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ABSTRACTS

CONFERENCE KEY-NOTE: Mechanobiology: A forceful player in health and disease
Viola Vogel, Chair of the Department of Health Sciences and Technology heading the Laboratory of Applied Mechanobiology, ETH Zurich (CH)

While commercial adhesins are typically rather non-specific when it comes to gluing objects together, and get weaker as the tensile load is increasing, bacteria have engineered a variety of highly specific adhesins with unique and unmatched mechanical properties. E. Coli evolved catch-bond adhesins that bind stronger to surfaces that are washed by fluid flow and can thereby colonize a range of technical and biological surfaces that are exposed to flow. S. Aureus specialized on entering host organisms through wound sites and have evolved adhesins that can distinguish health versus diseased tissue fibres. Understanding how such adhesins are designed at the nanoscale and operate far outside of equilibrium to fulfil their tasks is not only interesting academically, but can be exploited for a range of medical applications. Here we will discuss how macrophages take advantage of E. Coli's adhesins to hunt and capture their prey, how penicillin-derived antibodies might interfere with this process, and how the adhesins of S. aureus can be exploited as mechanosensitive probes to read out the tensional state of tissue fibres.

SESSION I: CARDIOVASCULAR RESEARCH

KEY-NOTE LECTURE: Early life stress: An emerging novel cardiovascular disease risk factor
by Jennifer S. Pollock, The University of Alabama at Birmingham (US)

The CDC Adverse Childhood Experiences (ACEs) project identified ELS as a unique risk factor for ischemic heart disease over traditional CVD risk factors, such as smoking and obesity. We demonstrated in young adults (median age 21, n=221) that higher ACEs is associated with elevated diastolic blood pressure, increased pulse wave velocity and total peripheral resistance. A longitudinal blood pressure study shows that higher numbers of ACEs were linked to an increasing trajectory of systolic and diastolic blood pressure elevation in adulthood. Further, we reported that young adults (median age 21) exposed to ELS have elevated circulating endothelin-1 (ET-1, vasoconstrictor and pro-inflammatory peptide), when compared to young adults with no ELS, linking ACEs to CV risk in young adults. In addition, results from our group show that in a subset of adults with high exposure to ACEs (>4) have reduced flow-mediated dilation as well as a loss of microvascular function measured with laser Doppler flowmetry compared to adults with no ACE exposures. These findings point towards that childhood adversity may initiate adulthood cardiovascular disease. Maternal separation with early weaning (MSEW) is a model of early life neglect (or ELS) without changes in growth rates. MSEW generates robust effects on anxiety and behavioural despair as well as immunological consequences in adulthood. These adult behavioural and immunological outcomes are similar to those observed in adult humans with childhood adversity. We found that MSEW mice have increased plasma ET-1, similar to young adults with ACEs. MSEW induces a pro-oxidant, pro-inflammatory vascular phenotype when compared to normally-reared mice linking MSEW to early origins of CVD. In conclusion, these findings in humans and animal models provide mechanistic insight for ELS induced cardiovascular disease risk.

BEST SUBMITTED ABSTRACTS

Characterization of the post-CLP microbial fluctuations in the blood and lavage by Next Generation Sequencing and bacterial cultures
Authors: Drechsler, Susanne; Walz, Madeline; Sohn, Kai; Ehling-Schulz, Monika; Osuchowski, Marcin
Presenter: Susanne Drechsler, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

After cecal ligation and puncture (CLP) bacteria spread from the peritoneal cavity into the systemic circulation. It remains unclear which/how rapidly bacteria can enter the blood stream. We aimed to characterize the dynamic pattern of microbial cell-free DNA (cfDNA) changes in blood and peritoneal lavage during acute CLP-sepsis and to compare it to standard microbiology cultures from the same samples. 3-month-old female CD-1 mice (n=24) underwent mild-severity CLP with and without antibiotic treatment. Mice were sacrificed at 0, 24, 48 and 72h for whole blood and peritoneal lavage. Body temperature and facial vein blood samples were collected repeatedly. cfDNA from plasma and total DNA from peritoneal lavages were analysed using next generation sequencing (NGS; high-throughput Illumina sequencing). Classified non-human reads were normalized and the sum of top five species per sample were calculated and correlated with CFU/ml of blood. Serial dilutions of samples were incubated under aerobic and anaerobic conditions for 24h at 37°C to determine total cell counts. Five colonies per sample were randomly picked and subjected to MALDI-TOF MS and FTIR analysis for bacterial species identification and subtyping. Overall, NGS data quantitatively matched with microbiology cultures especially in the lavage samples. In several mice, mainly those treated with antibiotics, positive NGS detection in plasma was not confirmed by bacterial culture of whole blood. The levels of cfDNA matched with CLP severity, i.e. decreased body temperature corresponded to higher bacterial load. Bacterial species identified in plasma from individual septic mice via NGS mostly matched qualitatively with those detected from microbiology cultures. The most abundant bacterial species detected in whole blood from septic mice and humans were identical, namely E. Coli, Enterococci, and Bacteroides. Isolated microorganisms did not develop resistance to the applied antibiotics (imipenem/cilastatin). This is the first characterization of microbiome fluctuations within the acute phase of CLP sepsis. NGS demonstrated higher sensitivity in detecting bacteria than standard microbiology cultures. NGS readout corresponds qualitatively to the microbial culture based approach. Supported by FWF T707-B13.

A-to-I editing of MicroRNAs alters Target Gene Selection and promotes Neovascularization after Ischemia
Authors: van der Kwast, Reginald; van Ingen, Eva; Parma, Laura; Peters, Erna; Quax, Paul; Nossent, Yael
Presenter: Reginald van der Kwast, Leiden University Medical Center, Leiden, Netherlands

Background: MicroRNAs play an important role in cardiovascular pathology. However, like other RNA species, microRNAs can be subject to adenosine-to-inosine (A-to-I) editing. Inosine residues act like guanosine residues in Watson-Crick base-pairing. Therefore, A-to-I editing of microRNAs can have drastic effects on microRNA function, including a complete shift in target gene selection. Methods and Results: We identified ischemia-induced A-to-I editing events in the seed sequences of 6 vaso-active precursor-microRNAs, both in muscle tissue of C57BL/6 mice following hind limb ischemia and in human arterial fibroblasts. One of these microRNAs, miR487b has very few conserved putative target genes. We hypothesized that A-to-I editing expands the targetome of miR-487b during ischemia. Using sequence specific endonuclease digestion, we both confirmed and quantified A-to-I editing in the seed sequence of miR487b. In the ischemic gastrocnemius muscle, primary-miR487b editing increased from 6.7±0.4% before to 11.7±1.6% (P=0.02) one day after ischemia. The edited primary-miR487b is processed into a mature microRNA, with a unique seed sequence, miR487b-ED, which is also upregulated following ischemia. In contrast to the wildtype miR487b's targetome, miR487b-ED's targetome is enriched for angiogenesis-associated pathways. Moreover, using whole exome microarray, we found that only miR487b-ED's targetome is actively repressed during neovascularization following hindlimb ischemia. Indeed, microRNA-overexpression experiments showed that miR487b-ED causes a 3-fold increase in neovascularization compared to miR487b in ex vivo aortic ring sprouting assays and it also increases migration of human endothelial cells and fibroblasts. We are currently investigating whether the other 5 microRNAs that show ischemia-induced A-to-I editing play a similar role in post-ischemic angiogenesis. Furthermore, we aim to study whether editing of microRNAs also influences other types of vascular remodelling, like atherosclerosis. Conclusions: Multiple microRNAs are edited in their seed-sequence following ischemia in both mice and human cells. Editing of miR487b is upregulated after ischemia and causes a shift in target gene selection, resulting in a novel, pro-angiogenic microRNA, miR487b-ED.

Evaluation of atrial flow patterns on flow field inside of the supported-ventricle

Authors: Ghodrati, Mojgan; Zonta, Francesco; Moscato, Francesco; Aigner, Philipp; Schima, Henrich
Presenter: Mojgan Ghodrati, Medical University of Vienna, Vienna, Austria

Usually simulation of the ventricular flow patterns is based on straight inflow conditions from the atrium without considering asymmetries arising from the contribution of pulmonary veins. In this study, the influences of the atrial flow conditions – including rotation and asymmetric flow profiles – on the intraventricular flow patterns were investigated via Computational Fluid Dynamics (CFD) simulations. A left ventricular model with a mechanical support device was used for three different simulations with laminar methods. At first was performed with normal velocity at the inflow as a validation study against PIV (pump speed: 2800 rpm, flow rate: 3.5 lit/min), secondly added a rotational component to the velocity field at the inflow (30 rpm) and third with an asymmetric inflow combining (60%/40% left/right flow ratio to replicate physiologic uneven flow distribution of the pulmonary veins'). Deviation of the velocity angle with respect to normal velocity at the inflow, as well as stagnation areas (velocity<0.01m/s) and instability of the vortical structures from the standard deviation (STD) of Q-value were calculated. The measured and calculated velocity angles at the ventricular inflow in the symmetric condition were comparable (PIV: 0.6°, CFD: 1.2°). By adding rotation or radial asymmetry these angles changed (CFDrot: 3.8°, CFDasym: -4.2°). With the rotational velocity at the inflow, less stagnation areas were detected (CFDrot: 3.4cm²,CFD: 4.9cm², CFDasym: 4.6cm²). Unstable vortex structures occurred when comparing inflow rotation and asymmetry with straight profile (STD of Q-value, CFD: 0.07 vs. CFDrot: 0.1, CFDasym: 0.09). Neglecting the atrial flow conditions could lead to inaccurate simulation of the ventricular flow pattern. Hence, reliable prediction of ventricular stagnation areas and recirculation zones requires also the consideration of the atrial inflow conditions.

Normalized vessel volume from quantitative computed tomography correlates with functional metrics and predicts survival in idiopathic pulmonary fibrosis

Authors: Pienn, Michael; Jacob, Joseph; Payer, Christian; Urschler, Martin; Kokosi, Maria; Devaraj, Anand; Olschewski, Horst; Wells, Athol U.
Presenter: Michael Pienn, Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria

Background: Recently, vessel volume normalized to the patient's lung volume has been shown to correlate with functional indices and can predict survival in idiopathic pulmonary fibrosis (IPF). Objective: To investigate whether normalized vessel volume determined by our new fully-automated artery/vein separation algorithm can reproduce the previous findings and whether separate analysis of arteries and veins can add information. Methods: 152 IPF patients had normalized vessel volume quantified from CT images by our algorithm. Separate quantification of vessel metrics in pulmonary arteries and veins was performed in 106 patients. Results were evaluated against readouts from lung function tests and survival data. RESULTS: Vessel volume expressed as a percentage of total lung volume was moderately correlated with functional indices on univariate linear regression analysis: forced vital capacity (R2=0.27, p<0.001); diffusion capacity of carbon monoxide (DLCO; R2=0.12, p<0.001); total lung capacity (R2=0.45, p<0.001); composite physiologic index (R2=0.28, p<0.001). There was no correlation with the transfer factor for carbon monoxide. Coefficients of correlation were weaker for normalized arterial and venous vessel volume than for the total vessel volume. Increased normalized vessel volume was associated with worse survival (Hazard ratio (HR): 1.92, p<0.001). Multivariate Cox regression analysis showed that normalized vessel volume was a stronger predictor of mortality (HR: 1.93, p<0.001) than DLCO (HR: 0.97, p=0.009) when adjusted for age, gender, smoking status and use of antifibrotic medication. When normalized vessel volume was replaced with normalized arterial or venous volume in this model, both were predictors of mortality (HR: 2.19, p=0.012, and HR: 3.77, p<0.001, respectively). Conclusion: Our study confirms previous observations of a predictive value of vessel volume for survival in IPF using our new vessel-analysis tool. The normalized vessel volume is correlated with results from lung function tests. While separate analysis of pulmonary arteries and veins after automatic segmentation did not enhance functional correlations when compared to total vessel scores, the predictive value of normalized venous volume seems to be slightly stronger than arterial volume.

SESSION II: MENTAL HEALTH, PSYCHOLOGY, PSYCHIATRY

KEY-NOTE LECTURE: **The development of novel treatment strategies for posttraumatic stress disorder (PTSD) using a translational approach**

By Ulrike Schmidt, University Medical Center Göttingen, Germany

Posttraumatic Stress Disorder (PTSD) occurs in a substantial percentage of individuals in the aftermath of exposure to a traumatic event such as violence, natural catastrophes, combat or sexual abuse. Trauma-focused cognitive behavioural psychotherapy is the current gold standard for PTSD treatment and is often combined with drug treatment, in particular with serotonin-reuptake-inhibitor (SSRI) antidepressants which are considered the most effective drugs for PTSD treatment. However, the facts that a significant proportion of PTSD patients does neither profit significantly from PTSD psychotherapy nor from drug treatment and, furthermore, that psychotherapies are time- and cost-consuming, urge the need for optimization of the current PTSD treatment options and development of novel PTSD treatment strategies. The latter requires the better understanding of PTSD pathobiology, ideally the identification of the molecular basis of the different PTSD symptoms which are aversive re-experiencing of traumatic memories, avoidance of trauma-related cues (e.g. avoidance of people reminding the patient of the traumatic incident), nervous hyperarousal and emotional numbing, i.e. the feeling to be unable to feel something. A translational research approach combines various experimental models and techniques. This talk focuses on the translational identification of molecular markers and factors for PTSD in PTSD patients in relation to healthy controls as well as in mouse models for PTSD-like syndromes. Animal experiments allow to elucidate the function and regulation of the molecules that we identified in the blood and saliva and hair of PTSD patients belonging to different cohorts (i.e. German soldiers, refugees settled in Uganda) and are, furthermore, a prerequisite for the development of drugs for human use. In summary, this talk presents distinct neuropeptides, as potential novel drug treatments for PTSD and several candidate molecules for PTSD pathobiology which belong to different molecule families, inter alia to the hypothalamic-pituitary-adrenal (HPA) stress hormone axis and might represent molecular targets for novel drugs for PTSD treatments. Furthermore, our studies support the hypothesis that the different symptoms of PTSD, and possibly of psychiatric syndromes in general, have different molecular underpinnings and might thus require different treatments.

BEST SUBMITTED ABSTRACTS

Using administrative data to learn about the uptake of mental health benefits by mentally ill parents: An analysis of Tyrolean health insurance data

Authors: Zechmeister-Koss, Ingrid; Tüchler, Heinz; Goodyear, Melinda; Paul, Jean
Presenter: Ingrid Zechmeister-Koss, Ludwig Boltzmann Institute for Health Technology Assessment, Vienna, Austria & Research group Village – How to raise the village to raise the child, Innsbruck, Austria

Background: Parental mental illness can have negative impacts on their children's health. Different approaches can be followed for the early identification and support of children of parents with a mental illness. One approach is to increase the opportunities for identification of children when parents come into contact with adult mental health services. To explore this strategy further, information on the users of adult mental health care is required. Aims and Methods: Data from the Tyrolean health insurance (TGKK) that covers 80% of the population are analysed addressing: a) use of physical mental health services (hospital, outpatient psychiatrists, psychotherapy, rehabilitation), b) medication, and c) sick-leave in order to identify benefit use characteristics of parents with a mental illness. Results: In 2017, 85722 persons (~15% of insured) used one or more benefits (co-) funded by the TGKK. Almost 60% of them were between 19-64 years old, thus potential parents of dependent children. Almost two third of them were females. 40% of them used a mental health service, 7% were treated in hospitals, while 50% received medication only. Discussion: Via the hospital setting, only a small proportion of parents can be reached, however these are likely to be those with the highest need for support for

themselves and their children. Through other mental health services, and even more by the primary healthcare system (where drugs are mainly prescribed), a far higher but less severely ill proportion of parents could be contacted. For a full picture data on further available adult mental health care services (e.g. funded by other payers) would be desirable. Conclusion: The proportion of parents with a mental illness that one may be able to reach via adult mental health care differs considerably according to the health setting chosen. Each setting has different pros and cons regarding disease characteristics of patients as well as logistics for implementing and evaluating support. For planning those activities, administrative data are a useful piece of information.

Lower Cognitive Reappraisal Capacity in Poly-Drug Use: An fMRI Study

Authors: Hiebler-Ragger, Michaela; Perchtold, Corinna M.; Unterrainer, Human F.; Fuchshuber, Jürgen; Koschutnig, Karl; Kapfhammer, Hans-Peter; Papousek, Ilona; Weiß, Elisabeth M.; Fink, Andreas

Presenter: Michaela Hiebler-Ragger, Medical University of Graz, Graz, Austria

Background: Insecure attachment, impaired personality structure and their relation to deficient emotion regulation are well documented in Substance Use Disorders (SUD). While negative emotions can trigger drug-use and relapse, cognitive reappraisal (CR) may reduce the emotional strain by promoting a change in perspective. In the present study, we explored behavioural and neural correlates of CR in Poly Drug Use Disorder (PUD) using a novel maximum performance test for CR. Material and Methods: 18 PUD inpatients and 16 healthy controls completed the Adult Attachment Scale, the Emotion Regulation Questionnaire, the Brief Symptom Inventory, the Wonderlic Personnel Test, the OPD (Operationalized Psychodynamic Diagnostics) Structure Questionnaire as well as two versions (during fMRI and outside the scanner) of the Reappraisal Inventiveness Test (RIT). In each form of the RIT, subjects are instructed to empathize with anger-eliciting situations and to consequently generate different reappraisals in order to downregulate anger. Results: PUD inpatients reported impairments with generally large effect sizes (eta2 = .12 to .58; p< .05) in personality structure, attachment and emotion regulation abilities compared to controls. Regarding CR, PUD inpatients showed less flexible and fluent reappraisals (botheta2 = .30; p< .01) as well as higher levels of induced anger than controls (eta2 = .15; p< .05). However, the reappraisal-related neural activation pattern for PUD and controls was remarkably similar. A conjunction analysis on voxels significantly activated in both groups revealed that PUD and controls activated the left inferior and superior frontal gyri, the right cerebellum as well as the right middle temporal cortex during CR. Conclusion: While the observed activation during CR fits almost perfectly with previous findings, the poorer task performance in PUD may point to an inefficient use of this activation, e.g. due to white matter impairments. Future research might also investigate automatic and largely unconscious processes of emotion regulation. From a more clinical perspective, our results highlight the importance of addressing inadequate emotion regulation strategies as the expression of insecure attachment and impaired personality structure in SUD treatment.

