, IL-5 and IL-13 without affecting the levels of IFN-γ or IL-2, when used on the re-stimulation of allergen specific T cells previously primed with wildtype peptide Art v 123-36. Furthermore, 4 APL were identified as bona fide antagonists (APL2, 16, 17 and 18), being able to reduce wildtype peptide-induced NF-kB activity up to 50%.

In summary, we have identified APLs effectively modulating T cell response on a relevant humanized murine allergy model.

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**P 158 IgE sensitisation to bacterial antigens in different forms of allergic disease.**

P 159 Peanut allergens Ara h 1 and Ara h 2 and peanut lipids impact on barrier function of human airway epithelial cells.

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Introduction: The airway epithelium forms a barrier to protect the body from inhaled harmful substances. Allergic reactions to peanut often occur after inhalation of airborne peanut allergens. Moreover, as peanuts contain about 50% lipids these might be involved in the sensitization process. We aimed to find out whether and how the major peanut allergens Ara h 1 and Ara h 2 together with peanut lipids affect the airway epithelial barrier.

Methods: The human bronchial epithelial cell line 16HBE14o- was cultured in a transwell system and treated with peanut allergens and/or lipids when transepithelial resistance reached (TER) >700 Ω cm². Barrier integrity was evaluated measuring the transepithelial passage of fluorescein isothiocyanate–dextran 4 kDa (FD4), 10 kDa (FD10), and 70 kDa (FD70) through the cell monolayer, co-administered with allergens and/or lipids. Polarized 16HBE14o cells were exposed to fluorescently-labelled peanut allergens for 1 hour and analysed by confocal microscopy. Results: Ara h 2 reduced TER by 45% in 16HBE14o cells after 6 hours (p

P 160 Brain-wide histopathological investigation of LGI1-encephalitis in cats


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Voltage-gated potassium channel complex (VGKC) encephalitis is an autoimmune limbic encephalitis (LE) with antibodies targeting various associated proteins, one of them being leucine-rich glioma inactivated 1 (LGI1). This type of encephalitis causes severe symptoms but patients respond well to immunotherapies and, therefore, histopathological investigations are difficult. However, a natural occurring animal model of LGI1-LE in cats has recently been shown to have high similarities with the human disease. Therefore, conclusions from the feline disease can be of high significance for human LGI1-LE. Consequently, we performed a brain-wide histopathological investigation in feline LGI-LE and could show that not only the hippocampus is affected but also the amygdala and the piriform lobe (n=15). Magnetic resonance images also showed a volume increase in the respective areas. Moreover, brain-wide inflammatory infiltrates were found. In the hippocampus an upregulation of glutamate receptor 1 subunit (GluR1) was found in the molecular layer of the dentate gyrus. We were able to show new aspects of LGI-LE and increase the knowledge about feline LGI1-LE and thereby contributing to a better understanding of the human pathology.

P 161 Depression in acute intermittent porphyria: pathogenic principles and neurobiological mechanisms


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Acute intermittent porphyria [AIP], an autosomal dominant inborn error of heme biosynthesis, is due to the half-normal activity of hydroxymethylbilane synthase [HMBS]. The disease is characterized by life-threatening acute neurovisceral attacks that are precipitated by various factors that induce the hepatic expression of aminolevulinic acid synthase 1 (ALAS1) resulting in the accumulation of the neurotoxic porphyrin precursors, aminolevulinic acid [ALA] and porphobilinogen [PBG]. While neuropsychiatric conditions such as depression and anxiety are reported in up to 50% of AIP patients, the underlying pathogenic mechanisms remain unclear. Here we aim to investigate the emotional disturbances in this disease and to elucidate the underlying neurobiological mechanisms using a severely affected AIP knock-in [KI] mouse model. These mice are homozygous for the human HMBS mutation, c.500 G>T (p.Arg167Glu), and have constitutively elevated ALA and PBG levels. Initial efforts will be directed towards analyzing depression-like and anxiety-related behavior in the KI mice.
using We will also evaluate the rate of proliferation and fate of newborn cells in the hippocampus of KI and wildtype [WT] mice as these characteristics have been strongly linked to depression and response to antidepressant treatment. Further, we will monitor neural function electrophysiologically in the hippocampus of the KI and WT mice and neuropathohistologically evaluate KI and WT brains.

In the final step we seek to unravel the molecular mechanism(s) involved in the behavioral and neurogenic deficits in AIP focusing on evaluating Gamma-aminobutyric acid [GABA]A receptor density, subtype expression and signaling as ALA has been shown to specifically interact with elements of the GABAergic neurotransmitter system, which is highly implicated in the pathophysiology of mood disorders.

**P 162 STAT3-dependent regulation of the serotonin transporter and its relevance for major depressive disorder**


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Major depressive disorder (MDD) is the most prevalent neuropsychiatric illness and one of the leading causes of disability worldwide, however a substantial proportion of patients show little or no response to current treatments. The serotonergic system, and the serotonin transporter [SERT] in particular, are widely accepted to play a fundamental role in the aetiology of MDD. In recent years, evidence for an interaction of this system with inflammatory processes in the brain has also been put forward, potentially contributing to pathophysiological mechanisms underlying MDD.

In this context, it has been shown that the cytokine interleukin 6 [IL6] induces depression-like behaviour in mice, and that it signals via STAT3 [signal transducer and activator of transcription 3] to regulate SERT expression and function. Further, systemically blocking STAT3 reduces depression-like behaviour in mice and modifies SERT expression. Here, the overarching aim is to investigate the regulation of SERT by STAT3 and its relevance in the pathophysiology of MDD at the molecular, cellular and systemic levels. In a first approach a conditional STAT3 knockout mouse, lacking STAT3 specifically in serotonergic neurons, was generated. This knockout mouse showed a reduction in depression-like behaviour compared to controls, while exhibiting otherwise normal behaviour. This suggests that the activity of STAT3 in serotonergic neurons may mediate the manifestation of depression-like behaviour in mice upon activation of the IL6 pathway. Further experiments using this mouse model will examine whether the STAT3 deletion confers resilience to chronic stress, and will probe expression levels of SERT and other relevant STAT3 targets.

Complementary experimental approaches will further examine the IL6-STAT3 pathway and its significance for MDD, expanding the current understanding of this disorder, and may provide valuable evidence for the identification of novel molecular drug targets.
**P 163 Characterizing agrin-dependent muscle specific kinase endocytosis**

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The neuromuscular junction [NMJ] connects a motor neuron and a muscle fiber. The receptor tyrosine kinase muscle specific kinase [MuSK] is the key signaling molecule at the NMJ required for the formation of a mature and functional NMJ. In mice lacking MuSK, motor neurons will overgrow the muscle and no postsynaptic specializations such as acetylcholine receptor [AChR] clusters are found. Activation of MuSK requires the motor neuron-derived proteoglycan agrin and the low density lipoprotein receptor Lrp4 localized at the muscle membrane. Agrin binds to Lrp4, which promotes the interaction with MuSK and the subsequent activation of MuSK. In general, receptor tyrosine kinases [RTK] are internalized rapidly upon ligand binding and routed intracellularly either for degradation or recycling. In addition, signaling from endosomes can activate specific pathways. Therefore, these processes influence signaling of RTKs.

The aim of the project is to characterize agrin-dependent MuSK endocytosis and its role in NMJ formation and maintenance. Using biochemical analysis of MuSK localization upon activation, pharmacological inhibition and/or RNAi-mediated knockdown of different components of endocytic pathways will allow to define the internalization properties of MuSK. Preliminary experiments suggest that pharmacological inhibition of endocytosis leads to an accumulation of phosphorylated MuSK upon agrin treatment. Microscopic studies on MuSK endocytosis will complement the obtained data. We have generated muscle cells expressing endogenously tagged MuSK via CRISPR/Cas9. These cells allow to track MuSK endocytosis and facilitate colocalization with distinctive vesicle markers. Additionally, the analysis of AChR clustering upon agrin stimulation and inhibition of endocytosis serves as a readout to determine the impact of MuSK internalization on its signaling. We expect by this to obtain new insights into the mechanisms regulating the development and the maintenance of the NMJ.

**P 164 A Novel Extra-Dimensional Attentional Set-Shifting Task for Rodents to Explore Prefrontal Networks**

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Attention aids adaptive usage of the limited capacity of neural processing systems. An innate part of information processing is the attentional set, which facilitates selection of
the relevant, and inhibits processing of distracting information. With assessing the
capability of attentional set-shifting, it is possible to measure cognitive flexibility and
executive functions. The most widely used neuropsychological task for the evaluation of
these functions in humans is the Wisconsin Card Sorting Test, which requires the subject to
alter the response strategy and use previously irrelevant information to solve a new set of
problems. The test has proven clinical relevance, as poor performance has been reported
for multiple neuropsychiatric conditions. However, similar tasks used for rodent models are
limited because of their manual-based testing procedures. In our novel task water-
deprived and head-fixed C57BL/6 mice were placed in a virtual environment and exposed to
a decision-making task to retrieve small water reward. The animals learnt to discriminate
two visual perceptual dimensions and they successfully switched their attention between
them. In addition, silicon probe recordings were performed in the medial prefrontal cortex
(mPFC) to address the underlying network mechanisms. We show that neuronal activity in
the mPFC is modulated by different temporally structured task episodes, as well as reward
delivery or reward omission. Furthermore, the rule switch induces changes in neuronal
activity. In conclusion, we demonstrate that our extra-dimensional set shift task for head-
fixed mice is an effective tool to study the molecular and cellular mechanisms within
neuronal networks underlying executive functions. The understanding of the role of
prefrontal network operations in cognitive flexibility is invaluable for understanding
numerous neuropsychiatric diseases, such as schizophrenia and depression.

**P 165 B-cells and CD8-positive tissue resident memory T-cells dominate the
inflammatory reaction in MS lesions**


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Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous
system. Active neurodegeneration in the MS brain is associated with the presence of
inflammatory infiltrates, consisting of T- and B-lymphocytes. In our study, we analyzed in
detail the phenotype and activation state of T- and B-lymphocytes in MS brain in
comparison to a large spectrum of controls with other inflammatory or neurodegenerative
diseases.

Studies were performed on archival formaldehyde-fixed and paraffin-embedded (FFPE)
autopsy material. It included 22 cases of MS comprising all the spectrum of the disease. As controls, cases of
acute disseminated encephalomyelitis (ADEM), Rasmussen’s encephalitis, viral
encephalitis diseases, Stroke, Alzheimer and age-matched controls without any detectable
brain disease were included. Immunohistochemistry was performed on paraffin sections
using markers for T-cells (CD3, CD4 and CD8) and for B-cells (CD20). To define their
proliferation state, T and B-cells were double stained with the proliferation marker PCNA.
For the activation state, the markers NFAT, CCR5, PD-1, CD103, CD69 and TCF7 were used.
We observed that the high number of B-cells distinguish MS from other inflammatory and non-inflammatory brain diseases. Moreover, the number of infiltrated T and B-cells in the MS lesions is related to activity stage. Proliferation was highly observed in ADEM and viral encephalitis diseases. Within MS, it was mainly seen in CD8+T-cells and B-cells in acute and relapsing remitting MS. We further characterized the phenotype of the CD8+ T-cells within the MS lesions and found that, depending upon lesions stage, the cells were either activated as effector T-cells or tissue resident memory T-cells.

We concluded that B cells and tissue resident memory T-cells are the most abundant subset present in MS lesions, and therefore new anti-inflammatory treatments should focus specifically on these cell types.

**P 166** Pre-limbic and infra-limbic cortex activity during a rule-switching task

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Adopting a flexible behavior is a crucial ability for animals to survive in a constantly changing environment. In this context, the medial prefrontal cortex (mPFC) is of particular interest, as it is involved in essential executive functions. However, anatomical and functional differences within the mPFC suggest that this brain region is not homogenous, but rather consists of distinct subdivisions. Notably, in rodents, the pre-limbic (PL) part of the mPFC is involved in the learning and expression of goal-directed behaviors, while the infra-limbic (IL) is important for maintaining the extinction of previously learned fears or motoric habits. The neuronal network dynamics and synchronicity of IL and PL during cognitive flexibility is yet to be explored.

Experimentally, cognitive flexibility can be assessed by a rule-switching task, during which animals have to stop following one behavioral rule and quickly learn another to maximise their amount of reward. We hypothesize that IL is involved in inhibiting the previously learned rule, while the PL is important for the learning and execution of the new goal-directed behavior.

To verify this hypothesis, we trained rats to perform a prefrontal-dependent task on a Y-maze, followed by an extra-dimensional rule-switching. The activity of multiple single units in the IL and PL divisions of the prefrontal cortex was simultaneously monitored using chronically-implanted tetrodes or silicon probes. We determined the resulting neural activity of putative pyramidal and interneurons, before and after the rule-switch and across different time episodes. Prefrontal neural activity seems to be correlated with multiple dimensions of the behavioral task.

**P 167** Rosai-Dorfman Disease: Case report of an isolated intracranial manifestation of this rare entity
Background: Rosai-Dorfman disease is a rare, benign, idiopathic histiocytosis. The characteristic morphology of the disease describes a proliferation of histiocytes of obscure etiopathogenesis with emperipolesis. Central nervous system involvement is exceedingly rare and is described in only 5% of the cases, among which 75% are located intracranial and 25% intraspinal.

Case Report: A 84-year-old woman complains initially about increased diuresis and is admitted to the neurological care unit due to sudden double vision. Furthermore, herpes zoster is reported in the area II and III innerved by the trigeminal nerve and treated with Zovirax antivirally. The result of the puncture of cerebrospinal fluid is inconspicuous. Neuroradiologically, a space occupying mass in the sellar region can be shown, which also infiltrates the sinus cavernosus and the optic chiasm. Laboratory investigations of blood and urine reveal high levels of prolactin (53ng/ml). A pituitary adenoma is first considered, however, neurosurgical biopsy confirms the diagnosis of Rosai-Dorfman disease. According to rare descriptions in literature a treatment with cortisone is initiated, at the same time polyuria is treated with minirin.

Result: In the following presentation a rare solitary manifestations of Rosai-Dorfman histiocytosis in the CNS is the true differential diagnosis of a pituitary gland adenoma. Based on an own literature research RDD with intracranial localisation or combined with solitary intracranial presentations, age distribution, as well as gender specificity will be investigated and presented in the following presentation.

Conclusion: Diagnoses of common diseases like adenomas of the pituitary gland should only be diagnosed if rare differential diagnoses are excluded. Rare clinical pictures state important differentially diagnosed substrates in clarifying lesions in pathognomonic intracranial regions.
located in the gingival part of any tooth. That will contribute to the objectification of differentiated diagnostic and therapeutic approaches in the dental treatment of various clinical variants of this defect localization.

The aim of the study - to develop the anatomical and functional classification for differentiated estimation of hard tissue damages located in the gingival part of any tooth, as the basis for the application of differential diagnostic and therapeutic approaches to the dental treatment of hard tissue defects disposed in the gingival part of any tooth.

Materials and methods of investigation: has been conducted the examination of 48 patients with hard tissue defects located in the gingival part of any tooth. To assess the magnitude of gingival destruction were used the periodontal probe and X-ray examination.

Results. The result of the conducted research is the classification of defects hard tissue located in the gingival part of any tooth using exponent power. The value of this indicator is equal to an integer number expressed in millimeters of distance from the epithelial attachment to the cavity’s bottom of defect.

Conclusions. The proposed classification fills an obvious gap in academic representations about hard tissue defects located in the gingival part of any tooth. Also it offers the prospect of consensus on differentiated diagnostic and therapeutic approaches in different clinical variants of location. This classification builds methodological "bridge of continuity" between therapeutic and prosthetic dentistry in the field of treatment gingival defects of dental hard tissues.

P 169 Clinical relevance of troponin measurement at the emergency department


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Background: Cardiac troponin is the preferred cardiac biomarker to detect cardiomyocyte necrosis and complements clinical assessment and 12-lead ECG in diagnosis of acute coronary syndrome (ACS). High sensitivity troponin (hs-TnT) assays permit the detection of very low levels of troponin, resulting in improved sensitivity and thus early detection. Increased sensitivity however comes at the cost of decreased specificity.

Aim of the study was to investigate causes and consequences of increased hs-TnT levels at the emergency department.

Methods: In a prospective, observational study we included all patients treated at the emergency department during a one month-period who received at least one hs-TnT measurement. Diagnostic and therapeutic procedures during hospital stay were documented. According to final diagnosis at hospital discharge patients were classified as: TnT negatives with non cardiac chest pain (nCCP TnT-), TnT positives with ACS (ACS TnT+), TnT positives without ACS (non ACS TnT+).

Results: Overall 1067 patients (591 (55%) male; age 54 (19) years) were enrolled, of which 719 (67%) were classified as nCCP TnT-, 60 (3%) ACS TnT+, 288 (27%) non ACS TnT+. In 84 hs-TnT positive patients (24% of total positive) coronary angiography and in 5 (1.4%)
coronary computed tomography was performed, resulting in 52 coronary interventions, 3 acute coronary bypass graft operations and 20 cases in which coronary artery disease was diagnosed, but no intervention was performed. Leading causes for hs-TNT elevation in non ACS TnT+ patients were hypertensive emergencies (37%), tachyarrythmia (32%), heart failure (31%) and renal dysfunction (27%).

Conclusion: Out of 35 hs-TnT tested patients per day, only one patient was diagnosed with MCI. Clinicians seem to be aware of the numerous non ACS-causes of elevated hsTnt, as only 24% of these patients received invasive coronary diagnosis.

P 170 VIABILITY AND VIRULENCE POTENTIAL OF STARVING LEGIONELLA BACTERIA


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Under adverse conditions legionellae can get non-culturable on standard media. They may be resuscitated by intracellular growth in host-organisms. Whether this viable but non culturable [VBNC] form of legionellae is of health relevance is not known. To better understand VBNC legionellae, we tried to induce, detect and characterize the VBNC state of six Legionella strains. At a concentration of 108 cells/mL they were starved in deionised, sterilised water at 45°C and monitored with respect to culturability, viability and virulence surface factors twice a week for 35 days and every 3 months for 1 year. L. pneumophila serogroup [SG] 1 cells became VBNC latest after 2 weeks, the SG 6 strain after 4 and the L. micdadei strain after 8 weeks of incubation. We could observe a slight reduction in viable cell numbers at the switch to VBNC state of up to 0.7 log numbers concerning esterase active cells and up to 1 log concerning membrane integrity. Among these populations different sub-populations dynamically developed as observed by flow cytometry like cells with high and low esterase activity. However, a certain part of the population showed stable signs of viability throughout the whole experiment. On the contrary, the incorporation rate of leucine was reduced by at least one order of magnitude already after one week of starvation and further decreased below detection limit after 3 weeks for all strains. Nevertheless, most of the membrane bound LPS- and protein structures were expressed at a high level throughout the whole experiment.

All these data indicate that starved Legionella populations enter the VBNC state and still express important virulence factors. Furthermore, a part of these VBNC cells remain viable at a low level of activity and don’t die off, as there are still dynamic changes occurring in viability parameters after longer periods of starvation. It thus cannot be excluded that starved legionellae are of relevance to human health.
P 171 Characterization of warm red blood cell autoantibodies

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Warm Autoimmune Hemolytic Anemia (wAIHA) is characterized by IgG autoantibodies against the patient’s own red blood cells (RBC), leading to complement activation, increased destruction of RBC and consequently anemia. For blood group serologists, IgG autoantibodies represent a difficult task particularly in terms of pretransfusion testing and selection of suitable blood products. RBC autoantibodies may lead to a positive direct antiglobulin test (DAT) and frequently to a positive indirect antiglobulin test (IAT), which impedes the detection of irregular blood group alloantibodies of clinical significance. The majority of IgG autoantibodies reacts with all RBC of common blood group phenotypes, therefore showing panreactive behavior in serologic tests. The second largest group appears to be directed against the Rh antigens (D, C, c, E, e) but it remains controversial if they are true or mimicking specificities. It is suggested that the target of IgG autoantibodies are high-frequency antigens on basic membrane proteins of the Rh complex (RhD, RhCcEe, Glycophorin A, Band-3, RhAG, LW and CD47) which are naturally occurring on all RBC. Flow cytometric protein expression studies are planned on different erythroid cells like RBC with selected phenotypes, erythroid progenitor cells and the erythroleukemia cell line K-562, which is known to lack Band-3 and the Rh complex. Genome editing by CRISPR/Cas9 will be used to obtain knockdowns of several membrane proteins in erythroid progenitors. Cells with complementary protein expression intensities will be used to test samples containing IgG autoantibodies. The results of this study may contribute to a better understanding of wAIHA and improve pretransfusion testing and blood product selection for affected patients. Moreover, the risk of misinterpretation of mimicking autoantibodies as dangerous allospecificities may be reduced. Such differentiation is of clinical relevance for safe transfusion.

P 172 Complement activation in relation to donor specific antibodies after kidney transplantation

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Introduction: Antibody mediated rejection (AMR) is the major immunological cause of kidney transplant failure also in the paediatric population. According to current knowledge,
late AMR is classically caused by the development of donor specific antibodies (DSA) and the complement system plays a critical role in its pathomechanism. The aim of this work was to determine the levels of various complement activation products in kidney transplant recipients.

Methods: 106 adult kidney transplanted patients who had detectable DSA after transplantation (DSA+, 189 days to 29 years post-transplantation) were involved in the study. 106 DSA-negative patients were matched as controls using 1:1 propensity score matching. Two patients were excluded due to poor sample quality. The levels of complement activation products (C3a, SC5b-9, C4d and Bb) were measured by enzyme-linked immunosorbent assay (ELISA) using EDTA plasma samples.

Results: Activation product levels were compared between the DSA-positive and negative groups. Mean C3a concentration in the plasma of DSA+ patients was 97.3 ng/ml (SD 38.9), compared with 93.86 ng/ml (SD 41.38) in the DSA- group (P>0.05). Mean SC5b-9 concentration in the DSA+ patients was 223.3 ng/ml (SD 86.8), compared with 222.8 ng/ml (SD 83.5) in the DSA- group (P > 0.05). Mean level of C4d in the DSA+/− groups was 2.7 µg/ml (SD 1.19) compared with 2.93 µg/ml (SD 1.65) (P > 0.05). Mean Bb levels of the DSA+/− patients found to be 1.3/1.15 µg/ml (SD 0.66/0.38) (P > 0.05).