Suicide Risk and Suicide Prevention in Austrian Patients with Chronic Skin Conditions from the Dermatologist's Point of View. An Online Survey Study

Authors: Pronizius, Ekaterina; Voracek, Martin

Presenter: Ekaterina Pronizius, University of Vienna, Vienna, Austria

More than 1,000 Austrian citizens take yearly their own life. One of the suicide risk factors is a chronic illness. The goal of my study was to estimate the rate of suicidal ideations in patients with atopic dermatitis, psoriasis, and acne from dermatologists' point of view. A link to the online ten-minute questionnaire that was specially developed for this study was emailed to 450 self-employed dermatologists of Austria. 45 (18 females) skin doctors have participated. The key results of my study are low rates of suicidal thoughts, suicide attempts, and suicides in Austrian dermatological practices. However, the majority of the sample (82%) are aware of the fact that these patients are at a higher suicide risk. 60% of the participants also believe, that it rather would not be a problem for them to recognize suicidal ideations in their patients. The intervention steps when facing patients in a suicide crisis are: Referring them to a specialist in a psychiatry and having a conversation about it. The most challenging about suicide is for the sample the lack of time and knowledge. The majority of participants are also interested in cooperation with mental health professionals, implementation of new intervention/prevention strategies, and are wishing for more suicide-related training programs. Statistical data analysis revealed several significant results. As expected, private specialists are having fewer patients but spending more time with them, compared to the contract physicians. Yet, these differences don't seem to influence the quality of treatment they provide. The quality of treatment was defined as the extent to which doctors tell their patients that an additional psychological treatment could be helpful and ask them about their emotional state. The two variables that have an impact on the quality of treatment are female gender and psychological background (e.g. a degree in psychology). The possible explanations for the low rate of suicidal ideations are an advanced Austrian health care system and/or dermatologists' underestimation of the suicide risk. Implications of the study are to promote the cooperation between dermatologists and mental health professionals and to address the problem of the suicidality from the first-person perspective (patients).

Psychotherapy – Effect factors and a German-speaking country comparison of legal regulations

Authors: Rosian, Katharina; Winkler, Roman

Presenter: Katharina Rosian, Ludwig Boltzmann Institute Health Technology Assessment, Vienna, Austria

Psychotherapy has become an independent scientific discipline in the German-speaking area. Since it is characterized by a multitude of effective factors, we were interested in the difference between general and specific effect factors. In addition, we provided a comparison of legal regulations in the German-speaking area with respect to education and training, as well as the recognition of methods in psychotherapy. In the context of this project, a narrative-descriptive synthesis of the literature was performed based on a systematic literature search and manual searches. Additionally, we conducted a semi-structured interview with three experts from the Federal Joint Committee (G-BA). Overall, general and specific effect factors are associated with therapeutic effects. General effect factors summarize all therapeutic variables that are present across all psychotherapeutic methods. The significance of general effect factors for positive therapeutic effects is considered decisive. In contrast, specific effect factors are explicit theoretic techniques that are anchored in psychotherapeutic methods and they serve a specific target achievement in psychotherapy. Regarding the legal regulations and requirements for state recognition of new methods in psychotherapy, numerous differences could be shown: In Austria and Switzerland there are criteria in guidelines that have to be met for the recognition of new psychotherapeutic methods. Whereas in Germany, the recognition is regulated by a benefit assessment procedure. As a result, there are varying numbers of psychotherapeutic methods eligible for reimbursement by national insurances in Germany (n=3), Switzerland (n=23), and Austria (n=23). Further, the requirements for the professional practice of psychotherapy are regulated differently in the German-speaking area. As a conclusion, it has transpired that general effect factors are attributed greater importance for achieving therapeutic effects than, for example, the application of specific therapy techniques. Looking at the results of legal regulations, the differences indicate that requirements and standards for the recognition of a new psychotherapeutic method differ substantially, which can be attributed to heterogeneous national legislations.

SESSION III: REGENERATIVE MEDICINE RESEARCH

KEY-NOTE LECTURE: Bridging the gap: Skeletal cell based strategies for bone repair

by Richard Oreffo, University of Southampton, United Kingdom

Advances in our understanding of skeletal stem cells and their role in bone development and repair, offer the potential to open new frontiers in bone regeneration. However, the ability to harness these cells to replace or restore the function of traumatised or lost skeletal tissue as a consequence of age or disease remains a significant challenge. We have developed protocols for the isolation, expansion and translational application of skeletal cell populations with cues from developmental biology informed by in vitro and ex vivo models as well as, nanoscale architecture and biomimetic niche

development informing our skeletal tissue engineering approaches. We demonstrate the importance of biomimetic cues and delivery strategies to directly modulate differentiation of human adult skeletal cells and, central to clinical application, large animal in vivo translational studies to examine the efficacy of skeletal stem and cell populations in innovative scaffold compositions for orthopaedics. These are exciting times in skeletal cell biology and tissue regeneration. While a number of challenges remain and will be reviewed, including the need to harness multidisciplinary approaches that integrate developmental and engineering processes as well as cell, molecular and clinical techniques for skeletal tissue engineering. Nonetheless, advances in our understanding of skeletal cells and the role of environmental cues offer the potential to open new frontiers across the hard tissue interface and exciting opportunities to improve the quality of life of an increasing ageing population.

BEST SUBMITTED ABSTRACTS

Skin and muscle connective tissue cells in axolotl bone healing

Authors: Polikarpova, Anastasia; Tanaka, Elly M.
Presenter: Anastasia Polikarpova, Research Institute of Molecular Pathology (IMP), Vienna, Austria

Large and complicated bone fractures fail to regenerate. In order to develop an effective treatment, a good understating of the molecular mechanism of fracture callus formation and bone healing is necessary. Axolotls have an exceptional ability to regenerate the amputated limb including all skeletal elements via formation of a transient progenitor cell mass, the blastema. In axolotl regenerating limb periskeletal cells build the proximal bone next to the injury, while soft connective tissue (SCT) cells migrate more distally and build the distal region of the bone. However, Axolotls cannot heal bone critical-size defects (CSD, 30% of the bone length), which can be potentially caused by diminished migration ability of SCT cells, leading to insufficient bone callus formation and fracture non-union. Here we aim to understand if SCT cells contribute to CSD healing in the axolotl. For this purpose, we have developed an axolotl femur fracture model comparable to mammalian bone fracture models. An external upper hind limb fixator was used to ensure better femur alignment upon osteotomy and constant distance between the bone fragments than the limbs without fixator. Importantly, CSD limbs without fixator collapse and heal as a small bone fracture. In small fracture samples, we observed callus formation and fracture bridging at 3-6 weeks, and woven bone formation at 12 weeks post-surgery. In CSD samples, fracture bridging was not observed at 12 weeks post-surgery. To trace SCT cells, full-thickness skin or muscle bundles from axolotls with SCT cell-specific fluorescent labelling using Prrx1 (limb bud and blastema-specific) and Col1a2 (cartilage progenitor marker) promoters, was transplanted to wildtype animals. Then, femur small fractures or CSD were created. After 3 weeks, the fluorescently labelled cells were observed in the fracture region. Further investigation of SCT cell proliferation and differentiation into cartilage/bone in fractures is necessary. This will shed light on the potential differences in SCT capacity to contribute to bone healing in limb regeneration, in small and critical-size bone fracture, offering processes which could be targeted by therapies.

BioImaging Austria: Correlated Multimodal Imaging Across Scales in Life Sciences

Authors: Walter, Andreas
Presenter: Andreas Walter, Vienna BioCenter Core Facilities GmbH, Vienna, Austria

The Correlated Multimodal Imaging Node Austria (CMI) is the official Austrian Euro-BioImaging initiative of leading imaging experts in Austria. It was established as a consortium of eight universities and research institutes in Austria (www.bioimaging-austria.at) to foster correlated multimodal imaging across scales in life sciences. Correlated imaging gathers information about the specimen with two or more complementary modalities that – combined – create a composite view of the sample. CMI offers a multitude of state-of-the-art imaging technologies from MRI to super-resolution microscopy, numerous multimodal imaging pipelines, and various support services, such as image analysis, to scientists on a national and international level. Imaging techniques at CMI span the entire resolution range of interest for preclinical and biological studies, and provide complementary sample information about structure, function, dynamics and chemical composition. More than 30 imaging techniques allow both in- and ex-vivo imaging and molecular analysis. CMI is developing entirely new multimodal workflows at the forefront of correlated imaging, which can involve more than two imaging modalities. CMI explicitly goes beyond Correlated Light and Electron Microscopy (CLEM) or (pre) clinical hybrid imaging – and hence significantly advances the field of correlated imaging with truly holistic approaches. In this talk, we will present first multimodal pilot projects to answer previously inaccessible biomedical questions mechanistically. We will focus specifically on characterizing and quantifying regeneration processes in osteonecrosis of the jaw bone by combining micro-Computed Tomography (microCT), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), and X-Ray Fluorescence Spectrometry (XRF). This multimodal pipeline allows us to assess and correlate density, mineralization, morphology, topology, mechanistic properties, such as Young's Modulus, and chemical composition of the jaw bone during the regeneration processes.

Laser strategies to overcome the repopulation challenges of dense articular cartilage matrices

Authors: Schneider, Cornelia; Rieder, Bernhard; Monforte, Xavier; Teuschl, Andreas; Heimel, Patrick; Zehetner, Hans; van Osch, Gerjo; Wolbank, Susanne; Redl; Nürnberger, Sylvia
Presenter: Sylvia Nürnberger, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Scaffold composition and architecture have a significant impact on cartilage repair, yet biomaterials currently in use do not mimic the characteristics of articular cartilage. Decellularised articular cartilage has often been proposed as optimal defect filling material, but the exceptionally dense matrix makes repopulation an ongoing challenge. In this study we propose laser engraving as strategy to repopulate decellularised articular cartilage with therapeutic cells. Incisions were engraved into human articular cartilage biopsies by CO2 or femtosecond laser, choosing settings for maximal depth and minimal thermal damage. Samples were then subjected to either devitalization or decellularisation and glycosaminoglycan (GAG) depletion for seeding tests. Furthermore, to evaluate the scaffolds' performance in a cartilage defect environment, an ectopic in vivo experiment was performed with osteochondral plugs as natural environment in nude mice. Histology, quantitative polarized light microscopy, µCT, SEM and mechanical testing were done to analyse the scaffolds and tissue formation in vitro and in vivo. Engraving via CO2 laser resulted in V-shaped incisions, while fem-tosecond laser created more narrow cuts. Both incision types enabled cellular ingrowth into deep scaffold layers and enhanced the cell/scaffold contact surface. Decellularised GAG depleted scaffolds were shown to be optimal for cell adhesion. The final scaffolds featured a compressive modulus several times higher than biomaterials currently in clinical use. Histological examination after in vivo testing showed complete filling of the defect with decellularised cartilage scaffold and newly formed matrix that stained positive for cartilage markers collagen type II and GAG. The new fibres were aligned in a way typical for deep cartilage zones. We were able to create recellularised articular cartilage scaffolds with promising properties for clinical cartilage regeneration. The scaffolds support neo-cartilage formation and their mechanical properties suggest a good performance under loaded conditions in vivo.

Microarchitecture variation in the vicinity of the standard transiliac biopsy site assessed by microCT

Authors: Blouin, Stéphane; Misof, Barbara; Berzlanovich, Andrea; Gruber, Gerlinde; Klaushofer, Klaus; Fratzl, Peter; Roschger, Paul
Presenter: Stéphane Blouin, Ludwig Boltzmann Institute for Osteology, Vienna, Austria

The transiliac bone biopsy sample is used for diagnosis and management of bone pathology. Its characterization is usually performed by histomorphometry and microCT. However, little is known about trabecular and cortical microarchitecture variations with age or in the region near to the standard biopsy site (2 cm dorsal from spina iliaca superior anterior and 2 cm caudal from iliac crest). Twelve 5x5 cm iliac crest necropsy samples were obtained from control women under 65 years (median=62 yrs; n=6) or over 90 years (median=94 yrs; n=6). MicroCT images (SCANCO 50, 90kV) were acquired on the whole sample (24 µm voxel resolution) for trabecular analysis and on a 10 mm broad vertical stripe including the standard site (7.4 µm voxel resolution) for cortical porosity analysis. We analysed the inner and outer cortices on a stack of µCT images

by setting a 500 µm thick region of interest starting from the periosteal surface. The trabecular bone volume (BV/TV -41%; p=0.004), trabecular thickness (Tb.Th -18%; p=0.04) and trabecular number (Tb.N -15%; p=0.01) were lower in the older age group. A general reduction in BV/TV (-22%; p=0.001), Tb.Th (-1%; p=0.024) and Tb.N (-6%; p<0.001) was measured in 6 mm caudal direction from the standard biopsy site, with progressive reduction with larger distances. The cortical porosity (Po.V/TV), due to mainly-vertical cortical canals, was higher in the inner cortex (+362%, p=0.015) and outer cortex (+337%, p=0.002) in the older individuals, while the interpore space was lower in the inner cortex (-11%, ns) and outer cortex (-24%, p=0.002). Irrespectively of age, an increased cortical porosity (+50%; p=0.012) and a decreased interpore space (-11%; p=0.034) was found in the outer cortex compared to the inner cortex. Moreover, the cortical porosity was decreased in inner (-59%, p=0.0015) and outer (-50%; p=0.0005) cortex at 1 cm beneath the standard biopsy site. These findings indicate a massive alteration of bone trabecular microarchitecture and an increased cortical porosity with age but also a high dependency to the bone site in the iliac bone.

SESSION IV: HAEMATOLOGY AND CANCER RESEARCH

KEY-NOTE LECTURE: Clonal heterogeneity in acute myeloid leukemia

by J.J. (Jan Jacob) Schuringa, Univeristy of Groningen, Nederlands

Intra-tumour heterogeneity caused by clonal evolution is a major problem in cancer treatment. To address this problem, we performed label-free quantitative proteomics on primary acute myeloid leukemia (AML) samples. We identified 50 leukemia-enriched plasma membrane proteins enabling the prospective isolation of genetically distinct subclones from individual AML patients. Subclones differed in their regulatory phenotype, drug sensitivity, growth, and engraftment behaviour, as determined by RNA sequencing, DNase I hypersensitive site mapping, transcription factor occupancy analysis, in vitro culture, and xenograft transplantation. Finally, we show that these markers can be used to identify and longitudinally track distinct leukemic clones in patients in routine diagnostics. Our study describes a strategy for a major improvement in stratifying cancer diagnosis and treatment.

BEST SUBMITTED ABSTRACTS

NUP98-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 lead to the expression of oncogenic fusion proteins that are associated with poor prognosis. More than 25 distinct rearrangements have been identified so far, however, targeted therapy of these patients is not possible as the molecular mechanisms underlying NUP98- fusion-protein-dependent leukemogenesis are unknown. We hypothesize that leukemogenesis induced by molecularly distinct NUP98 fusion proteins depends on common oncogenic mechanisms that are encoded in the fusion proteins' abilities to specifically alter gene expression. To identify the conserved network of critical genes that is essential for leukemogenesis we aimed to elucidate global effects of distinct NUP98 fusion oncoproteins on the transcriptome in a systematic manner. We established mouse AML models driven by four common NUP98 fusions proteins that allow tetracycline (Tet)-mediated control of oncogene expression in vivo. Despite expressing molecularly distinct NUP98 oncoproteins, mice developed a phenotypically similar, transplantable AML-like disease with full penetrance, characterized by 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-fusion-protein-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. ChIP-seq analysis showed that NUP98-fusion proteins were associated with the Cdk6 promoter region. Deletion of Cdk6 delayed NUP98-fusion-protein-induced leukemia in vitro and in vivo, establishing CDK6 as a target in NUP98-fusion expressing AML. In line with this, human and mouse AML cells transformed with NUP98-fusion-proteins were hypersensitive to treatment with the CDK4/6-inhibitor Palbociclib. Thus, inhibition of CDK6 could represent a promising approach to treat patients suffering from NUP98-fusion-protein-driven AML.