Conclusion: Levels of complement activation products in kidney transplant recipients were determined. No significant difference in plasma levels of activation markers for C3 (C3a), terminal pathway (SC5b-9), classical pathway (C4d) and alternative pathway (Bb), was observed in DSA positive and negative patients. Further analysis is necessary to identify association of complement activation with the histological signs of AMR.

**P 173 Lithium-related cytoprotection in experimental peritoneal dialysis**


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Exposure to peritoneal dialysis fluids (PDF) leads to injury of mesothelial cells associated with activation of glycogen synthase kinase-3β (GSK-3β). Lithium, a well-known GSK-3β inhibitor, applied as lithium chloride (LiCl) has been shown to improve the survival of immortalized mesothelial cells. The lithium-dependent cytoprotection, however, might be mediated by various mechanisms and their identification is desirable for clinical application of LiCl as cytoprotective additive to PDF. For this purpose a transcriptome- and proteome-wide study on primary human peritoneal mesothelial cells (HPMC) exposed to PDF without or with LiCl was performed. The transcriptome was investigated with gene expression microarrays (Affymetrix) and the proteome was analyzed using 2D gel-based approach. The pathways showing the PDF/LiCl dependence were characterized using the
PANTHER database. In an in-vivo model of PD, sections of the parietal peritoneum of C57BL/6 mice and effluent cells were characterized after treatment with PDF without or with LiCl. Cell injury triggered by PDF was associated with differential expression of transcripts of 601 genes as well as 17 protein spots. Pathway analysis showed an overrepresentation of 6 pathways related to stress, cytokine signaling and angiogenesis. PDF supplemented with LiCl caused lower cell injury and altered the expression of transcripts of 1003 genes and 24 protein spots. 62 transcripts showed an abolishment of the PDF effects. These genes are regarded as markers of LiCl-mediated cytoprotection. In-vivo LiCl addition leads to a decrease of PDF-induced thickening of the peritoneal membrane and increased Treg/IL-17 ratio of the effluent cells. The observed cytoprotective potential of LiCl addition, combined with the modulation of relevant mechanisms of cellular stress responses, fibrosis and inflammation suggests protective effects of this intervention. The molecular mechanisms of the improved outcome will be analyzed in ongoing studies.

**P 174 Multiple dosing of anti-C1s antibody TNT009 – Effect on HLA antibody-triggered complement activation in healthy volunteers and kidney transplant recipients with antibody-mediated rejection**


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**P 175 Off-Pump HeartWare HVAD Left Ventricular Assist Device Implantation**


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Objectives: Left ventricular assist devices (LVAD) are routinely implanted with cardiopulmonary bypass support. Patients with terminal heart failure are typically multimorbid and might therefore profit from a less invasive implant technique with avoidance of cardiopulmonary bypass. We report our experience with off-pump Heartware HVAD LVAD implantation.

Methods: We retrospectively reviewed data of 19 patients who underwent off-pump Heartware HVAD implantation from November 2012 to June 2016. Study endpoints were
patient demographics, operative outcome, long-term survival and incidence of adverse events.

Results: Mean patient age was 61±9yrs., 89.5% were male and underlying disease was of ischemic origin in 47.4%. Intermacs Levels at the time of HVAD Implantation were: Level I: 15.8%, Level II: 5.3%, Level III: 42.1%, Level 4: 36.8%. Implant strategies were Bridge to Transplantation (5.3%), Destination Therapy (31.6%) or Bridge to Candidacy (63.2%), respectively. Mean duration of support was 478±269 days (range 31–1105). The off-pump procedure was feasible in all patients with no on-pump conversions. We observed no perioperative stroke and only two surgical bleedings (15.4%). Both of which required surgical revision due to hemothorax and resulted in complete recovery. 30-day and inhospital mortality rates were 0% and 5.3%, respectively. One-year survival was 84.2%.

Conclusion: Our preliminary experience shows that off-pump Heartware HVAD implantation is a feasible and safe alternative to the standard implantation technique that is especially appealing in multimorbid high-risk patients.

P 176 Reflex to learning according to new method of reflective educational on anesthesia students: a qualitative study

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Background: In world’s advanced educational systems, continuously revise their objectives and activitiesto find new effective education method. Therefore this study was performed with aim of survey of Reflex to learning according to educational reflective method on anesthesia students.

Method: This Quasi-experimental qualitative study was conducted with 21 students of anesthesia at Sabzevar Medical University in Iran. In this study, we describe a process forembedding reflective skills into a semester program for anesthesia students. By asking students to explore reflection through complete a reflective journal after each session and a method of self-discovery supported by peer discussion in the beginning of each session. Methods of data analysis, was conventional content analysis.

Findings: The results showed that the average age of students who performed reflective journal was 19.62 ± 1.36. Aspect of gender, 2 Student (12.5%) male and 14 (87.5%) were female. In the last session of course, the students optionally made response about sentence of "I regularly or experience in what I learned in university, I responded ". About 17 (81%) subjects were agree and 4 (19%) strongly agreed. Also reflect to "I know the method of teaching reflex" included 2 (9.5%) strongly agree, 16 (76.2%) agree, 2 (9.5%) neutral and 1 (4.8%) disagree. we found that students were likely to recognize and value reflection as learning tool (a concept we term "contemplation for learning".

Conclusion: This Study showed that students who take the time to reflect on their daily experiences provide enhanced deep learning, have better actions in practice, which in
P 177 Relationship between Serum Levels of Total IgE Antibody and Percentage of Eosinophil Cells in Asthmatics pregnant women

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Background: Asthma is a hereditary disease associated with IgE-mediated reaction. Various factors influence perinatal IgE production (percentage of eosinophil cells). However, asthma development remains unclear. Thus, this study’s aim was to determine the relationship between serum IgE antibody level and the percentage of eosinophil in pregnant asthmatics.

Methods: This prospective cross-sectional cohort study was performed between August 2014 and April 2015. Women with a history of asthma referred to Mobini Hospital for prenatal care were enrolled. The blood samples of women and their neonates were drawn, after consent, and stored for measuring IgE and RAST. Spirometry with Spirolab S/N 000072 was performed in all asthmatics women. Cohorts were divided into two categories, according to IgE levels and the percentage of eosinophil. Descriptive and comparative statistical analyses were performed. Results were analyzed using SPSS version 20.

Result: The prevalence of asthma during pregnancy was 2.1% (34). Of 35.3% (12) of asthmatic patients were = 35 years of age. A significant relationship was found between maternal IgE level and age, p = 0.05. However, there was no significant correlation between maternal IgE and neonatal eosinophilia, p= 1.0 nor between neonatal IgE and neonatal eosinophilia, p =0.48 nor between maternal and neonatal eosinophilia, p= 1.0. As well, there was no significant relationship between maternal FEV1 and neonatal eosinophilia p=1.0 nor between asthma severity and maternal or neonatal IgE levels, p =0.94, p= 0.25, respectively. Circa 29% of subjects had IgE levels above normal.

Conclusion: Elevated eosinophil count was present in a few patients. There was not related between spirometry index and IgE levels, probably related to the small cohort. Further studies are needed, using a larger study group, to clarify the relationship between these markers asthma

Key words: IgE, eosinophil, asthma, pregnancy, FEV1

P 178 Association of Iron, Iodine, Zinc, Selenium and Manganese Status of Pregnant Women from Pakistan with Pregnancy Outcomes

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Pregnancy is a crucial stage for the mother and the fetus, because the requirement for the nutrients – micro and macro – increases rapidly for fulfilling the newly emerged requirements of the body, which are necessary for the normal function of the body. However, the micronutrient deficiency during this phase may lead to the worst consequences like maternal mortality, infant mortality, low birth weight, and the risk of premature infants. In this regard, various studies link the occurrence of the higher rate of maternal and neonatal deaths with the pregnancy related complications across especially in the low-income countries, which necessitate conducting the further research studies for the elucidation of the problem properly. The present study, therefore, will focus on the relationship between the micronutrient deficiencies and pregnancy related complications in Pakistan whilst choosing a blood sample of 80 pregnant women and 40 non-pregnant women of the same age for making a comparison of micronutrient status between the both, in addition to analysing the pregnancy outcomes and birth defects.

Self and interpersonal functioning of individuals at ultra-high risk for psychosis and with first-episode psychosis

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The continuum of stages of psychotic disorders is ranging from the ultra-high risk (UHR) to psychotic disorders via the first episode of psychosis (FEP) to recovery or schizophrenia with multiple episodes. It was found that the assessment of symptoms in the context of the patient’s sense of self and the quality of her/his object (interpersonal) relations is highly valuable for early stages in psychosis to detect the onset of the loss of personality structure and offer intervention strategies.

The aim of this research project is to empirically investigate the level of personality functioning, identity integration, and quality of object relations in patients at UHR and with FEP. Based on first findings in this field, it was hypothesized that there is a loss of personality structure during development of psychosis.

Using a cross-sectional design, 8 patients at UHR, 3 patients with FEP, 7 patients with borderline personality disorder (BPD) and 15 healthy individuals as a control group will be recruited. After identifying UHR people via the Comprehensive Assessment of At Risk Mental State (CAARMS) and measuring the strength of psychotic symptoms of FEP people
with the Positive and Negative Syndrome Scale (PANSS) DSM diagnosis were established by the Structured Clinical Interview for DSM Disorders (SCID I and II). All participants were assessed by the structured interview of personality structure (STIPO), level of personality functioning (LFPS), and self-disturbances (Examination of Anomalous Self-Experience, EASE). Self-rating instruments were applied for measurement of selected other psychopathological domains (childhood trauma, temperament).

The results of these investigations allow for a better understanding of self and interpersonal functioning in early stages of psychosis.

P 179 L-Mimosine and Hypoxia enhance Angiopoietin-like-4 Expression involving hypoxia-inducible factor-1 signaling: Insights from Monolayer and Spheroid Cultures of Human Dental Pulp-derived Cells and Tooth Slice Cultures

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Hypoxia-based strategies are a novel approach to stimulate angiogenesis and represent a potential new tool for regenerative endodontics. Angiopoietin-like-4 (Angptl-4) is a signaling factor that plays an important role in angiogenesis and osteoclastogenesis. Its role in the dental pulp is unknown. We hypothesized that hypoxic conditions can increase Angptl-4 production in the dental pulp and for this assessed if the hypoxia mimetic agent L-Mimosine (L-MIM) and hypoxia can stimulate the production of Angptl-4 in the dental pulp. Monolayer and spheroid cultures of human dental pulp-derived cells (DPC) and tooth slice cultures were treated with L-MIM or hypoxia. The production of Angptl-4 was assessed at mRNA and protein levels using RT-qPCR and ELISA, respectively. Additionally, inhibitor studies with echinomycin were performed in DPC monolayer cultures to assess the involvement of hypoxia-inducible factor (Hif)-1 signaling. L-MIM and hypoxia increased mRNA and protein production of Angptl-4 in DPC monolayer cultures. Similar effects were observed in spheroid cultures. This increase was downregulated after application of echinomycin suggesting an involvement of Hif-1. In tooth slice cultures no significant modulation of Angptl-4 production could be detected at mRNA or protein levels. Our results suggest that the hypoxia mimetic agent L-MIM and hypoxia stimulate Angptl-4 production in DPC, involving the Hif-1 pathway. The mechanism underlying the different responses in the cell and tooth slice culture models and how the increased levels of Angptl-4 contribute to regeneration will be assessed in future studies.
**P 180 Distal Femoral Reconstruction using Modular Megaprostheses in Non-Oncological Patients with Severe Bone Loss**


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Introduction: Revision joint arthroplasty of the distal femur is challenged due to severe bone defects and instability. Megaprosthetic reconstruction represents an ultimate limb salvaging treatment option.