The RAS/STAT5-regulated homing cell adhesion molecule CD44 triggers disease expansion in advanced systemic mastocytosis

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CD44 is a multifunctional adhesion molecule playing an essential role in homing and invasion of neoplastic stem and progenitor cells in various myeloid malignancies. Although mast cells (MC) reportedly express CD44, little is known about its prognostic impact, regulation, and function in neoplastic cells in systemic mastocytosis (SM). CD44 expression on primary neoplastic cells of 56 mastocytosis patients and its regulation on 8 native and 2 lentiviral RAS/STAT5-transduced human MC lines was analyzed by immunohistochemistry, immunocytochemistry, qPCR and flow cytometry. Soluble CD44 (sCD44) was measured in the sera of 129 mastocytosis patients by ELISA. In SM patients the CD44 surface expression on bone marrow-derived clonal CD34+/CD38− stem cells, CD34+/CD38+ progenitor cells, and CD117++/CD34− MC increased with the aggressiveness of SM and was found to correlate with overall survival. Correspondingly, higher sCD44 serum levels were measured in patients with advanced SM compared to indolent SM, cutaneous mastocytosis or healthy controls. Moreover, sCD44 levels and the mastocytosis WHO classification were found to be independent predictors for overall survival. All human MC lines examined (HMC-1, ROSA, MCPV-1) displayed cytoplasmic and cell surface CD44. In terms of regulation, we found that CD44 expression in neoplastic MC depends on RAS-MEK- and STAT5-signaling. To investigate the functional role of CD44, HMC-1.2 cells with an shRNA-mediated knockdown of CD44 or HMC-1.2 cells transduced with a control shRNA were subcutaneously injected into severe combined immunodeficient (SCID) mice. Human Alu-sequence-specific qPCR was performed to determine MC engraftment in our CD44 knockdown SCID mouse model. In these mice the knockdown of CD44 resulted in reduced MC expansion and metastasis formation as well as in prolonged survival. Together, our data show that CD44 is a RAS-MEK/STAT5-driven MC invasion receptor. In SM patients, the CD44 expression levels on neoplastic cells correlate with the aggressiveness of SM and have prognostic impact on patients' survival. Therefore, CD44 is an interesting new therapeutic target in advanced SM, which needs to be further investigated in forthcoming studies.

Intestinal failure and aberrant lipid metabolism in patients with DGAT1 deficiency

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Background: Congenital diarrheal disorders (CDDs) are rare disorders of the gastrointestinal system. Recently, mutations in diacylglycerol-acyltransferase 1 (DGAT1) have been identified in patients with CDD, but the underlying molecular mechanism remains elusive. Methods: We studied 10 patients from 6 pedigrees suffering from intestinal failure, including early-onset severe diarrhoea, vomiting, hypoalbuminemia, and/or faral protein-losing enteropathy. We performed whole-exome sequencing and analysed patient-derived fibroblasts and intestinal organoids. We confirmed the phenotype by exogenous expression of DGAT1 in patient fibroblasts and CRISPR/Cas9-guided deletion of DGAT1 in healthy control organoids. Results: We identified 5 novel biallelic loss-of-function mutations in DGAT1. DGAT1 catalyses the formation of triglyceride (TG) from diacylglycerol

(DAG) and acyl-CoA. DGAT1 mutations led to deficient protein expression and altered TG metabolism resulting in reduced lipid droplet formation. Exogenous DGAT2 expression and DGAT1 reconstitution rescued the lipid droplet formation in fibroblasts. In addition, we show that DGAT1-deficienct organoids were more susceptible to lipid-induced cell death. Conclusion: We identified the largest cohort of DGAT1-deficient patients to date and link DGAT1 deficiency to altered lipid metabolism and fat intolerance. For the first time, we show the importance of DGAT1 to fat metabolism and lipotoxicty in gut epithelium, and that exogenous DGAT1 and DGAT2 expression could rescue aberrant lipid metabolism in cells. A fat free diet can serve as first line of therapy in DGAT1-deficient patients. Our results highlight the importance of identifying known CDD-causing monogenic defects in sequencing of patients with intestinal failure for correct genetic diagnosis.

Five years of EMA-approved systemic cancer therapies for solid tumours – a comparison of two thresholds for meaningful clinical benefit

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Presenter: Nicole Grössmann, Ludwig Boltzmann Institute for Health Technology Assessment, Vienna, Austria

Objective: Several societies have proposed frameworks to evaluate the benefit of oncology drugs; one prominent tool is the European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS). Our objectives were to investigate the extent of European Medicines Agency (EMA)-approved cancer drugs that meet the threshold for "meaningful clinical benefit" (MCB), defined by the framework, and determine the change in the distribution of grades when an adapted version that addresses the scale's limitations is applied. Methods: We identified cancer drugs approved by the EMA (2011–2016). We previously proposed adaptations to the ESMO-MCBS addressing its main limitations, including use of the lower limit of the 95% confidence interval in assessing the hazard ratio. To assess the MCB, both the original and adapted ESMO-MCBS were applied to the respective approval studies. Results: In total, we identified 70 approval studies for 38 solid cancer drugs. 21% of therapies met the MCB threshold by the original ESMO-MCBS criteria. In contrast, only 11% of therapies met the threshold for MCB when the adapted ESMO-MCBS was applied. Thus 89% and 79% of therapies did not meet the MCB threshold in the adapted and original ESMO-MCBS, respectively. Conclusions: In a majority of cancer drugs, the MCB threshold is not met at the time of approval using both ESMO-MCBS scales. Since approval status does not translate into a MCB, stakeholders and decision makers should focus on the benefit/risk ratio of anticancer drugs to assure an appropriate allocation of resources in health care systems.

RAPID FIRE PRESENTATIONS

HAEMATOLOGY AND CANCER RESEARCH

CEBPA-mutated leukemia is sensitive to genetic and pharmacological inhibition of the MLL complexw

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The gene encoding the transcription factor C/EBPα is mutated in 10-15% of all patients with de novo acute myeloid leukemia (AML). N-terminal CEBPA mutations cause selective ablation of full-length C/EBPα (p42) without affecting the expression of a shorter oncogenic isoform, termed p30. As a balanced ratio of C/EBPα isoforms is crucial for hematopoietic homeostasis, depletion of p42 leads to increased cell growth and blocks myeloid differentiation, resulting in development of AML. However, the mechanistic basis of p30-induced leukemogenesis is not well understood. Here, we show that the SET/MLL histone methyltransferase complex represents a critical actionable vulnerability in CEBPA-mutated AML. The oncogenic C/EBPα p30 isoform and MLL show global co-localization on chromatin and core SET/MLL complex components exhibit robust physical interaction with p30 indicating a potential cooperative role in gene regulation. We further show that myeloid progenitor cells from a Cebpap30/p30 AML mouse model require the expression of an intact MLL protein, as targeted CRISPR/Cas9-mediated mutagenesis of Mll results in proliferation arrest and myeloid differentiation. In line with this, inhibition of the SET/MLL complex via small-molecule-inhibitors impairs proliferation, induces cell cycle arrest and causes apoptosis in mouse and human AML cells with CEBPA mutations. Global analysis of gene expression changes shows that inhibitor treatment results in myeloid differentiation of Cebpap30/p30 cells, which was confirmed by flow-cytometric analyses of myeloid maturation markers. Further, primary CEBPA-mutated leukemiacells are hypersensitive to pharmacological SET/MLL complex inhibition. Finally, we identify the transcription factor GATA2 as a direct critical target of the cooperative gene regulation between p30 and MLL in CEBPA-mutated AML. Taken together, we show that C/EBPα p30 requires the SET/MLL complex to regulate oncogenic gene
expression and that CEBPA-mutated AML is hypersensitive to perturbation of the SET/MLL complex either via genetic ablation of MLL or through pharmacological inhibition of the SET/MLL complex. These findings expand our understanding of CEBPA-mutated AML and identify the SET/MLL complex as a potential therapeutic target in this disease.

STAT3β is a tumour suppressor in acute myeloid leukemia

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Presenter: Petra Aigner, Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria

STAT3, a multifunctional regulator of transcription, is expressed in two alternatively spliced isoforms, STAT3α and truncated STAT3β. Although formerly postulated as a dominant negative form of full-length STAT3, STAT3β has been shown to have various regulatory functions and recently gained attention as a powerful anti-tumorigenic molecule in cancer. STAT3 has been found to be constitutively active in acute myeloid leukemia (AML) patients, causing a proliferative advantage and apoptosis
protection in AML blasts. Consequently, STAT3 became an attractive therapeutic target in AML, however results from early clinical studies only show moderate effectiveness. Thus, there is clearly need for improved understanding of the biological functions of STAT3 and its isoforms in AML. To gain better understanding of the function of STAT3β in leukemia we analyzed leukemic blasts derived from AML patients regarding STAT3 isoform mRNA expression. We observed a correlation between predicted prognosis and the STAT3β/α expression ratio. Specifically, a higher STAT3β/α ratio in patients was associated with
favorable prognosis and increased disease-free survival. Moreover, we generated a novel inducible Stat3β transgenic mouse model and crossed it with mice lacking PTEN, a previously described model for AML. In addition, we used an AML mouse model based on the MLL-AF9 fusion oncogene, which frequently occurs in AML patients. Here, fetal liver-derived stem cells from Stat3β transgenic mice were transduced with a retrovirus encoding for MLL-AF9 and transplanted into NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice. In both mouse models the elevated expression of STAT3β significantly delayed disease progression, impaired leukemic infiltration and increased overall survival. Although the underlying mechanisms of this anti-tumorigenic effect are still elusive, RNA-Seq analysis revealed around 70 genes specifically regulated in leukemic blasts upon Stat3β transgene expression, especially in pathways for cell surface interactions at the vascular wall. In conclusion, we demonstrated that STAT3β plays an important tumor-suppressive role in AML mouse models and that the balance of STAT3 isoforms could serve as a prognostic tool for AML patients.

Pharmacologic Targeting of Leukemic Stem Cells in Mast Cell Leukemia
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Presenter: Gregor Eisenwort, Ludwig Boltzmann Institute for Hematology and Oncology, Vienna, Austria

Leukemic stem cells (LSC) are increasingly recognized as promising targets of potentially curative therapies in various leukemias. Systemic mastocytosis (SM) is a rarem hematopoietic neoplasm characterized by an abnormal expansion of mast cells in the bone marrow (BM) and other organs. The role of LSC in the development and progression of aggressive SM (ASM) and mast cell leukemia (MCL) is poorly understood. We have recently shown that putative MCL LSC reside within a CD34+ fraction of the neoplastic clone. The aim of this study was to characterize the cell surface phenotype, frequencies and functional properties of LSC in patients with ASM and MCL and to explore the effects of various targeted drugs on the survival of these cells. LSC were purified from BM mononuclear cells by flow sorting and injected intravenously into NOD-SCID-IL-2Rg-/- miceexhibiting human membrane-bound SCF (NSGSCF). We found that the NSGSCF-engrafting LSC in MCL reside in a CD34+/CD38– fraction of the clone. As assessed by cell-dilution experiments, the frequency of LSC among all CD45+ cells ranged from 0.0003 to 0.0045%. Next we analysed the immunophenotype of LSCs in patients with MCL and ASM by flow cytometry. Compared to hematopoietic stem cells from the BM of healthy donors, MCL LSCs expressed higher levels of CD30 but lower levels of CD117. Furthermore, CD371 and CD33 were overexpressed on CD34+/CD38+ LSC in MCL compared to healthy BM. Application of the CD117-targeting drug midostaurin or the CD33-targeting drug gemtuzumab-ozogamicin (GO) did not induce apoptosis in primary MCL LSC. However, a combination of these two drugs produced a synergistic apoptosis-inducing effect on LSC. Moreover, pre-incubation with GO or a combination of GO and midostaurin inhibited the engraftment of MCL LSC in NSGSCF mice. Finally, we were able to demonstrate that treatment of MCL or ASM patients with midostaurin reduces the percentage of CD34+/CD38– cells in the BM of these patients, although LSC were never completely eliminated. Together, we have characterized the phenotype of MCL LSC and identified clinically relevant molecular targets in these cells. Since targeted drugs are able to induce apoptosis in MCL LCS, our studies may help to develop curative treatment approaches in this fatal leukemia.

Characterization of marker- and target expression profiles in CD34+/CD38− and CD34+/CD38+ stem- and progenitor-cells in AML and CML
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Presenter: Irina Sadovnik, Ludwig Boltzmann Institute for Hematology and Oncology, Vienna, Austria

In an attempt to identify novel markers and immunologic targets in leukemic stem cells (LSC) in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), we screened bone marrow samples of patients with AML (n=252), CML (n=96), and controls (n=233), including normal/reactive bone marrow and lymphoma staging samples, for expression of cell surface antigens on CD34+/CD38−and CD34+/CD38+cells by mul -color flow cytometry. In addi on, we examined mRNA expression profiles of highly purified CD34+/CD38−and CD34+/CD38+stem and progenitor cells by gene array- and qPCR analyses. Aberrantly expressed markers were iden fied in all pa ent cohorts examined. In CML, CD34+/CD38−LSC expressed an almost invariable aberra on profile defined as CD25+/CD26+/CD56+/CD93+/IL-1RAP+. By contrast, in AML, CD34+/CD38−cells variably displayed aberrant surface markers, including CD25 (IL-2RA) (55%), CD96 (Tac le) (35%), CD371 (CLL-1) (75%) and IL-1RAP (60%). With the exception of a subset of FLT3 ITD-mutated patients (40%), AML LSC did not exhibit CD26 (DPPIV). All other markers and targets identified on AML and/or CML LSC, including CD9 (Tspan29), CD33 (Siglec-3), CD44 (Pgp1), CD52 (Campath-1), CD105 (Endoglin), CD114 (G-CSFR), CD117 (SCFR/KIT), CD123 (IL-3RA), CD133 (AC133=Prominin-1), CD135 (FLT3), CD184 (CXCR4) and roundabout-4 (ROBO4), were also detected on normal hematopoietic stem cells (HSC). However, several of these cell surface targets, including CD33, CD52 and CD123, were expressed at substantially higher levels on CD34+/CD38−LSC compared to HSC. Moreover, an body-mediated immunotherapy using the CD33-targeted drug gemtuzumab ozogamicin or the CD52-targeted drug alemtuzumab resulted in LSC depletion in vitro and in a significantly reduced engraftment of LSC in NSG mice. By contrast, the CD26-targeting drug vildagliptin did not reduce engraftment of CML LSC in NSG mice. Moreover, we were able to show that the CD25-targeted drug denileukin diftitox induces apoptosis in the CD25+ CML cell line KU812 and in CML LSC. Together, we have established cell surface marker- and target expression profiles of AML LSC and CML LSC which should facilitate their enrichment, diagnostic LSC phenotyping, and the development of LSCeradicating
therapies.

MENTAL HEALTH, PSYCHOLOGY, PSYCHIATRY

A qualitative exploration of social connectedness as a resilience factor in early adolescents with and without a parent with mental illness: Focus on school transition
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Presenter: Susanne Schmalwieser, Research group D.O.T. – Die offene Tür, Krems, Austria

Objectives: Social connectedness represents the fundamental human desire for interpersonal relationships with others. It has its roots in attachment theory - in relation to maternal or paternal connectedness; and resilience theory - in relation to the protective influence that relationships with "significant others" may provide. In primary school, peer relationships begin to provide the social emotional support previously only available from the family, which makes the transition from primary to secondary education a period of increased risk of losing social ties. This may be particularly true for children of parents with mental illness (COPMI). Poor social connectedness in young people correlates with multiple negative long-term consequences, such as poor educational, social and emotional outcomes. However, up to date there is no consensus understanding of social connectedness as a resilience factor in early adolescents. The Austrian context, with relatively early transition from primary to secondary school constitutes a further specific influence on social connectedness in this age group. This qualitative study aims to capture the experiences and views of all relevant stakeholders to create an in-depth understanding of social connectedness, and related challenges and resources, around school transition in the Austrian context. Methods: Semi-structured interviews were conducted with older adolescents (14- to 16- year-olds) reflecting back on school transition, adult COPMI, parents with and without a mental illness, teachers, health and social service professionals. A thematic analysis approach was adopted for data analysis. Results: The resulting model of social connectedness shows a complex interplay of personal and environmental factors which differentially affect the quality and stability of relationships, depending not only on the properties of the interaction itself but also on the context in which interaction occurs, e.g. in school or out of school activities, at home or in online social networks. Conclusion: The dynamic model of social connectedness can serve as a basis to guide future interventions aiming to support adolescents to develop strong social relationships around school transition.

Decreased Activity of Heme Oxygenase in Cerebral Cortex and Hippocampus Two Weeks after Experimental Cardiac Arrest
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Presenter: Andrea Müllebner, University of Veterinary Medicine, Vienna, Austria

Ischemia/reperfusion injury, a consequence of cardiac arrest and resuscitation, results in acute neuronal damage, neuroinflammation and neurodegeneration as well as regenerative responses. The heme-degradating enzymes heme oxygenase (HO) and biliverdin reductase (BVR), which are important for neuronal function and memory formation, were shown to be affected in neurodegenerative disorders, such as Alzheimer's disease. We questioned whether neurodegeneration following cardiac arrest, is also associated with changes in the HO system. Therefore, we investigated the expression and activities of HO and BVR in motor cortex (mC), visual cortex, hippocampus (Hc), striatum, and cerebellum two

weeks after 8 min of cardiac arrest and subsequent resuscitation (CAR). Integrity of neuronal tissue of control and CARrats was analysed by histology (HE). In tissue homogenates we quantified mRNA expression of HO-1, HO-2 and BVR and determined activities of HO and BVR using an optimized enzyme-coupled spectrophotometric assay that yields the end product bilirubin (BR) in both cases. Additionally, we determined the mRNA expression level of the inflammation marker tumor necrosis factor-receptor 1 (TNFR1). Loss of neurons was determined only in the cornu ammonis 1 region of Hc. In homogenates of Hc we found increased expression of HO-1 mRNA, which was paralleled by increased levels of TNFR1 mRNA, indicative for ongoing inflammation in this region. We further found a decreased activity of HO in homogenates of Hc and mC, suggesting post-translational changes of HO-2. The other brain regions displayed unchanged HO activity. BVR activity was not affected. Our findings suggest that CAR induces sustained neuroinflammatory and neurodegenerative processes in Hc, which resemble those described in neurodegenerative disorders. Due to the relevance of HO for neuron survival and function, it is probable that the observed changes, are not only associated with, but also contribute to cognitive deficits frequently developed after cardiac arrest. We showed further that the determined changes of HO mRNA expression levels do not allow inferring the resultant heme degrading capacity of neuronal tissue. A.M. was supported by a grant by the Austrian research promotion agency FFG (project number 849090)

REGENERATIVE MEDICINE RESEARCH

Bone Organic Matrix Quality Significantly Correlates with Fracture Incidence

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Women with similar areal Bone Mineral Densities (BMD) may show divergent fracture incidence due to differences in bone quality. The hypothesis tested here is that postmenopausal (PM) women who have sustained osteoporotic fractures have altered organic matrix bone quality compared to those who have not. We used Raman microspectroscopy to analyze PMMA embedded transiliac biopsies (N=12) collected from fracturing (n=6, age 62.5 ± 7.4 yrs; Cases) and BMD-matched non-fracturing PM women (n=6, age 62.2 ± 7.3 yrs; Controls). Previous results show significant differences in intrinsic material properties by nanoindentation, along with lower mineral carbonate/phosphate ratio by Fourier transform infrared spectroscopic imaging (FTIRI), and no differences in bone tissue mineralization by digitized microradiography (DM). No differences between groups were seen by conventional histomorphometry. Spectra were acquired 2 μm from previously performed nanoindents, in cortical and cancellous compartments. Twenty such anatomical areas / biopsy / compartment were analyzed, the results averaged, and the mean value treated as a single statistical unit. The determined parameters were: mineral to matrix ratio (MM; positive correlation with bone stiffness), and tissue water (TW; positive correlation with bone strength), glycosaminoglycan (GAG; negative modulators of bone mineral homeostasis), lipid, pyridinoline (Pyd; trivalent enzymatic collagen cross-link; strongly associates with fracture incidence), N(6)-Carboxymethyllysine (CML; advanced glycation endproduct; negative correlation with bone strength), and pentosidine (PEN; advanced glycation endproduct; negative correlation with bone strength) content. The results showed no changes in MM in either cortical or cancellous compartments in general agreement with previous FTIRI and DM results. Cases had significantly lower TW and GAG content and significantly elevated Pyd, CML, and PEN content in both anatomical compartments analyzed compared to Controls. In conclusion, the results of the present study indicate significant differences in organic matrix quality in PM women that sustain fragility fractures versus age- and BMD-matched controls, and highlight its importance as an independent determinant of fracture incidence.

Physical Therapies – A Possible Saviour in Post-Antibiotic Era

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Presenter: Lisa Karner, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Chronic and infectious wounds do not only challenge patients and their families but also represent a massive socioeconomic burden. Already today, antibiotic resistances develop faster than research on new, antibacterially-active substances. Thus, the rapid development of new therapies for the treatment of infected wounds is a race against time. Photodynamic inactivation (PDI) is an innovative, physical approach that is already widely used in cancer therapy and is now intended to remedy the problem of infection control. Germs of the genera Escherichia and Staphylococcus play an important role in wound infection. Model germs of these genera were incubated with the photosensitizer (PS) methylene blue, which was then activated with pulsed red LED light. This treatment was compared to the effects of light alone at different wavelengths and settings. Bactericidal activity was assessed by evaluation of colony forming units and underlying mechanisms were investigated by molecular-biological methods. Furthermore, the effect of PS on eukaryotic cells, which are mainly involved in wound healing, was investigated. Regarding direct effects, only blue light could decrease the number of bacteria depending on the bacterial strain. This effect correlates with the activation of recA as a part of the cellular SOS mechanism. Red light had no germ-reducing effect but stimulated the proliferation of skin and connective tissue cells. However, in combination with the PS, red LED light irradiation significantly reduced the bacterial count by up to 6 log10 levels in each strain used. PDI also affected eukaryotic cells, however, these cells were damaged to a much lesser extent under the same conditions. In most cases, infections are mixed cultures, thus research focuses on the selection of the best PS for the respective strains and the corresponding treatment parameters, such as wavelength(s), incubation and irradiation time and light intensity. In times of increasing numbers of antibiotic resistances and chronic illnesses in an aging society PDI can provide a cost-effective, minimally invasive alternative. The further development of this therapy approach for wound treatment, the evaluation in pre-clinical models, as well as the establishment in the clinics are therefore of high relevance.

Discovery of Novel and Conserved Nociceptive Pathways in Congenital Insensitivity to Pain

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Chronic pain syndromes affect about 20% of the world’s population, creating an enormous financial burden to health care systems. Non-habit forming pain therapeutics are still lacking and by understanding the molecular and cellular basis underlying various pain syndromes we can identify novel targets for therapies. Congenital insensitivity to pain (CIP) syndromes are a group of rare genetic disorders of the peripheral nervous system marked by the absence of pain perception, due to dysfunctional or absent sensory neurons. Studies of CIP syndromes have been instrumental in uncovering molecular mechanisms underlining pain perception. For example, CIP is caused by mutations in sodium channels Nav1.7 and Nav1.9, neurotrophic growth factor β (NGFβ), and its receptor, neurotrophic receptor tyrosine kinase (NTRK1). We, and others, have additionally shown mutations in methyl transferase PR-domain containing member 12 (PRDM12) to cause a type of autosomal recessive CIP syndrome. PRDM12 is a transcription factor belonging to a conserved family implicated in cell fate decisions. PRDM12 plays an important role in the development of the neural crest in several species and plays a potential role in pathogenesis of chronic myeloid leukemia in humans. We showed that PRDM12 is a key regulator of sensory neuronal specification in Xenopus leavis and necessary for nocifensive behavior of Drosophila melanogaster. However, very little is known of the downstream targets of PRDM12 activity and of the potential role it might play in nociceptor physiology in vertebrates. We use various genetic mouse models to delineate the function of PRDM12 in pain perception, and uncover its downstream molecular targets. We find that PRDM12 is expressed in a specific subtype of nociceptors within the dorsal root ganglia, where it regulates their survival and function. We also uncover several novel downstream targets of PRDM12, previously unknown to participate in nociception. Here, we characterize homeobox protein MOX-2 (MEOX2) and its role in pain perception in a MEOX2-deficient mouse model. These studies enhance our knowledge of basic molecular mechanism underlying pain perception, as well as to provide novel research avenues for pain therapeutics.

CARDIOVASCULAR RESEARCH

Remote ischemic perconditioning attenuates adverse cardiac remodelling and preserves cardiac function following myocardial infarction in rats

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Objective: Remote ischemic conditioning is considered a potential clinical approach to reduce myocardial infarct size (MI). However, there is lacking evidence that RIC beyond MI size reduction also acts on post-infarct left ventricular (LV) remodeling. The activation of Neuregulin-1 (NRG-1)/ErbBs signaling provides significant anti-inflammatory and anti-remodeling effects. The aim of the study was to clarify the impact of RIC on the NRG-1/ErbBs pathway in association with the expression of pro-inflammatory cytokines, extracellular matrix (ECM) components and LV hemodynamic function. Methods: Male Sprague-Dawley rats were subjected to 30 min occlusion of left anterior descending artery (LAD) followed by 2 weeks of reperfusion (IR) and separated into three groups: (1) sham operated (Sham, without LAD occlusion; n=10); (2) IR (n=14) and (3) remote ischemic perconditioning group with three cycles of 5 minutes of I/R on hindlimb performed during myocardial ischemia (IR+RIPerc, n=15). Cardiac function was evaluated ex vivo on an isolated working heart model (WH) and in vivo by transthoracic echocardiography. Plasma levels of NRG-1 were measured by ELISA. mRNA expression of pro-inflammatory cytokines, ECM components, ErbB receptors were assessed by RT-qPCR. Results: IR resulted in a marked decline in cardiac output, external heart work ($P<0.01$ vs Sham respectively) in vitro and ejection fraction in vivo ($P<0.01$ vs Sham). This was accompanied with a subsequent ventricular dilatation and reduction in NRG-1 levels. In contrast, preserved LV cardiac function by RIC was associated with enhancement of NRG-1 levels and ErbB3 mRNA expression. Additionally, a decline in the expression of IL-1β, TNF-α, TNC and MMP2 ($P<0.05$, respectively) in RIC group represent anti-remodeling effects. Conclusions: We provided evidences that RIC improves cardiac remodeling and preserves ventricular function post-MI in association with NRG-1/ErbB3 signaling pathway.

Device specific differences between three contemporary LVAD types as risk factor for driveline infections

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Background: Driveline infections (DLI) are common adverse events in left ventricular assist devices (LVAD) patients, leading to severe complications and hospital readmission. The study aims at characterizing differences in demographics, clinical parameters and device types as risk factors for DLI readmission in the first 2 years post implant. Methods: This single-center study included 183 patients on LVAD support (n=43 HMII, n=29 HM3 and n=111 HVAD) following initial hospital discharge between January 2013 and July 2017. Demographics, risk factors, lab parameters, DLI related readmissions and survival were retrospectively analyzed. Additionally, experimental three-point bending tests of LVAD drivelines were performed to quantify the mechanical behavior and potential device specific influence to DLIs due to line movement. Results: 12.6% of patients were readmitted for DLI (RDLI), 14.8% experienced DLI but were treated in the outpatient setting (NRDLI) and 72.7% were free from DLI (NoDLI). Age, gender, BMI, strategy and INTERMACS were comparable between groups. Mean CRP ($p<0.001$), Leucocytes ($p<0.02$) and Fibrinogen ($p<0.001$) were higher in RDLI than in NRDLI and NoDLI patients. Elevated values were detected already up to 60 days before readmission. Freedom from any DLI readmission was comparable between HMII (98%) and HVAD (87%) but significantly lower in HM3 (72%) patients. Driveline bending tests indicated HM3 and HVAD (13.7 and 15.9N) as more rigid than the HMII (7.2N). 2-year survival of RDLI, NRDLI and NoDLI patients were similar (79%, 80% and 83%, $p=0.94$), without significant differences between the devices. Conclusion: DLI readmission remains a severe problem following LVAD implantation, but had no effect on survival. CRP, Leucocytes and Fibrinogen were elevated in readmitted DLI patients and might serve as risk factor already 60 days before. DLI readmissions were more common in HM3 compared to HVAD or HMII, possibly because of device specific differences in the driveline diameter, material and design.

"Herzensbildung [Heart literacy]" as an intervention to strengthen the health literacy of patients with cardiovascular diseases

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Presenter: Daniela Rojatz, Gesundheit Österreich GmbH, Vienna, Austria

"First heal with the word, then with the medicine and finally with the knife", said Asklepios, god of healing. The development and course of cardiovascular diseases can be significantly influenced by personal lifestyle and individual disease management. Due to demographic changes, an increase in patients with cardiovascular diseases and increased use of care services is to be expected. The project "Herzensbildung" of the Vienna Hospital Association aims to strengthen health literacy with regard to disease management and lifestyle of "heart patients". "Herzensbildung" includes two in-patient interventions "by healing with the word": a multilingual educational video and a workshop for patients. Methods: An impact and process evaluation was carried out. In the controlled trial (n=511), patients who participated in the intervention were compared with patients who received only standard care at three different times (after admission to hospital, before discharge and four to six months after discharge). The process evaluation included three focus groups with hospital staff involved and three interviews with project staff. Results: The impact evaluation shows a short-term increase in knowledge about the disease and lifestyle (e.g. blood pressure, cholesterol levels), which can also be proven in individual areas four to six months after discharge. In addition, patients in the intervention group more frequently report that they behave healthier after the intervention (e.g. less smoking, reduction of alcohol consumption). The process evaluation shows the importance of face-to-face interaction: 77% of patients are very satisfied with the workshop, 58% with the video. Challenges in the implementation of the interventions exist due to the short hospital stay (1.5 days) and staff shortage in the hospital. Conclusion: Today, the 3000 year old proverb has been reversed - the knife (read: heart catheter) and the medicine are used rather than ‘the word’. The ‘Herzensbildung’ intervention shows positive effects (health knowledge, health behavior). Nevertheless, consistent implementation of the measures is challenging under the given financial and structural conditions and requires a systematic approach to communication and information policy in the health system.

POSTERS

HAEMATOLOGY AND CANCER RESEARCH

Functional Dissection of the Conserved Interactome of NUP98-Fusion Proteins in Acute Myeloid Leukemia

Authors: Terlecki-Zaniewicz, Stefan; Schmoellerl, Johannes; Eder, Thomas; Parapatics, Katja; Müller, André; Grebien, Florian
Presenter: Stefan Terlecki-Zaniewicz

Oncogenic fusion proteins (FPs) caused by chromosomal translocations, are particularly prevalent in leukemia. FPs involving the Nucleoporin 98 (NUP98) gene are found in ~2% of acute myeloid leukemia (AML) patients and are associated with poor survival-rates. mThe NUP98 multi-partner translocation family (MPTF) comprises more than 25 different FPs, all harbouring an N-terminal fragment of the NUP98 gene that is fused to distinct C-terminal partner loci. Previous studies and preliminary data from our laboratory 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 critical leukemogenic pathways. Through mass spectrometry (MS)-based profiling of their interactomes, we aim to identify critical effectors of NUP98-fusion proteins. Affinity-tagged variants of five representative, yet distinct NUP98-FPs (NUP98-HOXA9, -JARID1A, -DDX10, -NSD1 and -PSIP1) were stably integrated and inducible expressed in human AML cells. Protein complexes were purified from cellular lysates and their composition was characterized by MS. Exogenous NUP98 and known NUP98-binding partners, such as RAE1 and RAN, were highly abundant in all

datasets. The processed NUP98-FP interactomes constitute a network of 453 proteins, of which 173 are linked with three or more NUP98-FP. Functional annotation revealed that conserved NUP98-FP-interactors are enriched for factors involved in DNA binding and mRNA splicing, indicating that NUP98-FPs have active roles in transcriptional control. Intersection of the conserved NUP98 FP interactome with published datasets from genome-scale CRISPR/Cas9 loss-of-function screens revealed 30 proteins with particularly high potential relevance in AML biology. We are currently validating these 30 interactors using a functional CRISPR/Cas9-mediated negative selection assay in NUP98-fusion-dependent cell lines. Future work will aim at characterizing molecular mechanisms that depend on critical effectors of NUP98-FPs. Altogether, this study provides the first comprehensive protein interactome of five NUP98-FPs and will significantly enhance our understanding of the molecular mechanisms of NUP98-FP driven AML.

Functional cooperation of CEBPA and TET2 mutations in Acute Myeloid Leukemia
Authors: Heyes, Elizabeth; Schmidt, Luisa; Eder, Thomas; Manhart, Gabriele; Volpe, Giacomo; Schmidt, Claudio; Chatziathanasiou, Konstantina; Frampton, Jon; Grebien, Florian
Presenter: Elizabeth Heyes

The gene encoding the transcription factor CCAAT-enhancer-binding protein (CEBPA) is mutated in 9% of Acute Myeloid Leukemia (AML) patients. Most of these mutations induce frameshifts in the CEBPA N-terminus, resulting in expression of a truncated variant of C/EBPα, termed p30. Mutations in the C-terminal basic leucine zipper domain (bZIP) disrupt the DNA-binding ability of C/EBPα. AML patients harbor mono- or biallelic CEBPA-mutations (CEBPAmo or CEBPAbi) and both genotypes are associated with concurrent mutations in other genes. Mutations in TET2 are among the most commonly co-occurring mutations in both groups (24.4% in CEBPAmo / 15.7% in CEBPAbi). We hypothesize that combinatorial effects of CEBPA mutations together with TET2 lesions specifically rewire transcriptional and epigenetic circuitries in AML cells, thereby strongly influencing disease outcome. We used the CRISPR-Cas9 technology in a mouse model of C/EBPα–dependent AML to establish novel cellular models harboring mutations in Cebpa and/or Tet2. Introduction of Tet2 mutations into previously established Cebpa-mutated murine cell lines induced a strong selective advantage of Tet2-targeted cells compared to non-targeted cells. To elucidate molecular mechanisms underlying the phenotypic changes that depend on particular genotypes, we profiled the genomic landscapes of Cebpa/Tet2-mutant cell lines using ATAC-Seq. This revealed that mutations in Tet2 were associated with reduced frequencies of accessible genomic regions. Gene ontology analysis showed that these regions were linked to myeloid leukocyte differentiation. To determine how these genomic changes translate to alterations in gene expression we performed RNA-Seq. In parallel, we are generating transplantation models to investigate the potential functional cooperation between Cebpa and Tet2 mutations during leukemogenesis in vivo. Detailed combinatorial analysis of ATAC- and RNA-Seq data from cell line and in vivo models will provide deeper insights into global epigenetic and transcriptomic changes that depend on CEBPA- and TET2 mutations in a physiologically relevant mutational context. This will enhance our understanding of gene cooperativity in AML and could provide entry points for the development of novel patient management strategies.