The aim of this study was to investigate the outcome of patients treated with distal femoral reconstruction due to severe bone defects without oncological primary disease of the bone.

Material & Methods: A retrospective data analysis detected 46 patients with the mean age of 71 years (range 42-95 years) treated with distal femoral replacement from 1996 to 2016.

Complications of megaprostheses were classified as Type I, softtissue failure, as Type II, aseptic loosening, as Type III, structural failure and as Type IV, infection according to Henderson classification.

Results: Indications for megaprosthetic treatment were infection in 21.7%, aseptic loosening in 23.9%, periprosthetic fracture 30.4%, traumatic fracture 2.2%, pseudoarthrosis 19.6%, and severe knee joint instability 2.2%.

At the time of the last follow-up 19 patients (41.3%) suffered at least one complication.

Revision free survival rates were 70% at one year, and 30% at 5 years. Complication were detected in 5 patients (9.6%) due to sofftissue failure, in 7 patients (13.5%) due to aseptic loosening and in 2 patients (3.8%) due to structural failure. 10 patients (21.7%) obtained revision due to infection. 5 patients (10.8%) developed primary infection after megaprosthetic reconstruction and 5 patients (10.8%) had recurrent infection after previously treated with prosthetic joint infection.

Conclusion: Distal femoral reconstruction may provide a limb salvaging technique with satisfying function and stability for non-oncologic patients with severe bone deficiency. High complication and revision frequencies detected may refer to a mostly elderly patient population treated with multiple surgeries resulting in insufficient soft tissue function, bone deficiency and recurrent infection.

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**P 181 Relation between various implant treatment protocols on long-term survival analysis and risk factors: a retrospective study**

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Background: Nowadays fast treatment approaches are more and widely used because of the availability of improved implant designs, surfaces and surgical techniques. However, long term follow up studies are rare.

Aim: Aim of this retrospective study was to compare survival rates among various treatment protocols and to evaluate influencing factors for implant failure.

Materials and Methods: This study was conducted on 3,338 patients with 9,832 implants between 2004 and 2012. The 10-year survival rates were computed using the Kaplan-meier method and patient- and implant-related risk factors were evaluated by Cox proportional hazards statistics.

Results: Overall implant survival rate was 96.8%. Cox regression analysis revealed the following significant factors associated with failure: single tooth gap (p = 0.02), Type 4 bone quality (p = 0.03), implant torque <30 N cm (p = 0.03), shorter implant (p<0.001) and protocol such as delayed placement with immediate provisionalization (DNN1ID, p<0.001), pre-implant bone grafting and immediate provisionalization (DPN1ID, p<0.001), pre-implant bone grafting with 1 stage healing and delayed loading (DPN1ND, p = 0.01), pre-implant bone grafting and delayed loading (DPN2ND, p<0.001), immediate placement and immediate provisionalization (INN1ID, p<0.001), immediate placement and delayed loading (INN1ND, p<0.001), immediate placement and 2 stage healing (INN2ND, p= 0.003). There was no significant association found regarding location (p = 0.17), jaw (p =0.81), and implant diameter (p= 0.3).

Conclusion: High long-term survival was observed in the traditional protocol (DNN2ND). Faster implant treatment protocol - immediate placement and immediate provisionalization (INN1ID) increased the risk of implant failure by 9.2 times compared to traditional protocols.

P 182 Influence of sagittal alignment on the outcome of posterior fixation of the thoracolumbar spine

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Introduction: Sagittal malalignment of the spine is associated with reduced life quality. Additionally, it has a predominant effect on the clinical outcome of the surgical management of spinal disorders. The goal of this study was to determine the role of the sagittal imbalance of the spine in the failure of the posterior fixation of the spine.

Materials and Methods: In this retrospective study, a study group (female n=23, male
n=15, age range: 15-83 years) who underwent a revision surgery due to breakage of the implants was compared with a control group (female n=14, male n=13, age range: 58-82 years) with intact implants. Whole spine lateral radiographies, obtained in standardized standing position, were investigated for lordosis gap (LG), pelvic incidence (PI), pelvic tilt (PT), sacral slope (SS), sagittal vertical axis (SVA), lumbar lordosis (LL), and thoracic kyphosis (TK). Data were analyzed using descriptive statistics, parametric and non-parametric inferential statistics, Pearson and Spearman correlation analyses.

Results: In the study and control groups, the sagittal spinopelvic parameters yielded the following results: LG (medians: 27.8 vs 13.5, p<0.05), PI (means: 66.7 vs 55.5, p<0.05), PT (medians: 31.7 vs 25.7), SS (medians: 34 vs 30.6), SVA (medians: 72 vs 65.6), LL (medians: 38.4 vs 46.1), and TK (means: 31.7 vs 32.6), respectively. Additionally, correlation analyses revealed significant relationships between LG (p=0.02), PI (p=0.003), PT (p=0.03), and SS (p=0.05) and breakage of the implants.

Conclusion: Considerable deviation from the normal values of sagittal spinopelvic parameters (in particular, LG, PI, PT, and SS) was associated with higher rates of breakage of the rods and screws in the posterior fixation of thoracolumbar spine.

P 183 Ultrasound anatomy of the infrapatellar branch of the saphenous nerve

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P 184 Immunohistochemical detection of Galectin-1 and Galectin-3 in the degenerative lumbar disc


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Introduction: A main cause of low back pain, affecting a large portion of the population, is the degeneration of the intervertebral disc (IVD). Recently, the carbohydrate-binding proteins Galectin-1 and Galectin-3 were shown to induce proinflammatory/degradative processes in osteoarthritic chondrocytes. This study aimed to localize Galectin-1 and Galectin-3 in the degenerated human IVD and to correlate their presence with the histological degree of degeneration.

Material and Methods: 18 clinical IVD specimens, obtained from 18 spondylochondrosis patients (32-80 years; Pfirrmann score 4, Modic score 2), were compared to 10 anatomical
IVD specimens from 6 donors (77-87 years). The specimens were graded using a histological score for degeneration (range 0 (healthy) to 10 (fully degenerated)). Then, specimens were immunohistochemically stained with antibodies against Galectin-1 and Galectin-3, followed by microscopic grading using a Quick score (range: 0 (no staining) to 20 (highest staining and cell positivity)). Data were analyzed using descriptive statistics and correlation analyses.

Results: Clinical IVD samples had a significantly higher total histological degeneration score (mean 9±1) than the anatomical specimens (3±1, p<0.01). Importantly, mean Quick scores of the clinical samples for Galectin-1 and Galectin-3 were 11±3 and 11±5, respectively, while those of the anatomical specimens were 0.2±0.4 (p<0.01) and 10±5 (p=0.7), respectively. The histological degree of degeneration had a strong correlation with the Quick score for Galectin-1 (p=0.02), contrary to the weak correlation with that for Galectin-3 (p=0.5).

Conclusion: The histological features of IVD degeneration are associated with an increased cellular presence of Galectin-1, but not of Galectin-3. This finding might point towards a functional role of Galectin-1 in degenerative processes of the IVD, and encourages further investigation of the IVD glycobiology.

**P 185 Galectin-3 cooperates with galectin-1 to induce inflammation and cartilage degeneration in osteoarthritis**


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The molecular mechanisms of cartilage degeneration in osteoarthritis (OA) are well described. However, the identification of effectors leading to the onset of OA remains challenging. In this study, we pursued initial evidence for an upregulation of members of the galectin family in the context of OA. Indeed, immunohistochemical localization in human cartilage sections (n=13 patients) revealed increasing amounts of galectin-3 (Gal-3)-positive chondrocytes in linear correlation with cartilage degeneration (p<0.001, Wilcoxon signed rank test). In primary human OA chondrocyte culture, Gal-3 was secreted (0.11±0.17 ng/ml) and surface binding was detected in a lactose-inhibitable manner using laser scanning microscopy. Exposure of cells to Gal-3 led to enhanced gene expression and secretion of functional disease markers such as chemokine (e.g. interleukin-8, interleukin-1β, tumor necrosis factor-α) and matrix degrading enzymes (e.g. matrix metalloproteinases-1, -3, -13). Genome-wide transcriptomic analysis broadened this result to reveal a pro-degradative/inflammatory gene signature under the control of the NF-κB pathway. Moreover, the activation profile of Gal-3 was found to overlap with that of
Gal-1 but also has distinctive supplementing features which remain to be elaborated in detail. When applied in a mixture, we found functional cooperation of the two galectins in the induction of inflammation and cartilage degeneration. In summary, our results suggest that galectins act in a network driving OA pathogenesis, and spark research on further members of the galectin family.

**P 186 Milk activates TGF-β signaling in periodontal cells: an in vitro approach**

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Objective: Transforming growth factor β (TGF-β) in milk is supposed to maintain intestinal mucosal homeostasis of the neonate. The impact of TGF-β in milk on cells of the periodontium, however, has not been investigated. To reveal the response of periodontal cells to milk is important for understanding possible clinical implications in the oral cavity.

Methods: Human periodontal fibroblasts were exposed to cow and mother milk followed by reverse transcriptase polymerase chain reaction of the TGF-β target genes interleukin 11 (IL11), proteoglycan4 (PRG4), and NADPH oxidase 4 (NOX4). An immunoassay was performed for IL11. Signaling was investigated with the TGF-β receptor type I kinase inhibitor SB431542 and phosphorylation of smad3. Studies with oral epithelial cells and on proliferation, migration and osteogenic differentiation are ongoing. Also fermented dairy foods will be investigated.

Results: We can report so far that cow and mother milk were potent inducers of IL11, PRG4, and NOX4 expression in oral fibroblasts. Consistent with its effect on TGF-β target genes, SB431542 completely prevented the expression of the gene panel. In the immunoassay, SB431542 blocked the increased production of IL11 in response to milk. Correspondingly, cow and mother milk increased phosphorylation of smad3.

Summary and Conclusions: These preliminary results demonstrate that milk-derived TGF-β targets oral fibroblasts.

**P 187 Effect of soluble CD14 on the response of human periodontal ligament stem cells to TLR2 agonist**

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Human periodontal ligament stem cells [hPDLSCs] do not express membrane-bound CD14, a co-receptor, which is important in the lipopolysaccharide [LPS] triggered toll-like receptor [TLR] 4 signaling. In a previous study we showed that triggering hPDLSCs with bacterial LPS in the presence of soluble CD14 [sCD14] enhances TLR4 signalling. If sCD14 also increases the signaling of other members of the TLR family in hPDLCs is not known. This study investigated the influence of sCD14 on TLR2 signaling in hPDLSCs. hPDLSCs were stimulated for 24 hours by synthetic TLR2/1 agonist Pam3CSK4 in concentrations from 0.1 to 1000 ng/ml. Stimulation was done in the presence or in the absence of sCD14 (0.2µg/ml). Stimulation was followed by measuring interleukin [IL]-6, IL-8, and monocyte chemoattractant protein [MCP-1] expression using qPCR and ELISA. hPDLSCs were positive for mesenchymal markers (CD29, CD90, CD105, CD146) and were lacking hematopoietic markers (CD14, CD34 and CD45).