STAT5-driven T cell neoplasia: a closer look at thymic T cell development
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Presenter: Tobias Suske

The recurrent activating point mutation in human STAT5B, N642H, has been found in more than 100 patients with aggressive mature or immature T cell neoplasias, and is linked to poor survival and increased risk of relapse. Accordingly, transgenic mice expressing hyperactive STAT5BN642H or STAT5AS710F under the vav promoter develop lymphoid neoplasia with massive expansion of mature CD8+ T cells in the periphery, leading to organ infiltration and organ failure. The diseased cytotoxic lymphocytes are fully functional and hypersensitive to cytokines, and the mice succumb to pulmonary infiltration and obstruction as the main cause of death. To understand lineage commitment of early T cell precursors (ETP) harboring STAT5 mutations, we analyzed thymi from diseased STAT5BN642H and STAT5AS710F mice and controls. We observed highly increased numbers of CD8+ single positive (SP) T cells and less CD4+ CD8+ double positive (DP) cells compared to wildtype (WT) controls, underlining the strong bias towards commitment to the cytotoxic T cell lineage in both models. Under physiologic conditions, murine CD4- CD8- double negative (DN) cells undergo maturation from stage DN1 to DN4, defined by expression patterns of CD25 and CD44, as a prerequisite to successfully pass T cell receptor selection. Here, we show that this development is perturbed in STAT5BN642H mice, as CD25 expression is downregulated. This trend was not observed in STAT5AS710F mice, which display less aggressive tumor progression. Further, well defined medullary and cortical structures in the thymus are disrupted in STAT5BN642H but not STAT5AS710F mice. Interestingly, in a Rag2-/- background, STAT5 hyperactive mice overcome arrest in DN stage and develop lethal thymic lymphomas. Diseased mice bear high numbers of thymic DP as well as CD8+ CD3- TCRβ- T cells, which are absent in Rag2-/- mice. Here, RNA-seq analysis of these populations is used to understand STAT5-driven transition from DN to DP thymocytes. Our results indicate that STAT5 hyperactivation 1) disrupts thymic T cell development in a mature T cell lymphoma model and 2) drives thymic lymphomagenesis of an immature T cell immunophenotype in Rag2-/- mice.

MeDALL-Chip-based IgE profiling in patients with mastocytosis
Authors: Smiljkovic, Dubravka; Kiss, Renata; Lupinek, Christian; Hoermann, Gregor; Greiner, Georg; Jilma, Bernd; Valenta, Rudolf; Valent, Peter; Sperr, Wolfgang R.
Presenter: Dubravka Smiljkovic

Mastocytosis is a heterogeneous disease that is characterized by accumulationof neoplastic mast cells in the bone marrow and other organs and tissues. The elevated burden of mast cells bears a high risk for allergen-induced life-threatening anaphylactic reactions. Therefore, an early detection of specific IgE is of particular importance in these patients. In this study, 42 mastocytosis patients (median age: 45.5 years) were analyzed for the presence of specific IgE using the MeDALL allergen-chip. Four patients had cutaneous mastocytosis (9.5%), 2 mastocytosis in the skin (4.8%), 23 indolent systemic mastocytosis (ISM, 54.8%), 5 smouldering SM (11.9%), 5 SM with an associated hematologic neoplasm (11.9%), and 3 aggressive SM (7.1%). An age- and sex-matched control cohort (n=42) was also analyzed. In 17/42 patients (40.5%) with mastocytosis, specific IgE was detected by MeDALL-chip profiling. Ves v 5, Phl p 4, Bet v 1, Aln g 1, Phl p 1, Phl p 5, Cor a 1.0401, and Pru p 1 were the most frequently detected allergens. Allergen reactivity was confirmed by demonstrating upregulation of CD203c on basophils by flow cytometry after allergen challenge. The MeDALL-chip was positive in 13/16 patients (50%) with mediator-related symptoms, and in 4/16 patients (28.6%) without mediator-induced symptoms. In patients with a known allergy, 9/9 cases (100%) were positive in the MeDALL chip. In the group without known allergies, the MeDALL-chip identified 8/31 positive cases (25.8%). The prevalence of MeDALL-positive cases (40.5%) was lower in the mastocytosis group compared to an age- and sex-matched control cohort (59.5%). The MeDALL allergen-chip represents a novel, simple, tool for early detection of mastocytosis patients at risk for IgE-dependent allergic reactions and thus for mast cell activation syndromes.

A universal 4-gene prognostic signature in acute myeloid leukemia: role of SOCS2 in leukemic stem cells and disease aggressiveness
Authors: Nguyen, Chi Huu; Glüxam, Tobias; Schlerka, Angela; Bauer, Katharina; Grandits, Alexander; Wieser, Rotraud; Heller, Gerwin
Presenter: Rotraud Wieser

Acute myeloid leukemia (AML) is a heterogeneous disease with respect both to its genetic and molecular basis and to outcome. Cytogenetic and mutational data have been used to classify patients into risk groups with different survival, but within-group heterogeneity is still an issue. A number of studies have explored gene expression patterns as an additional predictor of outcome in AML, but the clinical utility of the resulting signatures is hampered by the fact that they consist of relatively large numbers of genes. Here, we used a robust likelihood-based survival modeling approach that leads to the identification of a minimal number of genes whose combined expression values are prognostic of survival. The resulting gene expression

signature (4-GES) consisted of only 4 genes (SOCS2, IL2RA, NPDC1 and PHGDH) and was able to predict patient survival as an independent prognostic parameter in seven cohorts of AML patients with publicly available gene expression data. The top gene in this signature, SOCS2, a component of the JAK-STAT signalling pathway, was subjected to functional characterization, and found to promote leukemic cell growth in vitro and leukemogenesis and stem cell activity in a congenic mouse model of AML. Together, these data demonstrate that the 4-GES may serve as a useful prognostic parameter in AML, and contains genes with functional importance in this disease.

Development of 3-D tumour organoids as a preclinical model for colorectal cancer
Authors: Tran, Loan Egger, Gerda
Presenter: Loan Tran

Organoids are 3-D cell cultures that mimic the native organ microstructures and are derived from self-organizing mammalian pluripotent or adult stem cells in vitro. Here, we generated organoid cultures from tumorigenic and adjacent healthy tissue obtained from the same patient diagnosed with colorectal cancer. This approach enables the evaluation of the disease state while controlling for potentially confounding factors in the healthy specific genetic background. The derived organoid cultures were characterized on a histopathological level and reproduced the grade and differentiation capacity of their parental tumors. Further, the characterization of the organoids on a molecular level shows a stable mutational and DNA methylation profile. The analysis of the genetic and epigenetic composition of the organoids allow for correlations to be made between the organoid phenotype and a specific mutational and expression profile. Additionally, we established organoid-based orthotopic mouse tumor xenograft models for more complex tumor characterization and in vivo testing, including analysis of PET tracer uptake and specificity. Thus, a biobank of human organoids presents a platform for biomarker testing, as well as drug or small molecule screening. For translational-based cancer research, primary organoid cultures are ideal model systems, as they provide a means to showcase multi-lineage cellular differentiation of in vivo systems while maintaining the versatility of in vitro transformed cell lines. Eventually, organoids provide the possibility of high throughput analysis of samples from individual patients bridging the gap between basic research and personalized precision medicine.

Integrative functional analysis of the DEK-NUP214 fusion protein in acute myeloid leukaemia
Authors: Liberante, Fabio; Chatziathanasiou, Konstantina; Grebien, Florian
Presenter: Fabio Liberante

Acute myeloid leukaemia (AML), a blood cancer, is often driven by oncogenic fusion proteins that result from chromosomal rearrangements. A better functional understanding of AML fusion proteins is critical to improving treatment, as patients usually present with a very poor prognosis. The t(6;9) rearrangement in AML leads to the production of the DEK-NUP214 fusion protein, which drives malignant transformation. Little is known about the mechanistic basis of DEK-NUP214-induced leukaemia and, currently, there are no therapies targeting it. We used CRISPR/Cas9-mediated genome engineering to introduce epitope tags into the endogenous DEK- and DEK-NUP214 genes in leukaemia cell lines. Using Cas9/crRNA ribonucleoprotein complexes, we have successfully edited, isolated and validated sub-clones from cell lines to harbour Strep-HA-tagged DEK alleles. Characterization of protein complexes engaged by the DEK-NUP214 fusion proteins through affinity purification and mass spectrometry (AP-MS) revealed 415 unique, high-confidence interactors. The DEK-NUP214-interactome is highly enriched in RNA-binding and nucleolar proteins. In addition, we find interaction of DEK-NUP214 with protein complexes involved in epigenetic regulation of transcription. Interestingly, many of these interactors are regulated by MYC, which represents a known driver of leukaemia. We are currently developing functional genomics tools in murine and human DEK-NUP214-expressing cells to validate potential mechanistic contributions ofidentified gene targets as requirements for leukaemogenesis in vitro and in vivo. We are currently optimizing ChIP-seq protocols to investigate the global pattern of DEK-NUP214 interaction with chromatin, whether direct or through these newly-identified mediators. Integrative analysis and thorough validation of the combined data will identify critical effectors of the DEK-NUP214 oncoprotein, unearthing targets for novel therapeutic and diagnostic strategies for AML patients.

DFO* and oxoDFO*: Optimized new Chelators for Zirconium-89 ImmunoPET
Authors: Brandt, Marie; Aulsebrook, Margaret L.; Gasser, Gilles; Briand, Manon; Mindt, Thomas L.
Presenter: Marie Brandt

AIMS: The only chelator clinically applied used thus far for the imaging of 89Zr labelled antibodies by PET is the desferrioxamine (DFO), a siderophore. However, DFO does not fulfill the octadentate coordination preferred by 89Zr4+, which results in complexes that are not fully stable and consequently in the release and unspecific uptake of the radiometal in, e.g., the bones. This can impact the detection of bone metastases and results in unnecessary radiation doses to non-targeted tissues. The previously reported octadentate version of DFO, termed DFO*, provides 89Zr4+complexes with remarkably increased stability. However, its solubility in aqueous media could be improved to facilitate bioconjugation chemistry. Thus, an analogue containing an oxygen-containing carbon scaffold (oxoDFO*) with higher water solubility was developed. The modular synthesis of oxoDFO* and the possibility to increase its denticity also provides an opportunity to use the chelator for other radiometals, e.g., 68Ga . METHODS : DFO* was synthesized from commercial DFO in two steps. oxoDFO* was synthesized via solid support chemistry from 10-(benzyloxy)-1-(9H-fluoren-9-yl)-3,11- dioxo-2,7-dioxa-4,10-diazatetradecan-14-oic acid (4 steps). In order to yield bifunctional chelating agents (BFCA) for conjugation to antibodies via Lysine residues, the chelators were coupled to 4-isothiocyanatobenzoic acid. Water solubility tests were performed using the shake flask method. RESULTS : oxoDFO* was synthesized efficiently on solid phase with an overall yield of 77%. oxoDFO* and its BFCA derivative oxoDFO*-pBn-NCS exhibit an significantly improved water solubility compared to the previously reported DFO* (and DFO). CONCLUSION: oxoDFO* is the first octadentate, water soluble chelator for 89Zr and expected to provide complexes with the radiometal of high in vivo stability; radiolabelling experiments and preclinical evaluation are ongoing. Through bioconjugation to antibodies, oxoDFO* represents a valuable future tool for immunoPET imaging in cancer diagnosis.
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The role of the Metastasis Suppressor 1 (MTSS1) gene in the sensitivity of acute myeloid leukemia cells to chemotherapeutic drugs
Authors: Grandits, Alexander M.; Wieser, Rotraud
Presenter: Alexander M. Grandits

Acute myeloid leukemia (AML) is a malignant disease of the hematopoietic system with an annual incidence of ~1/25.000. Current therapeutic regimes achieve complete remissions in 50 – 80% of all cases, but most of these patients eventually relapse. Relapse is thought to arise from chemotherapy resistant leukemic stem cells and is associated with a poor prognosis for the affected patients. A genome wide gene expression screen revealed that the mRNA of MTSS1 was significantly down-regulated in relapsed AML as compared to paired diagnostic samples. MTSS1 down-regulation has also been implicated in chronic myeloid leukemia, and was associated with poor survival in AML as well as in ovarian, lung, breast, liver, and pancreatic cancer. The methylation status of the MTSS1 promoter was analyzed using methylation-specific PCR after bisulfite treatment of gDNA extracted from human myeloid cell lines. The promoter was methylated in cell lines without MTSS1 expression, and unmethylated in those with MTSS1 expression. MTSS1 was knocked out in the human AML cell line TF-1 using CRISPR-Cas9 technology, and complete suppression of MTSS1 in TF-1 clones was confirmed by immunoblot analysis. Metabolic activity was measured after treatment with daunorubicin (DNR) or cytosine arabinoside (araC) and results were confirmed by the AnnexinV/DAPI assay and by immunoblot analysis for cleavage of caspase-3. Loss of MTSS1 expression enabled TF-1 clones to tolerate higher doses of DNR and araC and the activation of caspase-3 in response to cytotoxic drugs was strongly reduced in MTSS1 knockout clones. The uptake of DNR and the generation of DNA double strand breaks during the incubation with cytotoxic drugs was not affected by the knockout of MTSS1. These results were confirmed in HL60 cells overexpressing MTSS1 in a

doxycycline inducible manner: induction of MTSS1 in this system increased cellular sensitivity to chemotherapeutic drugs. In conclusion, these data suggest that MTSS1 down-regulation contributes to chemotherapy resistance in AML.

Glycosylated 99mTc-(CO)3-Labeled Peptides for Improved Tumour Targeting
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Presenter: Carolina Giammei

Aim: Among the clinically relevant peptides, bombesin (BBN) derivatives are attractive candidates for targeting the Gastrin-releasing peptide receptors, which are overexpressed by various cancer cells, including prostate and breast cancer. The tumor-targeting peptide sequence of BBN has been radiolabeled with the single-photon emission computed tomography (SPECT) radionuclide technetium-99m (99mTc). A known drawback of the organometallic 99mTc-tricarbonyl core for the radiometallation of (bio)molecules is its increased lipophilicity in comparison to other 99mTc-cores and radiometals, which can interfere with the pharmacokinetic profile. A common strategy to overcome this limitation is to bind a polar pharmacological modifiers to the radioconjugates. Therefore, a glycosylated 99mTc-tricarbonyl-labeled BBN conjugate was synthesized and compared with a reference compound. Methods: The BBN derivative ([Nle14]BBN(7-14)) was prepared by solid-phase peptide synthesis and combined with the carbohydrate sorbitol as well as a tridentate chelator by the click-to-chelate approach. The radiometal labeling was achieved with a microwave oven. The lipophilicity (LogD) of the 99mTc-tricarbonyl-labeled BBN was evaluated by the "shake-flask method". Cell binding/internalization properties as well as receptor affinities (KD) of the conjugates were performed with GRP-receptor overexpressing PC-3 cells. Results:The radiochemical yield and purity of the final product was >95%. LogD values confirmed that the attachment of a glycosylated moiety led to a significantlymore hydrophilic radiolabeledpeptide conjugate. Besides the difference in lipophilicity, both compounds showed similar in vitro characteristics. Conclusions:Herein, we report that the attachment of a carbohydrate leads to a more hydrophilic peptide 99mTc-conjugate, which could improve its biological properties, e.g. by favoring fast renal excretion over slow hepatobiliary system. First in vitro results of the radiopeptides will be presented. Further investigations will include in vivo experiments with mice bearing PC-3 xenografts by biodistributions and small animal SPECT imaging studies. [1] K. Römhild, C. Fischer, T. L. Mindt, ChemMedChem, 2017, 12, 66 [2] C. A. Kluba, T. L. Mindt, Molecules 2013, 18, 3206-3226

Functional Investigation of SETD2 in Acute Myeloid Leukemia
Authors: Ebner, Jessica; Tröster, Selina; Skucha, Anna; Ringler, Anna; Kubicek, Stefan; Bajusz, Dávid; Keserű, György Miklós; Grebien, Florian
Authors: Presenter: Jessica Ebner

The methyltransferase SETD2 (KMT3C) is responsible for H3K36 tri-methylation. The H3K36me3 histone mark was implicated in transcriptional activation and elongation and has been associated with DNA mismatch repair, homologous recombination and regulation of alternative splicing. The role of SETD2 in cancer is controversial. SETD2 is frequently mutated in various cancers and heterozygous SETD2 loss caused chemotherapy resistance in cell lines and mouse models. In contrast, we and others found that SETD2 expression is critical in MLL-rearranged leukemia. SETD2 loss resulted in increased DNA damage, reduced proliferation and induction of differentiation in acute myeloid leukemia (AML) cells in vitro and in vivo. To clarify the molecular mechanism of SETD2 contributing to its cancer-specific role we plan to employ a set of complementary approaches. We are using the CRISPR/Cas9 technology to introduce various affinity- and degron tags into the endogenous SETD2 locus in human leukemia cell lines. Resulting new cell line models will allow functional investigation of SETD2-containing protein complexes and their association with chromatin, as well as global transcriptional and genomic consequences upon induced SETD2 degradation. Bioinformatic integration of these large-scale datasets will provide novel insights into the role of SETD2 in leukemia. In parallel, we aim to identify chemical structures that can be used as probes to study SETD2 function. We established an immunofluorescence-based read-out for monitoring the loss of the SETD2-dependent H3K36me3 mark. We screened a collection of compounds for cell-permeable inhibitors of H3K36me3. Additionally, we aim to identify small molecule SETD2 binders by a virtual screening approach. Top candidates from both experimental and virtual screens will be further validated in biochemical and cell biological assays to identify molecules with highest potency. These approaches will provide novel insights into the molecular mechanism of SETD2 in leukemia.