By stimulating hPDLSCs with only Pam3CSK4, a gradual increase in the expression of IL-6, IL-8 and MCP-1 was detected on gene and on protein level. The highest expression level was detected at a concentration of 1000ng/ml. The response of hPDLSCs to Pam3CSK4 at submaximal concentrations (1-100ng/ml) was significantly higher in the presence of sCD14. At the maximum Pam3CSK4 concentration (1000ng/ml) sCD14 had no significant influence on the hPDLSC response.

Local levels of sCD14 may have an important influence on the interaction between periodontal pathogens and the host immune response.


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P 189 Phase Imaging of Hyaline Cartilage in Ultra-Highfield Magnetic Resonance Imaging

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P 190 Functionalizing biomaterials with secretome of oral cells for applications in oral surgery


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P 191 Time to first treatment is associated with a refractory course of rheumatoid arthritis


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Background: It is an ongoing matter of research, whether the natural course of rheumatoid arthritis (RA) can be altered by an early intervention. So far, only short-term disease activity outcomes have been investigated, which are, however, inherently affected by the unknown rate of self-limiting disease. It is unclear, whether among those, who eventually develop RA, the disease course is really affected by the timing of their initial treatment.

Objectives: To explore whether the long-term course of RA is different according to the delay of initial treatment.

Methods: Based on a longitudinal observational dataset, we initially identified a group of patients with an observed refractory disease, defined with ongoing moderate or high disease activity (by SDAI), despite at least three courses of DMARDs with at least one biological compound. To ensure that sufficient time had been allowed for the previous treatments to be exert their non-effects, we also required these patients to have total treatment time of at least 18 months.

We could include 69 refractory and 282 non-refractory patients and then performed logistic regression analysis to assess the effects of different baseline characteristics on becoming refractory.

Results: By comparing patient characteristics, more of the patients, who later will become refractory, are female (94.2% Vs 73.4%, p>0.001), have higher baseline disease activity (SDAI of 25.5 vs 17.7, p<0.001), and longer delay of the initial treatment from symptom onset (3.17 Vs 1.34 years, p=0.001).
The multivariable logistic regression model confirmed that a longer delay of first treatment is independently afflicted with a higher probability of a refractory disease course at a later stage. This model was adjusted for disease activity at baseline and gender (p<0.001).

Conclusions: Our data suggest that delay to initial treatment in RA affects the long-term course of RA. Earlier treatment initiation thus may change the severity of RA.

**P 192 Trends in clinical oral implant research: systematical review and clinical analysis**

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The aim of the present work is to provide insights about thematic trends in clinical oral implant research.

Systematical review was performed to detect the current topics in clinical research publications of dental implants. Further clinical study was performed to check if the implant treatment protocol has changed over the years and whether the results happen to coincide with the literature findings.

Medline electronic searches as well as additional hand searches in the field were conducted. 2875 clinical studies published in the time period 2001-2012 met the inclusion criteria and were subjected to statistical analysis. Hot topics in dental implant literature were immediate loading (14.3%), bone substitutes (11.6%), cross-arch full bridges (8.0%), and immediate implant placement (7.5%). Significant increase of scientific interest was seen for immediate loading (+6.3%, p=0.001), platform switching (+2.9%, p=0.001), guided implant surgery (+1.9%, p=0.011), growth factors (p=0.014, +1.4%), piezoelectric surgery (+1.3%, p=0.015), and restorative materials (+0.7%, p=0.011).

Data from 16,062 dental implants, placed in the Academy for Oral Implantology, Vienna in the time period 2004-2014 was analyzed. Increasing use over the years was found for: short implants (+8.49%), immediate implant placement(+10.03%), transmucosal healing (+15.51%), immediate placement (+10.03%), immediate loading(+28.00 %). Declining trend was detected for onlay grafting (-0.62%), as well as the autologous bone substitutes(-5.85%).

The clinical results as well as the literature findings demonstrate increasing interest in techniques to avoid complicated procedures such as bone grafting and reduce the treatment duration.

**P 193 Anatomical footprint of the tibialis anterior tendon - Surgical implications for foot and ankle reconstructions**
This study aimed to analyze precisely the dimensions, shapes and variations of the insertional footprints of the tibialis anterior tendon (TAT) at the medial cuneiform (MC) and first metatarsal (MT1) base. Forty-one formalin-fixed human cadaveric specimens were dissected. After preparation of the TAT footprint, standardized photographs were made and following parameters were evaluated: the footprint length, width, area of insertion, dorso-plantar location, shape and additional tendon slips.

Twenty feet (48.8%) showed an equal insertion at the MC and MT1, another 20 feet (48.8%) had a wide insertion at the MC and a narrow insertion at the MT1 and 1 foot (2.4%) demonstrated a narrow insertion at the MC and a wide insertion at the MT1. Additional tendon slips inserting at the metatarsal shaft were found in two feet (4.8%). Regarding the dorso-plantar orientation the footprints were located medial in 29 feet (70.7%) and medioplantar in 12 feet (29.3%). The most common shape at the MT1 base was the crescent type (75.6%) and at the MC it was the oval type (58.5%).

The present study provided more detailed data on the dimensions and morphologic types of the tibialis anterior tendon footprint. The established anatomical data may allow for a safer surgical preparation and a more anatomical reconstruction.

P 194 Endotoxin tolerance in human periodontal ligament cells by stimulation with Porphyromonas gingivalis lipopolysaccharide

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Background: Endotoxin tolerance [ET] is a preventive mechanism developed by immune cells and other cytokine expressing cells, characterized by a state of hyporesponsiveness due to confrontation with endotoxins such as lipopolysaccharides [LPS] in low concentrations. According to recent studies human gingival fibroblasts do not show ET and hence may be crucial in the progression of periodontal disease. The aim of this study was to investigate, whether the fibroblast-like human periodontal ligament cells [hPDLc] fail to induce ET as well. The secondary goal was to assess the impact of the two most relevant LPS receptors Toll-like receptor [TLR] 2 and TLR4 on the magnitude of the immune response in hPDLc.
Materials & Methods: HPDLc of five donors were pretreated with Porphyromonas gingivalis [P.g.] LPS in a concentration that leads to a submaximal inflammatory response as determined in preliminary tests. After 24 hours, pretreated and non-pretreated cells were stimulated with P.g. LPS in a higher concentration. Further stimulation with Escherichia coli LPS (TLR4 agonist) and Pam3CSK4 (TLR2 agonist) was performed to allow a separate evaluation of either receptor.

The protein expression of cytokines was analyzed with ELISA, whereas the gene expression of cytokines and TLRs was measured by PCR.

Results: The gene expression of the cytokines and TLR2 showed no significant difference between the pretreated and non-pretreated group in response to all stimuli. Similarly, no significant difference in the cytokine protein production was observed. TLR4 gene expression was significantly decreased in the pretreated cells compared to non-pretreated cells.

Conclusion: The results show that hPDLc do not develop ET, suggesting an important role in the progression of periodontal disease by excessive cytokine expression. Furthermore, this study demonstrates that TLR4 expression does not affect the amplitude of the inflammatory response in hPDLc, indicating a less considerable contribution in ET.

P 195 The surprising link between tRNA splicing and selenocysteine biosynthesis

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Splicing of intron-containing tRNAs is a two-step process involving endonucleolytic cleavage by the tRNA splicing endonuclease (TSEN) complex and re-ligation of the exon halves by the tRNA ligase complex, with RTCB as its essential catalytic subunit. We used a bioinformatics approach to identify proteins in the same cluster of orthologous groups as the tRNA ligase complex, which often suggests co-evolution and thus a functional link. Surprisingly, we found a close connection to a set of proteins and enzymes involved in the biosynthesis and incorporation of the 21st amino acid, selenocysteine.

Using RNAi we depleted the enzymatic machinery that produces selenocysteine. Among them, the absence of PSTK (O-phosphoseryl-tRNAsec kinase) caused the strongest impairment in the ligation of tRNA exon halves. Together with previous results showing that the tRNA ligase becomes inactive in the presence of oxidants, this result led to the hypothesis that defects in the selenocysteine pathway should impact on the synthesis of selenoproteins, in turn needed to counteract oxidative stress. In line with this idea, we could inhibit the tRNA ligase complex by using chemical inhibitors against the Thioredoxin-Thioredoxin reductase (Trx/TR) system, essential to keep the redox balance of the cell. Curiously, we also noticed that the genome of the nematode Caenorhabditis elegans
encodes the entire selenocysteine biosynthesis and incorporation machinery to provide a selenocysteine residue to a single protein, Thioredoxin reductase 1 (TR1). Experiments are currently being conducted to deplete TR1 in C.elegans and monitor tRNA ligation activity in an in vitro system or by northern blots to detect the accumulation of a tRNA fragment, diagnostic of inhibition of the tRNA ligase.

Taken together, this project aims at dissecting a hitherto unanticipated and crucial connection between the most powerful antioxidant cellular system and the splicing of tRNA molecules.

**P 196 Identification of a novel RNA 2′,3′-cyclic phosphatase activity in human cells**

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Defects in RNA processing are increasingly being linked to numerous pathologies. In particular, the correct processing of RNA 3′ ends determine RNA stability and function. Our group finds it critical to identify and characterize the responsible enzymes. Yet to be studied in depth, RNA terminal 2′,3′-cyclic phosphates play essential roles in RNA metabolism as intermediates in tRNA splicing, non-canonical mRNA splicing during the unfolded protein response and as products of endonucleolytic cleavage by ribonucleases.

Interestingly, RNA terminal 2′,3′-cyclic phosphates can also be generated de novo by the RNA 3′-terminal phosphate cyclase, RTCD1 (RtcA in bacteria). By characterizing RTCD1 we detected a seemingly associated, new enzymatic activity that further converts terminal 2′, 3′-cyclic phosphates into nucleotides displaying 2′, 3′ OH groups. We purified the enzymatic activity from HeLa cytoplasmic extracts using a classical chromatographic approach. A highly-purified fraction was analyzed by Mass Spectrometry and genes encoding candidate proteins were silenced by RNAi. At this point we are confident in having identified the responsible gene.

We are currently expressing the recombinant enzyme in E.coli and in HEK293 cells as a myc-tagged enzyme. The activity and functions of the novel enzyme in RNA processing, repair and decay will be thoroughly investigated both in vitro and in vivo.

**P 197 Three-Dimensional Coculture Model to Analyze the Cross Talk Between Endothelial and Smooth Muscle Cells.**

The response of blood vessels to physiological and pathological stimuli partly depends on the cross talk between endothelial cells (EC) lining the luminal side and smooth muscle cells (SMC) building the inner part of the vascular wall. Thus, the in vitro analysis of the pathophysiology of blood vessels requires coculture systems of EC and SMC. We have developed and validated a modified three-dimensional sandwich coculture (3D SW-CC) of EC and SMC using open µ-Slides with a thin glass bottom allowing direct imaging. The culture dish comprises an intermediate plate to minimize the meniscus resulting in homogenous cell distribution. Human umbilical artery SMC were sandwiched between coatings of rat tail collagen I. Following SMC quiescence, human umbilical vein EC were seeded on top of SMC and cultivated until confluence. By day 7, EC had formed a confluent monolayer and continuous vascular endothelial (VE)-cadherin-positive cell/cell contacts. Below, spindle-shaped SMC had formed parallel bundles and showed increased calponin expression compared to day 1. EC and SMC were interspaced by a matrix consisting of laminin, collagen IV, and perlecan. Basal messenger RNA (mRNA) expression levels of E-selectin, angiopoietin-1, calponin, and intercellular adhesion molecule 1 (ICAM-1) of the 3D SW-CC was comparable to that of a freshly isolated mouse inferior vena cava. Addition of tumor necrosis factor alpha (TNF α) to the 3D SW-CC induced E-selectin and ICAM-1 mRNA and protein induction, comparable to the EC and SMC monolayers. In contrast, the addition of activated platelets induced a significantly delayed but more pronounced activation in the 3D SW-CC compared to EC and SMC monolayers. Thus, this 3D SW-CC permits analyzing the cross talk between EC and SMC that mediate cellular quiescence as well as the response to complex activation signals.