Data mining approaches to investigate the biological roles of established biomarkers
Authors: Sheibani Tezerji, Raheleh; Egger, Gerda
Presenter: Raheleh Sheibani Tezerji

One of the challenges in medical science is how to translate scientific findings into better clinical results. Information and data are the main input for such a translational process. In the era of postgenomics, public databases such as the Cancer Genome Atlas, have contributed to the development of various Omics data types from 33 cancer types. Omics analysis have facilitated the discovery of effective cancer biomarkers. Aside from biomarker discovery, access to such a big database provides significant insight into understanding biological changes, modifications and functions of established biomarkers in certain patients. The purpose of this study is to provide an independent analysis of public cancer databases from primary or metastatic tumor samples to investigate the biological roles of known biomarkers and add novel perspectives to biomarker discovery and application. Existing public cancer databases from TCGA and GEO were used for data mining and to evaluate biological response in cancer patients. We analyzed the correlation between PSMA (and CXCR4) gene expression levels and DNA methylation in two groups of patients with high and low expression of PSMA (and CXCR4). Both biomarkers are well established for PET imaging of prostate cancer and colorectal cancer with liver metastasis in advanced patients. Our results showed unique and shared biological roles of PSMA and CXCR4 in two groups of prostate and colorectal patient samples, respectively. We illustrated the differences between the patients on different levels of data especially on gene expression and pathway analysis. We also showed the correlation between gene expression and DNA methylation data from colorectal patient samples. We present here data mining of publicly available cancer databases to develop and provide additional support for further use of known biomarkers such as PSMA and CXCR4 and to assess the positive predictive power of known biomarkers as predictor of prostate and colorectal cancers. These findings will help us to translate our biological knowledge into medical practice for disease prevention, diagnosis or treatment. Translational biology can support personalized medicine to construct predictive models from early diagnosis to monitoring prognosis and predict response to treatments.

Inhibition of the WNK/SPAK-OSR1 signaling pathway leads to cell growth arrest and induces apoptosis in the human colon cancer cell line HT-29
Authors: Kloesch, Burkhard; Reiff, Markus; Lohberger, Birgit; Steiner, Guenter
Presenter: Burkhard Kloesch

Background: With No lysine kinases (WNKs) belong to the mitogen-activated protein kinases (MAPKs) and modulate cell proliferation upon different MAPK cascades (ERK1/2, ERK5). WNKs activate the two highly related STE20/SPS1-related Pro/Ala-rich kinase (SPAK) and oxidative stress responsive 1 (OSR1) proteins by phosphorylating a threonine residue (SPAK Thr233 and OSR1 Thr185) within their catalytic T-loop motif. SPAK and OSR1 in turn bind and activate the Na-K-2Cl cotransporters NKCC1 and NKCC2 and K-Cl cotransporter KCC3. There is growing evidence for roles of WNK kinases in various signaling cascades related to cancer. For example, in glioblastoma, NKCC1 expression levels were significantly higher than in grade II glioma and normal cortex. Pharmacological inhibition of NKCC1 by bumetanide and shRNA-mediated knockdown of NKCC1 expression led to decreased cell migration and invasion in vitro and in vivo [1]. In the high metastatic hepatocellular carcinoma cell line MHCC97H blockage of NKCC1 attenuated the proliferation and invasion ability of this cell line in vitro and in vivo [2]. Aim: In the present study, we investigated the effects of the WNK pan inhibitor WNK463 on proliferation and cell viability of the human colon cancer cell line HT-29. Results: We observed that in HT-29 cells, SPAK-OSR1 is hyper-phosphorylated at S373 and S325, respectively, and NKCC1 is overexpressedin an atypical manner. Treatment of the cells with WNK463 reduced proliferation and induced apoptosis in a concentration-dependent manner. Apoptosis was initiated by cleavage of

the apoptotic marker proteins caspase-3/-9 and PARP. Moreover, WNK463 reduced phosphorylation of SPAK-OSR1, Akt, p38 MAPK and CREB. Expression of p27 Kip1, a putative tumor suppressor, and a regulator of drug resistance in solid tumors was highly upregulated in HT-29 cells after treatment with WNK463. Interestingly, direct blockage of NKCC1 by bumetanide and bumetanide analogs did not affect the viability of HT-29 cells. Conclusion: We found that the WNK pan inhibitor WNK463 reduced cell growth and induced apoptosis in the human colon cancer cell line HT-29. We conclude that inhibition of the WNK/SPAK-OSR1 pathway could be an interesting therapeutic option for the treatment of colorectal cancer.

Targeting JAK1/2 in preclinical models of KRAS driven non-small cell lung cancer
Author: Mohrherr, Julian
Presenter: Julian Mohrherr

Lung cancer still represents the leading cause for cancer related deaths worldwide. Several genetic alterations have been associated with lung cancer e.g loss of tumor suppressor genes such as p53, INK4a, LKB1 and mutations/amplifications in several oncogenes like KRAS, EGFR or c-MYC. Development of KRAS inhibitors has been unsuccessful so far. Approaches to treat KRAS-mutated tumors are currently based on interfering with either KRAS downstream effectors or with key signaling pathways in tumorigenesis, such as the JAK/STAT pathway. Within this project, we aimed to: (i) Validate JAK1/2 as a prognosis marker for human KRAS-mutated NSCLC (ii) Assess the effects of a JAK inhibition on preclinical models of NSCLC. In our preliminary studies, we discovered so far that KRAS driven lung tumors upregulate expression of JAK1 and JAK2 during cancer progression. In addition, we demonstrated that JAK1/2 inhibition in preclinical models attenuates successfully tumor growth. Taken together, our data point out a beneficial effect JAK1/2 inhibition in KRAS driven NSCLC tumorigenesis.

Interaction of cell signaling, lipid metabolism and epignetics in cancer – a rich resource for novel therapies
Authors: Grunt, Thomas; Wagner, Renate
Presenter: Thomas Grunt

Development of resistance to anti-cancer drugs is caused by the high degree of regulatory plasticity in malignant cells. This plasticity is supported by the intricate architecture of networks that consist of crucial pathways, including mitogenic signaling, metabolic homeostasis, and epigenetic control of gene expression. Important functional interaction has been identified between growth signaling and lipid metabolism, growth signaling and epigenetics as well as between lipid metabolism and epigenetics. Elucidation of the molecular mechanisms of this relationship is required for understanding the promotive function of metabolism and its disorders (e.g. hyperphagia, diabetes, metabolic syndrome) for development of cancer and progression to resistant disease. Cancer-specific perturbations of signaling, metabolism and epigenetics can be cause and/or consequence of malignant transformation. Evidence indicates that these regulatory systems interact with each other to form highly flexible and robust cybernetic networks that promote malignant growth and confer treatment resistance (1). Deciphering these plexuses using holistic approaches known from systems biology can be very instructive for the future design of novel anticancer strategies. Recent findings allow a deep understanding of the multiple molecular interdependence between cancer-specific signaling, cell metabolism and epigenetics in order to provide insight into how major cancer machineries interact with each other during cancer development and progression, and how this knowledge can be used for future co-targeting strategies. Funding from the Medical Scientific Fund of the Mayor of the City of Vienna, from the ‘Initiative Krebsforschung’ of the Medical University of Vienna, and from the Herzfelder Familienstiftung, Vienna, Austria, is highly acknowledged. (1) Grunt TW. Trends Endocrinol Metab. 2018;29:86-98.

Identification of biomarkers for prostate cancer by DNA methylation analysis
Authors: Dillinger, Thomas; Egger, Gerda
Presenter: Thomas Dillinger

Prostate cancer is the second leading cause of cancer related mortality in men. Elevated levels of the biomarker prostate specific antigen (PSA) are detected in serum of prostate cancer patients. However, PSA levels increase not only in patients with prostate cancer but also under certain benign conditions, resulting in a high level of false positives and makes more specific biomarkers necessary. DNA-hypermethylation of CpG-islands in the promoters of tumor suppressor genes frequently occurs in tumors, resulting in epigenetic silencing. Changes in the DNA-methylation can be measured, making hypermethylated genes promising new biomarkers for prostate cancer detection. The genome-wide DNA-methylation profiles of primary prostate cancers were compared to normal adjacent tissue, using the Infinium HumanMethylation450 BeadChip from Illumina®. Principal component analysis of the obtained data clustered the samples into two distinct groups, based on the differentially methylated CpG sites. We identified several promoter regions of genes that were hypermethylated in prostate cancers. These were successfully validated in an independent cohort using methylation-specific qPCR (qMSP). Among the top significant hypermethylated genes we found SERPINB1, a serine protease inhibitor shown to play a role for invasiveness in other tumor entities. Using Monolayer and spheroid invasion assays, we identified a negative correlation between SERPINB1 expression and the invasive potential of different prostate cancer cell lines. Differential methylation between prostate cancer and normal prostate epithelium allows for the clear classification of prostate cancer and normal tissue and provides insight into biologically relevant targets for prostate cancer development. As a next step we aim to design assays for non-invasive detection of our epigenetic biomarker panel in liquid biopsies and urine samples of prostate cancer patients. Furthermore, we plan to do xenograft models with the SERPINB1 knockout cell lines.

Comparative evaluation of algorithms and normalization strategies for improved detection of differential chromatin occupancy patterns in ChIP-seq datasets
Authors: Eder, Thomas; Grebien, Florian
Presenter: Thomas Eder

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a widely used in the global investigation of protein-DNA interactions. One main application of this technology is the analysis of differential chromatin binding patterns of proteins of interest in varying biological states. Various algorithms can be used to compare between ChIP-seq datasets. Computational tools apply different normalization strategies strongly influencing the results of the analyses. Most researchers do not know which built-in normalization methods are appropriate for the ChIP-seq data to be analysed. Using inappropriate tools can lead to erroneous outcomes. To overcome this limitation, we assessed tools for differential ChIP-seq analysis and we provide recommendations which tools to use. We created standardized reference datasets by in-silico simulation of ChIP-seq data representing different biological scenarios. That include up- and down-regulation of equal proportions of genomic regions in both samples and global reduction of genomic regions in one sample. We used these scenarios to evaluate the performance of 22 tools for differential ChIP-seq analysis by identifying unique and overlapping regions, which were used to calculate Precision-Recall curves. We found enormous differences in precision and recall with the outcome depending on the size and shape of simulated peaks as well as on the regulation scenario. We are currently verifying our findings with publicly available and unpublished ChIP-seq datasets. Our analysis provides unbiased recommendations which tools to use for particular biological scenarios. The application of appropriate analysis tools will greatly improve the outcomes of ChIP-seq studies, ultimately leading to improved identification of molecular mechanisms.

CARDIOVASCULAR RESEARCH

Riboflavin mediated UV-crosslinking of extracellular matrix conduits to improve vascular graft characteristics

Authors: Schneider, Karl Heinrich; Rohringer, Sabrina; Kapeller, Barbara; Grasl, Christian; Walter, Ingrid; Wojta, Johann; Bergmeister, Helga
Presenter: Karl Heinrich Schneider

Introduction: Naturally-derived materials have been proposed as an alternative source to create small diameter vascular grafts. Although, the extracellular matrix (ECM) supports various cell functions, the required decellularization process, to remove cellular antigens and to reduce immunogenicity often leads to a decrease in mechanical strength of the material. Furthermore, preservation induced collagen exposure on the luminal surface mediates platelet adhesion and may favours graft occlusion without further graft modification steps. Methods: In this study, placental arteries with an inner diameter of 2 mm were decellularized using either Triton X-100 or SDS. After decellularization, UV-irradiation in combination with riboflavin (vitamin B2) was used to crosslink the collagenous ECM fibers of the small diameter vascular grafts and heparin molecules were covalently cross-linked to the matrix to further improve mechanical strength and hemocompatibility of the grafts. Graft characteristics and biocompatibility with and without UV-crosslinking (UV-CL) were studied in vitro and in vivo. Results: The UV-CL ECM-grafts showed significantly improved mechanical strength and complete smoothening of the luminal graft surface compared to untreated grafts. Cell seeding using human endothelial cells and human fibroblasts confirmed no cytotoxic effects of the UV-CL treatment. Short-term orthotopic implantation of ECM and UV-CL ECM grafts (n=28 +/- UV-CL) in a rat model showed similar performance of both graft types. Histological examination revealed undisturbed cell migration and matrix degradation of both UV-CL ECM-grafts. Using a myograph system functional graft remodeling was detected in all grafts indicated by contractile responses to increased levels of potassium chloride. Conclusion: UV-crosslinking is a preferable tool to improve graft characteristics of decellularized matrix grafts.

The role of Neuropeptide Y receptor Y1 in hypoxia induced pulmonary hypertension

Authors: Mutgan, Ayse Ceren; Crnkovic, Slaven; Holzer, Peter; Wilhelm, Jochen; Kwapiszewska, Grazyna
Presenter: Ayse Ceren Mutgan

Background and Objective: Disturbance in neuronal signaling is increasingly recognized as underlying mechanism in vascular remodeling. Neuropeptide Y receptor Y1 (NPY1R) has a higher expression in the pulmonary arteries of PH patients compared to donors. NPY mediates vasoconstriction and proliferation of vascular smooth muscle cells. However, little is known how NPY1R is involved in progression of pulmonary hypertension (PH). Thus, here we investigated the role of NPY1R in PH pathogenesis. Methods: NPY1R knockout mice were used to assess the role of NPY1R in PH by using chronic hypoxia mouse model. Right ventricular systolic pressure (RVSP) and ratio of right ventricular vs left ventricular plus septum (RV/LV+S) were measured to asses severity of PH. Pulmonary vessel thickness was analyzed by semi-automated software analysis. Transcriptome profiling was performed on lung tissue from hypoxia exposed NPY1R and WT littermates. Results: NPY1R knockout mice were protected from hypoxia induced PH. NPY1R knockout showed reduced levels of RVSP and reduced ratio of RV/(LV+S) after 3 weeks of hypoxia. Analysis of pulmonary pathology by double immunohistochemical staining of vWF/aSMA indicated that NPY1R did not involve in muscularization of pulmonary vessels. Transcriptome analysis revealed that myogenesis pathway (FDR= 0.0037) was up-regulated in NPY1R knockout mice. Among many others, transcription factor MEF2B and leiomodin 1 , vascular smooth muscle cell (VSMC) specific contractile genes that are regulated by the Myocardin/Serum Response Factor (SPC) complex, were up-regulated in NPY1R knockout mice. Conclusion: NPY1R is involved in PH possibly by meditating vasoconstriction and vascular smooth muscle differentiation. Further investigation will be conducted to underpin the exact molecular mechanisms.

Moderate activation of endoplasmic reticulum stress can reduce cardiac injury after traumatic haemorrhagic shock

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Presenter: Andreia Luís

Traumatic hemorrhagic shock (THS) impairs tissue perfusion causing hypoxia and inflammation, which has shown to induce endoplasmic reticulum (ER) stress. ER stress triggers the unfolded protein response (UPR) aiming at resolving ER stress or inducing cell death if ER stress cannot be solved. Thus, the activation of the UPR can either contribute to rescue damaged organs or further aggravate organ dysfunction. We questioned if UPR modulation can influence the developing of organ damage following THS. THS was induced in male Sprague Dawley rats by a median laparotomy and blood withdrawal until the mean arterial pressure dropped to 35-40 mmHg. The severity of shock remained controlled using blood gas parameters. The animals were treated at the beginning of resuscitation with an activator (Tunicamycin) or an inhibitor (TUDCA) of ER stress. Blood and tissue samples were collected 24 hours after shock for determination of organ damage and quantification of UPR markers: GRP78, spliced X-box-binding protein 1 (XPB1s) and CHOP. In the THS group, the UPR markers were neither up-regulated at mRNA, nor at the protein level. In the serum of THS animals, we observed an increase in markers of liver damage (ALT and AST), cell/muscle injury (CK and LDH), as well as in cardiac troponin I (cTnI), a specific marker of cardiac injury. In contrast, the treatment with Tunicamycin strongly increased mRNA levels of GRP78, XBP1s and CHOP in liver, kidney, and lungs but not in heart tissue. Interestingly, the treatment with Tunicamycin resulted in a decrease of cTnI levels and a consistent trend in decreasing the other organ injury parameters (ALT, AST, CK, and LDH). Additionally, we found an inverse correlation between the release of cTnI and the expression of CHOP and XBP1s in liver and kidney tissues. Our findings suggest that the treatment with Tunicamycin may protect the heart from damage either through an UPR-independent mechanism or via the UPR activation observed in the liver, kidney, and lungs. The splicing of XBP1 into XBP1s appears to be an essential factor mediating the reduction of cardiac injury. Our data further suggest that the IRE1α-XBP1-XBP1s pathway can be a potential mechanism underlying the beneficial effect of ER stress activation in THS.