**P 198 Inhibition of activated protein C and formation of Neutrophil Extracellular Traps in non-traumatic hyperfibrinolysis**

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**P 199 The regulation of vascular barrier function by tight junction adaptor cingulin in response to inflammatory stimuli**

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Endothelial barrier function is critical for the maintenance of blood flow and tissue homeostasis. Vascular barrier dysfunction in response to agonist-induced activation of Rho-GTPase signaling aggravates pathologic processes including atherosclerosis, brain and lung edema and pulmonary hypertension. We have shown that the cytoplasmic adaptor protein cingulin is part of the tight junction complex and regulates vascular permeability. The most important RhoGTPases regulating barrier functions are RhoA and Rac1. Cingulin connects tight junction proteins to these Rho signaling pathways. RhoGTPases exist in active (GTP-bound) and inactive (GDP-bound) states and can be controlled by activating guanine-nucleotide exchange factors (GEFs) and inhibitory GTPase-activating proteins (GAPs). Recently, our in vivo studies showed that in cingulin-deficient mice GDP/GTP-exchange factors GEF-H1 and p114RhoGEF were absent from the endothelial surface. However, the mechanism of how cingulin regulates Rho activity and endothelial barrier function remains unclear. Cingulin may be required for a critical balance of permeability responses and space and time-specific activation of RhoGTPase regulators. Therefore, we aim to investigate GEF localization during inflammation and show GEF-H1-CGN colocalization in response to inflammatory stimuli. We propose that the effect of cingulin on Rho activity is limited to a defined area at the junction.

P 200 A novel 3D angiogenesis assay for studying the role of stromal Wnt2 in blood vessel formation of colorectal cancer


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The so-called tumor stroma participates in tumor cell growth, invasion and metastasis and is now widely accepted to be responsible for bad prognosis. A gene specifically elevated in cancer associated fibroblasts within large intestine cancers is Wnt2. Wnt2 induction has been shown in a wide variety of carcinomas and functional experiments have demonstrated that Wnt2 actively participates in cancer progression and to act as an angiogenic factor in hepatocellular carcinomas. However, if there is a role for Wnt2 in colon cancer angiogenesis is not clear. To address this we developed and further characterized a novel model, which recapitulates the establishment of blood vessels in a three-dimensional cell culture environment. With this method we combined the formation of vessel-like structures in fibroblast co-cultures with the simulation of angiogenic sprouting from existing endothelial structures, which are mimicked by endothelial cell (EC)-coated beads. In this model the extracellular matrix (ECM) required for endothelial cell tube formation is provided by densely seeded fibroblasts without further addition of external ECM such as fibrin or collagen gels. The tubular structure of sprouts was verified by confocal microscopy. Important characteristics of angiogenic sprouting – the number of sprouts,
sprout length, the number of branch points and overall EC tubular structure area can be quantified. We could show that in this assay angiogenesis can be inhibited by bona-fide anti-angiogenic treatment (using bevacizumab and nintedanib) or genetic interference in fibroblasts, but can also be increased by exogenously adding angiogenic factors (bFGF, VEGFA). This method will be used in our study to elucidate the role of Wnt2 in blood vessel formation in colorectal cancer and might also be used for screening of pro- an anti-angiogenic agents.

**P 201 Prevention of Neointimal Hyperplasia in Vascular Grafts**


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Coronary bypass lifetime is diminished due to various graft diseases. One of these is intimal hyperplasia which is characterized by excessive proliferation of smooth muscle cells. In a healthy blood vessel endothelial cells form the innermost layer which is in constant contact with the blood circulation. In order to provide mechanical stability smooth muscle cells wrap around the endothelial cells in multiple layers. In case of hyper proliferation the lumen of the grafted artery is decreasing until the blood flow is restricted.

In order to simulate human graft disease phenotype a mouse model of bypass grafting is applied. The inferior vena cava is grafted into the carotid artery to simulate a venous bypass exposed to arterial circulation patterns. In addition to this, the responses of blood vessels to individual stimuli which occur regularly during vascular surgery are assessed to identify the critical factors which trigger intimal hyperplasia. Besides the characterization of causative factors, also a pharmacological intervention using a multi kinase inhibitor is tested.

The short term inflammatory reaction pattern of a vein graft exposed to arterial circulation was characterized by RNA sequencing for up to 24h following grafting. Additionally, the responses of arterial vessels to different noxious influences such as mobilization, clamping and ischemia-reperfusion injury were analyzed. By doing so a characterization of various inflammation markers was performed during the first 24h post-surgery. Furthermore, a multi kinase inhibitor was used to block a wide array of inflammatory pathways which dampened the inflammatory response.

Our results give a broad overview of the inflammatory gene expression pattern in reaction to various sorts of noxious vascular stimuli and how to pharmacologically interfere with this inflammatory response pattern.
Inter-individual donor properties mediating the aging process of human packed red blood cells in transfusion units


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Packed red blood cells (PRBCs) undergo so called storage lesions with time, including redox stress, changes in energy metabolism, and reduced deformability. Meta-analyses mostly report no overall association between PRBC storage duration and adverse effects in transfusion recipients. Despite this, PRBCs towards the end of shelf life can elicit negative pathophysiological reactions like e.g. in transfusion-related acute lung injury.

In this pilot study we assessed if variations in biochemical properties of individual PRBCs bags reflect intrinsic biological differences among individual donors which might limit storage duration for particular PRBC units. Qualitative stratification was done with 3 volunteers donating blood 3 times each within 18 months. PRBCs were analyzed for hemolysis, PS exposure, vesiculation, and plasma membrane redox activity at weekly intervals. Another 42 single donor PRBC units were analyzed similarly.

Changes in hemolysis and PS exposure at the outer membrane were employed as aging markers. PS exposure appeared to increase earlier (week 4) than hemolysis which rises significantly by week 5. It is unclear at present whether this makes the onset of PS exposure an predictor for PRBC aging. Both parameters differed in a donor-dependent and clearly reproducible manner and thus may represent individual donor trait. In contrast, evolving redox stress was independent of donor, possibly due to PRBC preparation or current donor status.

These results serve as basis for a clinical study to corroborate which inter-individual donor-specific molecular signatures evolve at different rates during PRBC storage to reduce the risk of adverse transfusion reactions.

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P 202 Inter-individual donor properties mediating the aging process of human packed red blood cells in transfusion units

P 203 Retinal oxygen extraction in patients with type 1 diabetes
P 204 The Role of G0S2 in Melanoma Progression

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P 205 Gender differences in heart failure with preserved ejection fraction – insights from a prospective registry

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Background. Approximately half of all heart failure (HF) patients present with a near normal left ventricular ejection fraction (HFpEF). Even though gender is thought to play an important role in this disease, studies elucidating gender-differences are scarce.

Materials and Methods. 260 consecutive patients with HFpEF were included in our study. Patients underwent clinical assessment including 6-minute walk test (6-MWT), left and right heart catheterization and cardiac magnetic resonance imaging. Prospective follow-up of study participants via outpatient visits or telephone calls was performed. The primary outcome was a composite endpoint of HF hospitalization or cardiac death.

Results. Median age of the total cohort was 73.0 years, 181 (69.6%) were female, median N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was 1169 pg/mL and 170 (65.4%) study participants were in NYHA class =III. Men were clinically more compromised and had a higher burden of co-morbidities.

Men had a worse event-free survival, both for the combined endpoint (Fig. 1A, p=0.031) and cardiac death (Fig. 1B, p=0.011). No difference could be detected for all-cause death (Fig. 1C, p=0.629). Also, men were more likely to die from cardiac death as compared to women (p=0.008) and less likely to die from non-cardiac death (p=0.030). NT-proBNP, mean pulmonary artery pressure and exercise capacity were predictors of outcome in both genders. However, right ventricular function and co-morbidities such as atrial fibrillation, diabetes mellitus or COPD only predicted event-free survival in female HFpEF patients.

Conclusion. Among a well-characterized HFpEF cohort, marked gender-differences were
Men had worse exercise capacity, hemodynamics, and a higher burden of co-morbidities, which was accompanied by a shorter cardiac event-free survival. Interestingly, the clearly higher burden of co-morbidities hardly affected outcome in the male cohort, but strongly did so in women.

**P 206 Characterisation of engineered vascular networks derived from endothelial cells and adipose-derived stem cells in a fibrin matrix**


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Co-cultures of endothelial cells with mesenchymal stem cells currently represent one of the most promising approaches in providing oxygen and nutrient supply for microvascular tissue engineering. Still, to translate this model into clinics several in vitro parameters including growth medium and scaffold degradation need to be fine-tuned. We recently described the co-culture of adipose-derived stem cells with endothelial cells in fibrin, resulting in capillary formation in vitro as well as their perfusion in vivo. Here, we aimed to further characterise microvascular tube formation in fibrin by determining the role of scaffold degradation, thrombin concentration and culture conditions on vascularisation. We observed that inhibition of cell-mediated fibrin degradation by the commonly used inhibitor aprotinin resulted in impaired vascular network formation. Aprotinin had no effect on laminin and collagen type IV deposition or formation of tube-like structures in plasminogen-free fibrin or scaffold-free co-culture, indicating that poor vascularisation of fibrin clots is primarily caused by inhibition of fibrinolysis. Furthermore, we demonstrate that thrombin negatively affects vascular network density at high concentrations. However, only transient activation of incorporated endothelial cells by thrombin could be observed, thus excluding a long-term inflammatory response in tissue-engineered micro-capillaries. Finally, we show that vascularisation of fibrin scaffolds in basal medium is undermined because of increased fibrinolytic activity leading to scaffold destabilisation without aprotinin. Taken together, our data reveal a critical role of fibrinolysis inhibition in microvascular tissue engineering as it reduces cell-mediated vascularisation of fibrin scaffolds but is required for culture in basal medium.

**P 207 IκB Kinase 2 impairs GPIIb/IIIa Activation in Platelets**


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Megakaryocytes can sense inflammatory signals, but little is known how this might change platelet function. Most inflammatory signaling pathways converge at the kinase IKK2 (IκB kinase 2) activating the transcription factor NF-κB. Our aim was to determine the effect of chronic inflammation on platelets by using a conditional transgenic mouse model that alters NF-κB activity in megakaryocytes. The aim of this study was to determine the effect of persistent inflammation on platelet function, by altering NF-κB activity in megakaryocytes with a constitutively active IKK2. Mice with a megakaryocyte-specific constitutively active IKK2 were compared to littermate controls. Platelet count and lifespan was determined and function was tested in vitro by agonist-induced degranulation and aggregation and in vivo by tail bleeding and intra vital microscopy of ferric chloride induced thrombus formation. Platelet count and lifespan is unaltered, however degranulation and GPIIb/IIIa activation were decreased in platelets with constitutively active IKK2 upon stimulation with ADP and PAR4 receptor agonist peptide. Consistently, in vitro platelet aggregation is reduced and intra vital microscopy of ferric chloride induced thrombus formation is impaired in mice with constitutively active platelet IKK2, leading to increased bleeding time. Taken together our data indicates that active IKK2 or NF-κB interferes with platelet activation, either directly through kinase activity in platelets or via constitutively active NF-κB signaling in megakaryocytes.

**P 208 Identification of novel interaction partners of the Lysine Methyltransferase SETDB2**

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SETDB2, a member of the SUV39 lysine methyltransferases (KMTs), plays an important role in chromatin modifications and immune responses. In Schliehe et al. it was shown that SETDB2 acts as an interferon-stimulated regulator, modulating the expression of NFB target genes via H3K9me3, affecting influenza virus-induced susceptibility to bacterial superinfection. A similar mechanism was later also shown to play a role in gastric cancer. Despite possessing a methyl-CpG binding domain which allows for binding to methylated DNA, it remains unclear how SETDB2 is recruited to its histone or potential non-histone protein targets. We hypothesise that the recruitment of SETDB2 to the promoter of target genes is facilitated via transcription factors and/or chromatin-associated factors. Here, we try to identify potential interaction partners of SETDB2 by a mass spectrometry based approach. Candidate chromatin associated factors and transcription factors will be further investigated by CRISPR Cas9 mediated loss-of-function experiments in Hoxb8 progenitor derived murine bone marrow macrophages. These cells will allow us to follow SETDB2 effects and interactions in a primary myeloid system. Ultimately, the establishment of
human cell lines will add to a comprehensive understanding of SETDB2 and its interactions on a molecular level. As it has been shown that SETDB2 plays a key role in the immune response, it would be interesting to identify its binding mechanisms and potential partners which will help to unravel SETDB2’s biological functions in the immune system even further.

P 209 Identification of keratinocyte differentiation-associated IL-1 family members


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Members of the interleukin (IL)-1 family of cytokines, including the prototypical representatives IL-1-alpha and IL-1-beta, are central regulators of inflammatory reactions. Here, we performed a comparative genomics study of the IL1 family in vertebrates and and determined the expression of IL1 family genes during the terminal differentiation process of human epidermal keratinocytes, which establishes the skin barrier to the environment. We found that the IL1 family gene cluster is largely conserved in terrestrial mammals whereas the genes IL36A, IL36B, IL37, and IL38 have been lost in whales and dolphins, in which the epidermal differentiation program has degenerated during the acquisition of a fully aquatic lifestyle. When human epidermal keratinocytes were stimulated to undergo differentiation in vitro the transcription of IL36A, IL36B, IL37, and IL38 was strongly upregulated. Western blot analysis showed a increase in IL37 protein abundance in differentiating human keratinocytes, and immunohistochemistry demonstrated expression of IL37 in the granular layer of normal human epidermis. These results suggest that the expression of IL37 and three other IL1 family members in differentiated keratinocytes contributes to the homeostasis of normal epidermis.

P 210 The Role of Osteopontin in Liver Regeneration and Dysfunction.


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Background: Osteopontin [OPN] is known to be involved in inflammation, fibrogenesis and carcinogenesis. In regard to liver regeneration, it was shown, that OPN knock-out mice demonstrate reduced liver regeneration after partial hepatectomy. On the other side, OPN
overexpression during rat liver regeneration was shown to lead to hepatic necrosis and augmented inflammatory injury. However, to date no data on the influence of OPN on human liver regeneration is available.

Methods: A total of 48 patients undergoing hepatic resection for liver malignancies was included in this study. Blood was taken preoperatively, and on the first [POD1] and fifth postoperative day [POD5]. Plasma preparation was accomplished, and patients were followed up for morbidity and postoperative liver dysfunction [LD]. OPN levels were assessed using commercially available ELISA kits and were further correlated to patients’ outcome. Further, liver biopsies were taken prior to resection as well as from the regenerating liver and gene Expression of OPN was evaluated using RT-PCR.

Results: Patients with reduced liver function as well as HCC patients displayed a stronger OPN expression in the liver at baseline. Circulating levels of OPN increased on POD1 (P=0.001) and remained elevated up to POD5 (P=0.012). Interestingly, patients suffering from LD in the postoperative time course showed significantly higher levels of OPN on POD1 (P=0.039). A similar tendency was observed for patients with postoperative complications (P=0.072). Ultimately, ROC analysis for OPN on POD1 showed a highly accurate predictive potential for postoperative LD with an AUC of 0.704.

Conclusion: We here present the first data on the role of OPN in human liver regeneration. While OPN seems to be important for liver regeneration, we observed that overexpression of OPN might lead to postoperative LD, maybe due to its pro-inflammatory properties. Further, we were able to document the value of OPN as a marker for postoperative LD.

P 211 New targets for by-passing acquired drug resistance in non-small cell lung cancer HCC827 cells


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Acquired resistance to receptor tyrosine kinase (RTK) inhibitors (TKI) limits efficacy of targeted cancer therapy. Thus, treatment of patients harboring activating epidermal growth factor receptor (EGFR) mutations frequently leads to acquired EGFR inhibitor resistance. This acquired insensitivity is often based on amplification of the Met gene believed to cause hypersensitivity towards Met inhibitors. Non-small cell lung cancer HCC827 harbors an activating EGFR mutation raising sensitivity towards EGFR inhibitor treatment. In this study, HCC827-derived sublines with acquired resistance to EGFR inhibitors (Erlotinib and Gefitinib) were generated, both exhibiting amplification of the Met gene. Accordingly, combination with Met inhibitor Crizotinib re-sensitized the cells towards EGFR inhibitors. To identify whether this synergism might be targeted by acquired
resistance, the cell models were further selected for resistance to Crizotinib. mRNA expression array analysis identified members of the ErbB/HER family and fibroblast growth factor receptor 1 (FGFR1) to be selectively overexpressed. For confirmation, Western blot analysis, cell viability and clone formation assays using specific TKI were performed. Afatinib (irreversible EGFR/HER2 inhibitor) selectively reduced viability of Crizotinib-selected sublines. Additionally, high expression of FGFR1 was observed in EGFR/Met inhibitor-resistant sublines. Accordingly, a combination of Afatinib with FGFR1 inhibitor PD173074 was tested, revealing synergistic growth inhibition. Sapitinib (a reversible EGFR/HER2/HER3 inhibitor) restored sensitivity to Crizotinib in the resistant sublines. Interestingly, the higher anti-proliferative activity of Afatinib compared to Sapitinib suggests that targeting HER2 is more effective than HER3. To conclude, HER2 and FGFR1 seem to contribute to acquired EGFR/Met inhibitor resistance. Hence, these RTKs might represent promising targets for combination approaches in EGFR-driven lung cancer.

P 212 Algorithm developement for fast and robust heart localization

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Objectives: Respiratory motion is a major obstacle for cardiovascular magnetic resonance (CMR). Methods like breath-holding, synchronized breathing1 and measurements in prone position can decrease motion artifacts, but have their limitations. Real-time navigator gating (NG) works independently of subject cooperation. Current methods for NG include pencil shaped beams2 and cross correlation to a reference3. This work evaluates an alternative fast, robust approach for detecting cardiac motion. 1:A.Hock et al.DOI:10.1002/nbm.3069 2:S.Kozerke et al.DOI:10.1002/mrm.10182 3:D.Piccini et al.DOI:10.1002/mrm.23247 4:D.Firmin et al.PMID:11816615

Methods: Sagittal images in the cardiac region were acquired from 4 healthy subjects (3 male, 1 female, age: 25±2y, BMI: 26±3kg/m2) in supine position using Magnetom Prisma 3T and 7T scanners (Siemens, Erlangen, Germany). Different MR sequences - gradient echo (GRE) and turbo flash (TFL) - and variation of sequence parameters and slice positions were investigated. The images were smoothed by a Gaussian kernel. Then the average value in phase-encoding (AP-O) direction followed by the derivative values along the readout (HF-O) direction was computed. Cranial of the global maximum of the average values a global minimum of the corresponding derivative can be observed, as well as a global maximum in caudal direction representing the heart’s edges.

Summary of presented data: GRE and TFL sequences, slice close to the apex, worked out equally well. ECG triggering, alignment of the heart-liver border in phase encoding direction and a saturation slab at the chest wall were used to increase the detection accuracy.

Conclusion: Localizing the heart using the algorithm is possible in 3T and 7T data-sets. Rotated images enable the possibility to derive motion components in HF and AP direction.
The presented method is a promising step towards the implementation as navigator in CMR. Funded by FWF P 28867-B30

**P 213 Methane modulates nitric oxide metabolism under ischemic conditions**


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Methane (CH4) inhalation decreases tyrosine nitrosylation after an ischemia-reperfusion injury, a process which involves nitric oxide (NO) and peroxynitrite formation. In recent pilot experiments intestinal tissue NO levels were decreased in CH4-treated rats under ischemia. These data may suggest an influence of CH4 on the biological processes regulated by NO, but to date, the interplay between CH4 and NO - or other gases - has not yet been investigated in mammals. Our aim was to examine the in vitro consequences of CH4 - NO interactions to shed light on the details of the in vivo mechanism. Rat liver and ileum homogenates were incubated under N2 atmosphere with or without 2.2% CH4 to continuously monitor the release of NO purged from a liquid sample using a highly sensitive chemiluminescence-based NO detection method. We observed that CH4 significantly decreased the rate of NO release in rat liver and ileum homogenates (17% and 6% reductions, respectively), a phenomenon which was in line with the effect observed in ischemic tissues in vivo. In further in vitro studies, we identified xanthine oxidoreductase (XOR) as the major NO generating nitrite reductase under anoxic conditions, accounting for up to 98% of NO measured. In experiments with XOR and xanthine as electron donor, however, we detected 26% increase in the NO release in the presence of CH4, but in the next series CH4 again reduced NO levels, if XOR was co-incubated with denatured tissue homogenates. This opposing CH4-induced effect might be attributed either to an increased incorporation of NO in tissues (e.g. protein nitrosylation) which results in less free NO in the medium or alternatively, to a shift in the substrate preference of XOR. Future research is definitely needed to elucidate both possibilities. Supported by NKFI K120232 and GINOP 2.3.2-15-2016-00015.