Alternative activation induces tissue factor in macrophages

Authors: Mayer, Julia; Thaler, Barbara; Fischer, Michael B.; Wojta, Johann; Hohensinner, Philipp
Presenter: Julia Mayer

Background: Macrophages can be polarized differentially according to their surrounding environment leading to different function and behavior of macrophages. Proinflammatory polarization is associated with increased tissue degradation and propagation of inflammation whereas alternative polarization is associated with wound healing and angiogenesis. Aim: To identify changes in macrophage coagulatory function upon polarization. Methods: Primary human macrophages were polarized towards a proinflammatory state (M1) using LPS and IFN-gamma, alternatively polarized macrophages (M2) were generated using IL4 and IL13. Results: Unpolarized macrophages constantly produce microvesicles. Upon proinflammatory polarization however, microvesicle production is significantly reduced whereas alternative activation leads to increased shedding of microvesicles. This increased shedding of microvesicles is accompanied by increased expression of tissue factor (TF) via a STAT6 dependent signaling pathway in M2 macrophages. In addition, tissue factor activity in the microvesicle fraction was highest in M(IL4+IL13) macrophages followed by M(LPS+IFN) macrophages and unpolarised macrophages. In contrast to monocytes, macrophages from the same donor do not increase TF expression after M1 polarization Epigenetic changes were observed in the promoter region of TF required for NF-kB signaling after macrophage maturation. This blunting of TF induction was not dependent on maturation of monocytes to macrophages with similar results for both MCSF and GMCSF derived macrophages. Previous proinflammatory polarization did not change the capability of alternative polarization induced tissue factor induction. Conclusion: Alternative polarization induces microvesicle production together with an increase in tissue factor and tissue factor bearing microvesicles. Proinflammatory activation leads to opposite behavior culminating in reduced tissue factor acitivity together with reduced microvesicle shedding. This suggests that alternative activation of macrophages leads to a procoagulatory macrophage phenotype.

The role of fibrinolysis inhibition in engineered vascular networks derived from endothelial cells and adipose-derived stem cells
Authors: Mühleder, Severin; Pill, Karoline; Schaupper, Mira; Labuda, Krystyna; Priglinger, Eleni; Hofbauer, Pablo; Charwat, Verena; Marx, Uwe; Redl, Heinz; Holnthoner, Wolfgang
Presenter: Severin Mühleder

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. Specifically, co-culture in fibrin can be exploited as a system for cell and growth factor delivery to assist cells in situ in regenerating tissues. 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 and tranexamic acid resulted in impaired vascular network formation. Aprotinin had no effect on laminin and collagen type IV deposition or formation of tube-like structures in scaffold-free co-culture, indicating that poor vascularisation of fibrin clots is primarily caused by inhibition of plasminogen-driven fibrinolysis. Co-culture in plasminogen- and factor XIII-depleted fibrin did not result in different vascular network density compared to controls. 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. This suggests that inhibition of scaffold degradation may have beneficial effects when using co-culture in serum- and growth factor-free conditions, thereby fulfilling an important prerequisite for clinical translation of prevascularised tissues. Taken together, our data reveal a critical role of fibrinolysis inhibition in in vitro cell-mediated vascularisation of fibrin scaffolds.

Characterization of cardiac and vascular function in Duchenne Muscular Dystrophy in mice
Authors: Szabo, Petra Lujza; Hamza, Ouafa; Inci, Milat; Hilber, Karlheinz; Ebner, Janine; Podesser, Bruno Karl; Kiss, Attila
Presenter: Petra Lujza Szabo

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive, progressive muscle wasting disease. Besides skeletal muscle degeneration, increasingly important source of morbidity and mortality is dilated cardiomyopathy leading to heart failure, arrhythmias and vascular dysfunction. There are several animal models for this human disease are developed. The most widely used is the dystrophin deficient mdx mouse, which develops severe symptoms at late onset (9-14 months old). Therefore, we aimed to assess the development of cardiac and vascular dysfunction in young mice (3 and 6 months old) to investigate the pathophysiologic processes in the early stage of the disease. Male mdx and BL/10 mice were used (3 and 6 months old). Echocardiography was used to assess left ventricular (LV) ejection fraction (LVEF) and hemodynamic function was monitored by an invasive method involving the determination of LV systolic pressure (LVSP) and the rate of LV pressure development (+dP/dt). Vascular reactivity was performed by wire myography. We observed the early signs of the cardiac dysfunction as a slight LV dilatation in mdx mice at age 6 months old in compared to wt littermates and 3 month old ($p<0.05$, respectively). Of importance, endothelial dysfunction on aorta segments were observed at age of 3 and 6 months in mdx mice in compared to wt littermates ($p<0.05$). Our study demonstrated the early sign of endothelial dysfunction a mouse model of DMD, which may contribute to the progression of cardiomyopathy. Thus, targeting the preservation of endothelial function in DMD might be a therapeutic approach for preserving cardiac function.

The opposing effect of TMEM16A on the proliferation of pulmonary vascular cells
Authors: Skofic-Maurer, Davor; Nagaraj, Chandran Zabini, Diana; Sharma, Neha; Kwapiszewska, Grazyna; Olschewski, Andrea
Presenter: Davor Skofic-Maurer

OBJECTIVE: TMEM16A is a Ca2+-activated Cl- channel implicated mainly in the growth and invasion of various types of cancers. The channel is ubiquitously expressed in different organs particularly in the lung, where it is predominantly localized to the epithelium and vasculature. Endothelial cell dysfunction plays a central role in the pathogenesis of pulmonary hypertension (PH), promoting sustained vasoconstriction, and remodelling. However, nothing is known about TMEM16A in endothelial cell homeostasis, precisely its contribution in regulating the resting membrane potentialand [Ca2+]i of pulmonary artery endothelial cell (PAECs). Therefore current study investigates the role of TMEM16A in the PAEC with the emphasis of its differential effect on the PAEC and pulmonary artery smooth muscle cell (PASMC). METHODS: Cas3/7 activity Apoptosis assay, Matrigel tube-formation assay, 3H-Thymidine incorporation proliferation assay, DiBAC4(3) resting membrane potential determination assay. RESULTS: TMEM16A is localized in the pulmonary vasculature. Upon overexpression, resting membrane potential (Em) is significantly depolarized in both PASMC and PAEC, while depolarization is more pronounced in the PAECs. Furthermore, the change in the Em is followed by a differential effect on the proliferative potential of these cell types, augmenting the proliferation of PASMC on the one hand and decreasing the proliferation of PAEC on the other, with no effect on the apoptosis. Additionally to proliferative effect, TMEM16A-overexpressing PAEC also have a disrupted angiogenic response as seen in reduction of the branching of a newly formed tubular network. CONCLUSIONS: TMEM16A is present in both PAEC and PASMC, where it has different effects on the cellular physiology. The interplay between endothelial cells and smooth muscle cells in the pulmonary vasculature is a well-known, yet vaguely understood process. Our findings suggest that increased TMEM16A expression and activity is an important pathologic mechanism possibly underlying vasoconstriction and remodelling in PH. Potential downstream effectors that mediate different responses in these cell types are yet to be determined.

Epigenetic modulation of Tenascin C in the heart: Implications on myocardial ischemia, hypertrophy and metabolism
Authors: Acar, Eylem; Fonseca Gonçalves, Inês; Costantino, Sarah; Szabo, Lujza; Hamza, Ouafa; Tretter, Eva Verena; Klein, Klaus Ulrich; Inci, Milat; Paneni, Francesco; Hallström, Seth; Kiss, Attila; Podesser, Bruno Karl
Presenter: Eylem Acar

Tenascin C (TN-C) is considered to play a pathophysiological role in adverse left ventricular remodeling. Yet, the mechanism underlying TN-C-dependent cardiac dysfunction remains elusive. The present study was designed to investigate the effect of hypoxia and hypertrophic stimuli on TN-C expression in H9c2 cells and its putative regulation by epigenetic mechanisms, namely DNA promoter methylation. In addition, rats subjected to myocardial infarction (MI) were investigated. H9c2 cells were subjected to oxygen glucose deprivation (OGD), incubated with Angiotensin II (Ang II) or human TN-C (hTN-C) protein. Hypertrophic and fibrotic markers, TN-C promoter methylation were assessed by RT-qPCR. TN-C protein levels were assessed by ELISA. Tnc mRNA was markedly increased by both OGD and Ang II ($p<0.01$, respectively). In addition, Ang-II-dependent TN-C upregulation was explained by reduced promoter methylation ($p<0.05$). Cells treated with TN-C displayed upregulation of BNP, Mmp2, β-MHC, integrin α6 and integrin β1. Furthermore, TN-C treated cells showed a significant reduction in AMP and ATP levels. In vivo, plasma and myocardial TN-C levels were increased 7 days post myocardial infarction (MI; $p<0.05$, respectively). In conclusions, both hypoxia and hypertrophic stimuli lead to epigenetically-driven TN-C upregulation and subsequent impairment of cellular energy metabolism in cardiomyoblasts. These findings might enlighten our understanding of left ventricular remodeling following MI or hypertension and indicate towards a strong participation of TN-C.

Development of an in vitro phosphate buffer induced calcification model
Authors: Kapeller, Barbara; Hager, Pia; Rohringer, Sabrina; Bergmeister, Helga; Podesser, Bruno Karl; Schneider, Karl Heinrich
Presenter: Barbara Kapeller

Introduction: Calcium mineral deposition (calcification) in tissues is a multifactorial process that frequently accompany atherosclerosis or valvular heart disease. Patients suffering from hyperphosphatemia, chronic inflammatory diseases or thrombocytosis are facing increased risk to develop symptoms of vascular calcification due to steady administration of corticoid or anticoagulative drugs. We developed a calcification model to mimic and compare several pathological risk factors in vitro. Cardiovascular tissue specific cells can be stimulated by elevated levels of inorganic or organic phosphates, corticoids and ascorbic acid to trigger calcification of the cells. Materials & Methods: To establish a phosphate buffer induced calcification model with high reproducibility using endothelial cells (EC) and fibroblasts (FB), cells were seeded in 24 well plates and stimulated with calcification media (CM). The calcification media (CM1 and CM2) consisted of regular cultivation medium for each cell type (DMEM for FB and Medium 200 for EC) supplemented with inorganic 2 mM phosphate (CM1) or organic 10 mM b-Glycerophosphate, 50 mg/ml Ascorbic acid and 10 nM Dexamethasone (CM2). Cells cultivated with the regular cultivation medium were used as control. Culture plates were incubated for 6, 24, 48, 72, and 96 hours before histological staining was performed to determine proliferation rates and calcium deposition. Results: Both cell types showed a change in proliferation rates when incubated in both of the calcification media. As expected, a significantly increased reaction to both of the calcification media was observed in EC culture compared to FB. Furthermore, the CM1 medium was more competent to induce calcification in both cell types compared to CM1 medium. Each approach was performed three times and showed little divergence within each group. Conclusion: We established a simple and reproducible in vitro test for investigating the influence of different proteins or drugs on cell types involved in calcium deposition. Creating high technical repetitive accuracy within our model was an important factor to enable future calcification studies.

Development of a fibre control apparatus for the production of electrospun vascular grafts
Authors: Desch, Michael; Grasl, Christian; Stoiber, Martin; Bergmeister, Helga; Schima, Heinrich
Presenter: Michael Desch

For the treatment of cardiovascular diseases, including coronary artery and peripheral vascular pathologies, there is a clinical need for small diameter (<6mm) vascular protheses. Current used synthetic materials often lead to vascular occlusion due to their surface thrombogenicity and the development of intimal hyperplasia caused by their inferior biomechanical properties. Electrospinning offers an interesting alternative to these materials because of the close match of electrospun materials to the biomechanical and structural properties of native vessels. In this study, an attempt was made to control the highly instable fiber jet in the electrospinning process with an additional electric field. This was done by adding two auxiliary high-voltage electrodes which were applied with a time-varying square wave potential generated by a newly developed electrical switch. Thereby, the electrospinning jet was periodically deflected between the electrodes, which leads to an aligned fiber-deposition. Furthermore, three types of vascular grafts (random- (rand), longitudinal- (long) and circular (circ) orientated; wall thickness: 100µm) were spun and later tested for their biomechanical properties. A quasistatic uniaxial tensile test was performed, and the stress/strain curves were recorded. With this setup it was possible to produce deflection potentials with voltages up to 18kV and alternation frequencies of 5-150Hz. In spinning experiments, it was found that the amplitude of the deflection exponentially decreases with increasing frequency. Furthermore, a linear correlation between the extent of the deflection and the voltage was found. The tensile tests showed that the fiber orientation affects the maximum tensile strength (long: 0.85±0.16, rand: 3.63±0.78 and circ: 2.77±0.08N), the compliance (long: 10.1±1.09, rand: 9.1±3.26 and circ: 5.6±1.27%/100mmHg and maximum strain of the grafts (long: 129.75±27.29, rand: 316.27±43.45 and circ: 332.20±25.66%). With the newly developed fiber control apparatus higher switching frequencies compared to the previous setup could be achieved and vascular grafts with different fiber orientations could be produced.

Coronary perfusion pressure as a predictor of CPR outcome in a VF CA rat model
Authors: Berger, Teresa; Schemm, Raphael; Clodi, Christian; Schriefl, Christoph; Weihs, Wolfgang; Holzer, Michael; Warenits, Alexandra; Schober, Andreas; Ettl, Florian; Magnet, Ingrid
Presenter: Teresa Berger

INTRODUCTION: High-quality cardiopulmonary resuscitation (CPR) aims to re-establish blood flow to vital organs until restoration of spontaneous circulation (ROSC). Coronary Perfusion Pressure (CPP) is an indicator for CPR effectiveness in the experimental setting. Direct CPP measurement from the coronary artery is not possible in small animal models. Several methods for calculating CPP from systemic blood pressure have been published. This study evaluates the reliability of calculated true mean CPP in assessing CPR quality in terms of ROSC rate in our ventricular fibrillation (VF) cardiac arrest (CA) model in rats. METHODS: Thirty-Four male Sprague-Dawley (400g) with 8 minutes of VF CA followed by CPR with mechanical chest compressions, ventilations, defibrillation and epinephrine were included in this study. Mean CPP was calculated from continuous mean arterial pressure (MAP) and central venous pressure (CVP). CPP, MAP and end-tidal CO2 (ETCO2) were calculated in two-minute intervals during CPR in animals without and with ROSC. Results are reported as mean±SD. Details are presented in the graphs. RESULTS: CPP, MAP and ETCO2 were not different during the first two minutes of CPR. After two minutes or CPR, CPP (20 ±8 no-ROSC vs. 28 ±8 ROSC p=0.033), MAP (31 ±4 vs. 37 ±6 p=0.11) and ETCO2 (20 ±2 vs. 24 ±3, p=0.003) became significantly higher in ROSC animals. Statistical significance disappeared for CPP after Bonferroni-correction for multiple testing, but not for MAP and ETCO2. CONCLUSION: CPP as calculated from MAP and CVP during CPR correlates with ROSC in rats, but so do MAP and ETCO2.

Therapeutic efficacy of human adipose-derived stem cell secretome (ASC-Sec) in emergency setting: In vitro and in vivo studies
Authors: Jilge, Julia; Ashmwe, Mostafa; Jafarmadar, Mohammad; Banerjee, Asmita; Wolbank, Susanne; Keibl, Claudia; Redl, Heinz; Bahrami, Soheyl
Presenter: Julia Jilge

Recently we have shown that the systemic administration of rat ASC-Sec ameliorates the inflammatory reactions and the resulting organ damage caused by hemorrhagic traumatic shock (HTS) in rats (Ashmwe et. al. 2017). The aim of this pilot study was to examine the efficacy of human ASC-Sec in the same animal model. In order to minimize in vivo experiments, efforts are made to establish in vitro test systems for further quality controls of human ASC-Sec. Human ASC were isolated from the fatty tissue (after liposuction), characterized, cultured and the supernatant (ASC-Sec) was extracted after 24 hours. In vivo; rats were subjected to HTS, receiving human ASC-Sec or vehicle 20 minutes after onset of reperfusion. Shock intensity, inflammatory response (IL-6, IL-10, MCP-1, GRO-α, Rantes, Leptin) and cell/organ injuries were evaluated. HTS induced an inflammatory response e.g. reflected in an IL-10 increase, peaking at 24 hours in both groups with no difference. Circulating markers of cell injury (CK, LDH) and organ function (ALT, UREA, CREA) increased in both groups similarly up to 24 hours after shock. In vitro; considering the anti-inflammatory effects of stem cells, whole blood or isolated white blood cells stimulated with LPS are tested to validate and optimize the biological activity of human ASC-Sec. In contrast to the rat ASC-Sec, human ASC-Sec did not show any efficacy in vivo. Ongoing studies aim to improve human ASC-Sec quality by preconditioning of the ASCs.

Epinephrine during CPR significantly increases organ perfusion in a rat VF cardiac arrest model
Authors: Schemm, Raphael; Berger, Teresa; Clodi, Christian; Weihs, Wolfgang; Holzer, Michael; Warenits, Alexandra; Schober, Andreas; Magnet, Ingrid; Ettl, Florian
Presenter: Raphael Schemm

INTRODUCTION: Epinephrine is an established vasopressor and the primary drug administrated during cardiopulmonary resuscitation (CPR). This study evaluates the influence of epinephrine on coronary perfusion pressure (CPP), mean atrial pressure (MAP)and end-tidal CO2 (ETCO2) in our rat VF CPR model. METHODS: Adultmale Sprague-Dawley rats (400g) were randomized to CPR with (n=15)and without epinephrine (n=10). After 6 minutes of untreated cardiac arrest (CA) study animals were resuscitated with mechanical chest compressions, ventilations and defibrillations. In the epinephrine group, epinephrine 10 micrograms per kilogram were administered every two minutes. Mean arterial pressure (MAP), central venous pressure (CVP) and ETCO2 were continuously monitored and calculated for the first two minutes of CPR. Mean CPP was calculated as difference between MAP and CVP. RESULTS : During CPR the mean CPP ± SD was significantly higher in animals resuscitated with epinephrine than without epinephrine(38.13 ± 8.29 vs. 19.55 ± 9.29 $p < 0.01$). The epinephrine group showed also significantly higher MAP values(45.53 ± 8.29 vs. 26.70 ± 6,67 $p < 0.01$). The mean ETCO2 was higher in the no epinephrine group (42.11 ± 8.11 vs.34.30 ± 8.15 $p = 0.052$). Detailed course of parameters are presented in the graph. CONCLUSION: As seen in given literature epinephrine increases organ perfusion in our 6 minutes VF cardiac arrest rat model. Interestingly, ETCO2 reacted inversely and was lower in the group without epinephrine.