**P 214 Investigating the role of STAT3 isoforms in acute myeloid leukemia cell lines**

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Signal Transducer and Activator of Transcription 3 (STAT3) is part of a family of transcription factors mediating cytokine signalling in cell survival, proliferation, migration and tissue differentiation. In several cancer types, STAT3 has been shown to be abnormally expressed and inconclusive results suggested a role either as oncogene or tumour suppressor. STAT3 exists in two alternatively spliced isoforms, STAT3a (770 amino acids) and a shorter STAT3ß isoform (720 amino acids), with a truncation in the C-terminus and an addition of seven unique amino acids. Preliminary data from our laboratory suggests a tumour-suppressive role of STAT3ß in acute myeloid leukemia (AML). In an adjacent master thesis, the role of STAT3 in human AML cell lines should be analysed in detail by overexpression and knock-out of either STAT3a or STAT3ß. Expecting STAT3ß to act as a tumour suppressor, we are assuming that cells will exhibit changes in proliferation and migration after knock-out or overexpression of either isoform. Currently, we aim at knocking out STAT3 by CRISPR/Cas9 in six selected AML cell lines, followed by insertion of one of the two isoforms back into the cells by lentiviral infection. In a second approach, parental cells will be infected with lentiviruses carrying constructs of either STAT3a or STAT3ß to induce overexpression. The successful modification of cells will be analysed by R-T qPCR and Western Blotting. Furthermore, cells will be monitored for the effect of these alterations on cell proliferation, migration and ability for stimulation. Both setups, overexpression and knock-out with introduction of one variant back into the cells, will be examined in detail by RNA sequencing to further determine target genes of STAT3a and STAT3ß. Using these cell lines we have valuable tools for elucidating the role of STAT3 isoforms in acute myeloid leukemia.

**P 215 Gender-related Differences in Neurological Outcome After Out-of-hospital Cardiac Arrest**


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BACKGROUND: Out-of-hospital cardiac arrest (OHCA) is still one of the main reasons of death in the western world, caused by cardiovascular diseases in 70%. Recent research implies significant differences in outcomes between women and men. Whereas overall survival is higher in male victims of OHCA, females tend to have better neurological outcome. Current research does not explain these discrepancies.

AIM: We aimed to analyse factors (demographic, medical history and directly OHCA-related) associated with good neurological outcome after OHCA regarding patients’ gender. We assessed both differences in occurrence of these factors between women and men, and
differences in effect of these factors between sexes.

METHODS: From September, 1st 2013 to August, 31st 2015, we prospectively collected data of all patients treated for OHCA due to cardiac origin at our high-volume tertiary care centre. Data consisted of detailed information on cardiac arrest according to Utstein criteria, as well as clinical characteristics, details on medical history, diagnostic findings and therapy. Logistic regression modelling was used to analyse differences in factors associated with good neurologic outcome between women and men, as well as possible interaction of those factors with gender.

RESULTS: We enrolled 169 patients, 31 (19%) of them women, mean age 60 [+/-] 11 yrs. Risk factors didn’t vary too much between the sexes, except smoking habits (f<m) and previous MCI s (f<m). Female OHCA was witnessed significantly more often (88% vs. 68%), but an AED was rarely (3% vs. 21%) used on women. Overall women survived less (47% vs. 53% at 30 days), but those who did survive had a better neurological outcome.

CONCLUSIONS: A higher rate of witnessed cardiac arrest, and thus a better chain of survival might explain women’s better neurologic outcomes. Further analyses of possible influences of past medical history on outcomes are needed.

P 216 Variability of Cytokine Profiles in Bronchoalveolar Lavage Fluids of Clinically Stable CF-Patients


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Cystic fibrosis (CF) is a monogenetic multisystem disease. The major cause for morbidity and mortality of CF continues to be the progressive chronic pulmonary inflammation. Elevated levels of cytokines in CF lungs have been detected in previous studies upon clinical exacerbation, but also at baseline. This work assesses the variability of cytokine levels in CF-patients over time. Bronchoalveolar lavage fluid (BALF) was longitudinally obtained from 51 children and adolescents with CF at the Department of Pediatrics and Adolescent medicine at the Medical University of Vienna as part of a surveillance program. Each patient contributed at 2 to 7 samples. At each visit, the patients were categorized on whether their disease was stable or exacerbated. Cytokine levels were measured via multiplex enzyme linked immunosorbent assay (ELISA). The results showed that, IL-1β, IL-5, IL-17A and IL-12p70 were significantly upregulated in the airways of exacerbated patients (p<0.05). When considering all repetitive measurements and their connection with individuals, a regression curve (x-axis=age and y-axis=cytokine level) was calculated for IL-5 (p<0.05, nLevel1=148), IL-13 (p<0.01, nLevel1=148) and IL-12p70 (p<0.01, nLevel1=148).
Interestingly, do the levels of key inflammatory cytokines (IL-1β, IL-5, IL-6, IL-13, IFN-γ, IL-12p70 and TARC) suggest visually a stability over the course of the visits. In conclusion, this data shows elevations of IL-1β and IL-17A (TH17) and IL-5 (TH2), but also IL-12p70 (TH1) in exacerbated patients and supports partly the notion of worse health in patients with a TH17 and TH2 skewed cytokine profile. Further a relationship between cytokine trends and age is demonstrated in line with disease progression. Nevertheless, there is also some evidence for a stability of cytokine levels over time that suggests an ongoing inflammation that may allow individual risk predictions over time.

P 217 Presence and cell-type expression of IL-1β, IL-1Receptor and Caspase-1 in Rasmussen Encephalitis

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Rasmussen Encephalitis (RE) is a rare progressive epileptic disorder with unknown etiology. It is characterized by unilateral hemispheric inflammation and seizures. Inflammatory mediators, such as cytokines, have been shown to be highly epileptogenic in animal models, among them interleukin 1β (IL1β).

During inflammation, IL1β can be released after formation of the inflammasome, a multiprotein complex which includes Caspase1, a key enzyme for activation of IL1β. Little is known about the role of inflammasomes and IL1β in the course of RE. To analyze the induction and presence of inflammasomes and IL1β, we used formalin-fixed paraffin-embedded (FFPE) tissue and cryo tissue from human RE patients (n=18) and controls (n=11). We measured IL1β and its receptor, interleukin 1 receptor 1 (IL1R1), by qPCR and analyzed gene expression differences along the progression of the disease. Moreover, we investigated which cell types in the CNS express IL1β, IL1R1 and Caspase-1 by double and triple confocal fluorescence stainings with markers for astrocytes (GFAP), microglia (Iba-1 and CD68) and neurons (NeuN).

Our qPCR data revealed that RE patients of all stages have a significantly higher expression of IL1β and IL1R mRNA compared to controls. We showed the presence of IL1β protein in RE by immunohistochemical (IHC) stainings and quantification of IL1β+ cells. Our confocal fluorescence stainings showed that IL1β and Caspase1 were expressed in microglia but not in astrocytes, with Caspase1 positive cells being more abundant than IL1β positive cells. IL1R1 on the other hand was expressed in microglia as well as in astrocytes. This study gives first insights in the possible role of IL1β in RE and shows the upregulation of the cytokine in the disease. Moreover, it provides a better understanding of the cells in the CNS that express IL1β, IL1R1 and Caspase1 and therefore contributes to a better understanding of the function of these cells in human pathology.
The role of the low-density lipoprotein receptor-related protein 4 in muscle-specific kinase expression and signaling during neuromuscular junction formation

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The neuromuscular junction [NMJ] transfers signals from a motor neuron to muscle fibers and thereby regulates skeletal muscle movement. The low-density lipoprotein receptor-related protein [Lrp4] is one of the key players in the formation of the NMJ. It localizes at the NMJ and forms a complex with the muscle-specific kinase [MuSK] that represents a receptor tyrosine kinase expressed selectively in skeletal muscles. The binding of the heparansulfate glycane Agrin to Lrp4 results in the activation of MuSK. Agrin is secreted by motor neurons and deposited in the basal lamina of the synaptic cleft. N-Agrin does not interact directly with MuSK but rather binds Lrp4. MuSK activation leads to the clustering of acetylcholine receptors [AChRs] at the NMJ. Patients with myasthenia gravis, a severe disorder of neuromuscular transmission, develop either antibodies to AChRs, to MuSK or to Lrp4. Cross-talk between endocytosis and signaling raised the interest to study MuSK trafficking and its role in the formation of the NMJ. At this stage, it is unclear what the turnover rate of MuSK protein is and how endocytosis is regulated (e.g. together with Lrp4). The aim of this thesis is to characterize the role of Lrp4 in MuSK trafficking/endocytosis upon Agrin treatment. MuSK endocytosis is studied in an in-vitro muscle cell system. In order to determine the influence of Lrp4 in MuSK endocytosis, trafficking and signaling, an Lrp4-knockout line in mouse-derived myoblasts via the targeted genome editing technique CRISPR/Cas9 has been generated. Biochemical analyses to determine the amount of surface MuSK in WT and KO cells upon different treatments will be the method of choice. Furthermore, we will study the effect on MuSK activation and downstream signaling. With this study we expect to elucidate how Lrp4 affects MuSK endocytosis.

Mechanism of Immunglobulin G - Uptake in Megakaryocytes and Platelets


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Background: Circulating antigen-specific immunoglobulin G (IgG) proteins play a central part for humoral immune response against viral infections. Platelets have been shown to
contain intracellular IgGs that can be released during platelet-activation. However, it is unknown how IgGs are taken up into megakaryocytes (MK) and/or platelets.

Aim: The aim of the study was to investigate the basic mechanism of IgG uptake into megakaryocytes and platelets.

Methods: IgG content and localisation of murine megakaryocytes (mMK) and platelets was measured by flow cytometry and by immunofluorescence microscopy, respectively.

Results: Surface IgG levels of mMK incubated for 3 hours with wildtype or receptor binding-deficient IgG showed that IgG binding to mMKs was largely independent of Fc[gamma]R, but partially depended on the neonatal Fc receptor (FcRn). In line with this observation, cultured MKs from FcRn-deficient mice showed a significant decrease in IgG uptake potential. Also, isolated and cultured MKs but not plasma cells from beta-2-microglobulin (b2m)-deficient mice showed significantly reduced IgG content compared to wildtype mice. Further, intracellular IgG levels were reduced in both FcRn- and b2m-deficient platelets.

Conclusion: Our data show that both FcRn and b2m play crucial roles in antibody uptake from the microenvironment into megakaryocytes, which might rescue IgG from degradation.

P 220 Impact of IKK2 on platelet degranulation


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Platelets are key players and master regulators in primary haemostasis and prevent excessive blood loss upon vessel injury. Presence of extracellular matrix proteins leads to platelet activation, degranulation and aggregation. It was previously reported that the platelet specific deletion of inhibitor of NF-κB kinase (IKK2) inhibits their granulation to a large extent, and leads to severe bleeding. The authors used a PF4 Cre IKK2flox/flox with a deletion of exon 7, resulting in a kinase-dead version of IKK2.

Interestingly, although also using a PF4 Cre IKK2flox/flox mouse, we were not able to reproduce this striking phenotype, as the mouse we use lacks exon 3. In order to investigate whether IKK2 is truly deleted in our mouse line and how this affects platelet function, we simulated and compared the deletion of exon 3 and exon 7 of IKK2 in silico. Furthermore, the conditional knockout will be verified by Western Blot of platelets and their progenitor cells, megakaryocytes. Moreover, we will test the functional loss of IKK2 by measuring NF-κB activity upon stimulation. Additionally, platelet degranulation, aggregation and adhesion will be investigated, and in vivo assays, like tail bleeding, will be conducted to assess platelet function. In conclusion, the difference between the two mouse models is apparently the exon deletion; our mouse line may lead to a truncated, rapidly degraded IKK2 protein with a premature termination codon. The published kinase-dead protein on the other hand, may act dominantly negative instead. This could result in diverging platelet
activation behaviours. It can be therefore assumed that the interplay of IKK2 and platelet degranulation is more complex than it seems at a first glance.