Characterization of a novel closed chest model of ischemic mitral regurgitation in pigs
Authors: Hamza, Ouafa; Kiss, Attila; Kramer, Anne-Margarethe; Trojanek, Sandra; Tillmann, Katharina Elisabeth; Goncalves, Ines; Acar, Eylem; Tretter, Verna; Klein, Klaus Ulrich; Abraham, Dietmar; Podesser, Bruno
Presenter: Ouafa Hamza

Background: Surgical treatment of secondary mitral regurgitation remains a subject of controversy and still doesn’t show a clear impact on the mortality. In addition, there is unmet need to establish less invasive approaches in patients with secondary mitral regurgitation. Aims: establish and characterize a clinically reliable large animal model of mitral valve regurgitation. Methods: Young female domestic pigs were used (n=12, weight =60 +/- 12kg)). The induction of mitral valve regurgitation was performed by a localized posteromedial papillary muscle (PMPM) myocardial infarction. The PMPM irrigating branches were first identified by selectively injecting contrast media into the circumflex branches and 2ml of pure Ethanol were injected. The evaluation of the mitral valve regurgitation and cardiac function was assessed by echocardiography. After 6 weeks observation period, pigs euthanized and tissue samples from the papillary muscles and the mitral leaflets were taken for further analysis. Results: Seven pigs survived the 6 weeks follow up period. Ethanol injection resulted in postero-inferior wall and PMPM dyskinesia. Mitral regurgitation jet area significantly increased (jet area at baseline 0.03±0.015 cm^2 vs at 6 weeks 3.22 ± 0.53cm^2). A significant tenting area developed over the follow up period (Tenting area at baseline 0.35 ± 0.21cm^2 vs 2.17 ± 0.63cm^2 at 6weeks; $p<0.001$). Significant left ventricle enlargement was noticed (End diastolic diameter at baseline: 50,04 ± 4.34mm vs at 6 weeks 62,12 ± 3.92mm; $p<0.001$) as well as left atrium enlargement (left atrium area at baseline: 7.75 ±0.95cm^2 vs at 6 weeks 17.65 ± 3.2cm^2; $p<0.001$). These functional changes were accompanied by an increase expression levels of TGFß, MMP9, IL1ß as well as TLR2 and 4 in the posterior mitral valve leaflet while TNC expression was upregulated in the myocardial tissue but not in the valvular tissue.Conclusion: We established a novel, reproducible and clinically relevant model of ischemic mitral regurgitation in pigs. The functional changes of MVR were associated with a moderate remodeling on valvular level. Collectively, our large animal model gives a platform to test novel pharmacological and device based therapeutic approaches for treatment and reconstructed MVR.

Native T1 mapping of the anterior right ventricular insertion point is a strong predictor of outcome in heart failure patients with preserved ejection fraction: Insights from a cardiovascular magnetic resonance study
Authors: Nitsche, Christian; Mascherbauer, Julia; Bonderman, Diana; Hengstenberg, Christian; Kammerlander, Andreas; Loewe, Christian; Beitzke, Dietrich
Presenter: Christian Nitsche

Background: In pulmonary hypertension (PH), increased afterload for the right ventricle (RV) is reported to induce fibrosis at the RV insertion points (RVIPs), detectable by cardiac magnetic resonance (CMR) using late gadolinium enhancement (LGE). In contrast to LGE imaging, T1-mapping, as a relatively new CMR technique, allows quantitative assessment of myocardial native T1 times and extracellular volume (ECV). However, the prognostic value of T1-mapping and ECV of the RVIPs in heart failure patients is unknown. Methods: We prospectively investigated 167 consecutive patients with heart failure and preserved ejection fraction (HFpEF), a patient population frequently suffering from PH, who underwent CMR including T1-mapping. Of these, 155 (92,8%) underwent right heart catheterization (RHC) for hemodynamic assessment. Native T1-times were measured at the anterior and inferior RVIP and ECV was calculated. The prognostic value of T1-mapping of the RVIPs was investigated by multivariable Cox regression analysis. Results: Native T1-times were 995±73ms at the anterior and 1040±90ms at the inferior RVIP and ECV was 30.3±5.8% and 34.3±7.7%, respectively. RVIP T1 times were correlated with pulmonary artery pressures (PAP), pulmonary artery wedge pressure (PAWP) and right atrial pressure (RAP), by linear regression analysis (p for all<0.05). Patients were followed for 43.2±22.6 months. In total, 30 (18.0%) subjects died during follow up. By Kaplan-Meier analyses, T1 times at both RVIPs (log-rank, p-values: 0.002 and 0.039 for anterior and inferior RVIP, respectively) were associated with mortality while for ECV this was only the case for the anterior (log-rank, p=0.020), but not the inferior RVIP (log-rank, p=0.063). By multivariable Cox regression analysis, including imaging, invasive hemodynamic, and clinical parameters, NTproBNP serum levels (p=0.021), sPAP (p=0.016), native T1 time of the anterior RVIP (p=0.029), and RVEF (p=0.021) remained significantly associated with outcome. Conclusion: Fibrosis of the anterior RVIP as detected by T1-mapping is associated with increased PAP, PAWP and RAP, and appears to be independently related with prognosis in HFpEF.

Early Warning System for Adverse Events in Left Ventricular Assist Devices
Authors: Maw, Martin; Schlöglhofer, Thomas; Gross, Christoph; Schima, Heinrich ; Moscato, Francesco
Presenter: Martin Maw

Left Ventricular Assist Devices (LVADs) provide circulatory assistance to end-stage heartfailure patients. Adverse events like pump thrombosis remain a challenge in LVAD treatment. An early detection of these adverse events could supply caregivers with sufficient time to act before clinical symptoms become apparent. The purpose of this work is to develop a method to detect pump thrombosis from pump data only. Development was based upon retrospective patient logfile data of the HVAD LVAD system. 8 patients, which were rehospitalized for pump thrombosis and 8 propensity score matched patients without pump thrombosis were analysed for a total of 6292 patient days. The existing "2 Watt elevation from baseline” Alarm Algorithm was compared to the newly developed Normality-Deviation Score (NoDeS) algorithm. The NoDeS compares live data to statistical properties of the previous day, and can thereby quantify abnormal changes. The clinical standard "2 Watt elevation from baseline” Alarm was activated in 4/8 thrombus patients on the day of readmission. The NoDeS was able to detect pump thrombosis in 7/8 patients. It was able to anticipate readmission in 2 cases by 2 days and in another 2 cases by 3 days. Elevated NoDeS was measured on 8 additional days in the control dataset, whereas 3 alarms were not explainable by clinical records. The other algorithm did not produce any false positives. Additionally the NoDeS was able to detect 3 bleeding events in the dataset. The presented NoDeS algorithm is easily implementable and offers the benefit of anticipation of pump thrombosis before evident clinical symptoms. This may lead to improved clinical outcome and reduced burden on the caregiver. If the NoDeS could also reliably detect other forms of adverse events remains to be investigated more thoroughly.

Inadequate pump flow response contributes to exercise limitation in patients with a left ventricular assist device
Authors: Gross, Christoph; Marko, Christiane; Schlöglhofer, Thomas; Wiedemann, Dominik; Zimpfer, Daniel; Schima, Heinrich; Moscato, Francesco
Presenter: Christoph Gross

Left ventricular assist devices (LVAD) implanted increase perfusion, restore hemodynamics at rest and improve submaximal physical capacity as well as quality of life of end-stage heart failure patients. The relevant contribution of cardiac output provided by the LVAD and ejected through the aortic valve for different exercise intensities has been barely investigated. The hypothesis of this study was that different responses in continuously recorded LVAD parameters occur according to exercise intensity and that the response in LVAD flow has an impact on the oxygen uptake at peak exercise (pVO2). Cardiac and LVAD parameters such as estimated LVAD flow-rate (QLVAD), heartrate (HR) and aortic valve (AV) opening were analyzed from continuously recorded LVAD data during physical exercises of maximal (bicycle ergometer test) and sub-maximal intensities (6-min walk test and regular trainings). A hemodynamic lumped parameter model was used to reproduce the different individual responses in LVAD parameters during maximum bicycle ergometer test and explain the underlying hemodynamic mechanisms. Cardiac and LVAD parameter responses of 16 patients correlated with exercise intensities for HR: +20.4±15.4 vs +7.7±5.8 bpm (p<0.0001) and AV-opening with 71% vs 23% of pats (p<0.0001) though QLVAD were similar with +0.89±0.52 vs +0.59±0.38 L/min (p=0.07). Multi-regression analysis with pVO2 (R^2=0.77) showed relation to workload normalized by bodyweight (p=0.0002), HR response (p=0.001), AV-opening (p=0.02) and age (p=0.06) whereas the change in QLVAD was irrelevant. Constant speed LVADs provide inadequate support for maximum intensity exercises. AV-opening and improvements in HR show an important role for higher exercise capacities and reflect exercise intensities. Changes in LVAD flow do not impact pVO2 and contribute to the exercise limitation of LVAD patients. An LVAD speed control may lead to adequate left ventricular support during strenuous physical activities.

The presence of non-significant coronary artery atheromas vs. completely normal coronary arteries as demonstrated by CAG in patients with Tako-Tsubo syndrome has no impact on clinical outcome
Authors: Piackova, Edita; Weihs, Valerie; Geppert, Alexander; Nürnberg, Michael; Wesely, Emil; Smetana, Peter; Huber, Kurt
Presenter: Edita Piackova

Background: With respect to the Mayo Clinic diagnostic criteria, Tako-Tsubo (TTS) patients have coronary arteries either completely normal or with non-significant luminal narrowing of less than 50% in all epicardial coronary arteries, confirmed by coronarography (CAG). The aim of this study was to investigate potential differences in in-hospital and one-year mortality in TTS patients with completely normal coronary arteries and such with nonsignificant stenoses (luminal narrowing ≤ 50%) in CAG. Methods: Data from 99 consecutive TTS patients, who were admitted between 2006 and 2015 into the Wilhelminenhospital, in Vienna were analyzed. Aim: The study population was divided into two groups of patients, such presenting with completely normal or with one or more coronary artery stenoses ≤ 50%. Differences in variables such as patient´s characteristics, levographic findings, in-hospital mortality, and one-year mortality were investigated. Multivariate regression analysis was performed in order to correct for significant confounders in univariate analysis. Results: No differences in patient characteristics such as age, incidence of hypertension, hyperlipidemia or ejection fraction were found (Table). Midventricular dysfunction measured by means of acute levography, although rare, was only present more often in Tako-tsubo patients with normal CAG. Patients presenting with nonsignificant stenosis had more frequently diabetes mellitus, history of previous myocardial infarction, or percutaneous coronary intervention, respectively. Interestingly, in-hospital mortality (3,4% vs 1,7%) and one-year mortality (8,4% vs. 4,2%) tended to be higher in the group with normal coronary arteries by unadjusted (in-hospital mortality:HR=0,689; CI= 0,126-3,759, p= 0,667; one-year mortality: HR=0,769; CI= 0,258-2,296, p= 0,638, respectively) or by multivariate analysis adjusted for confounders (in-hospital mortality: HR=0,735; CI= 0,128-4,233, p= 0,731; one year mortality: HR=0,883; CI= 0,288-2,710, p= 0,828, respectively). Conclusion: CAG proven existence of visible wall irregularities vs. completely normal looking coronary arteries in patients presenting with TTS syndrome was associated with a higher cardiovascular risk profile, but had no influence on in-hospital or one-year mortality.

Release of mitochondrial DNA is Associated with Mortality in Severe Acute Heart Failure
Authors: Krychtiuk, Konstantin; Wurm, Raphael; Lenz, Max; Huber, Kurt; Wojta, Johann; Heinz, Gottfried; Huelsmann, Martin; Speidl, Walter
Presenter: Walter Speidl

BACKGROUND: Inflammation is regarded as an important trigger for disease progression in heart failure (HF). Particularly in severe acute heart failure (AHF), tissue hypoxia may lead to cellular damage and the release of intracellular mitochondrial DNA (mtDNA), which acts as an activator of the immune system due to its resemblance to bacterial DNA. It therefore may serve as a mediator of disease progression. The aim of this study was to determine circulating levels of mtDNA and its association with mortality in patients with HF in different presentations. METHODS: Plasma levels of circulating mtDNA were measured in 90 consecutive patients with severe AHF admitted to our medical ICU as well as 109 consecutive chronic heart failure (CHF) patients. RESULTS: In patients admitted to our medical ICU (median age 64 (49-74) years, median NT-proBNP 4986 (1525 – 23842) pg/mL, 30-day survival 64.4%), mtDNA levels were significantly higher in patients that died within 30 days after ICU admission and patients with plasma levels of mtDNA in the highest quartile had a 3.4-fold increased risk (p=0.002) of dying independent from renal function, vasopressor use and NT-proBNP, TnT, lactate levels or CardShock and APACHE II score. Patients with severe AHF showed significantly higher mtDNA levels (p<0.005) as compared to patients with CHF. In these patients, mtDNA levels were associated with NYHA functional class but were not associated with outcome.CONCLUSIONS: mtDNA release into the circulation is associated with mortality in patients with severe AHF but not in patients with CHF. Release of mtDNA may therefore play a role within the pathophysiology of AHF.

Impact of ST-segment elevation pattern in patients with TakoTsubo syndrome
Authors: Kaufmann, Christoph; Piackova, Edita; Weihs, Valerie; El-Razek, Abd; Geppert, Alexander; Nürnberg, Michael; Wessely, Emil; Smetana, Peter; Weiss, Thomas; Huber, Kurt
Presenter: Christoph Kaufmann

Background and Aim: The clinical significance of the ST-segment elevation pattern in patients with TakoTsubo syndrome is unknown, while in patients with anterior STEMI, both convex and straight type ST-segment elevation are associated with worse left ventricular function and a higher rate of cardiovascular complications. Accordingly, we sought to investigate the implications of ST-segment elevation pattern in admission ECGs of patients with TakoTsubo syndrome. Methods: This single-center study included 53 consecutive patients diagnosed with TakoTsubo syndrome between 2006 and 2017. The study population was divided into three groups based on the pattern of ST-segment elevation in leads representing the anterior wall of the heart, mainly V2-V4, on 12-channel ECG at admission: Group A (concave type, n=35), group B (straight type, n=8) and group C (convex type, n=10), respectively. Baseline characteristics, cardiac biomarkers, LVEF, in-hospital major adverse cardiovascular events (MACE) rate (cardiogenic shock, cardiac decompensation, cardiac arrhythmias) as well as in-hospital and long-term all-cause mortality were compared among the groups. The mean follow-up time was 5,4 years. Results: Baseline characteristics were largely comparable between groups with the exception that patients of the straight type ST-segment elevation group had lower history of hypertension compared to the other groups. There was no statistically significant difference in left ventricular ejection fraction or cardiac biomarkers between groups. Straight type ST-segment elevation was associated with a higher incidence of in-hospital MACE (p = 0,002), while patients with convex type ST-elevation were hospitalized for a longer time (p= 0,010). The survival analysis showed significantly higher in-hospital all-cause mortality in patients with straight type ST-segment elevation (log rank, p= 0,031), but no difference in long-term all-cause mortality between groups(log rank, p= 0,801). Conclusion: The present study indicates that different ST-segment elevation patterns in patients with TakoTsubo syndrome have an impact on in-hospital cardiovascular complications, in-hospital mortality, and length of hospitalization but not on long-term mortality.

Copeptin Plasma Level in Type 1 and Type 2 Myocardial Infarctions
Authors: Kassem, Mona; Andric, Tijana; Tajsic, Milos; Soysal, Hatice; Tscharre, Maximilian; Vargas, Kris; Huber, Kurt
Presenter: Mona Kassem

Background: During the last years, distinguishing between type 1 (T1MI) and type 2 myocardial infarction (T2MI) by use of biomarkers became a matter of clinical interest. This study aimed to investigate whether copeptin plasma levels can help to differentiate between T1MI and T2MI. Methods: In a retrospective analysis, 959 unselected consecutive patients with chest discomfort and suspicion of acute MI were evaluated. Patients diagnosed with ST-elevation MI were excluded from the analysis. The remaining patients were classified into T1MI, T2MI, and no-MI, using clinical assessment and coronary angiography. Copeptin concentrations were measured using Thermo Scientific BRAHMS Copeptin ultrasensitive Kryptor assay and compared between both MI subtypes. Furthermore, univariable and multivariable regression analyses for significant confounders were performed. Results: After exclusion of 848 patients (747 no MI and 102 STEMI), 111 (11.6%) subjects with NSTE-ACS were included in the analysis. Of those, 62 (55.9%) were classified by clinical means as T1MI and 49 (44.1%) as T2MI. The Mann-Whitney-U test revealed a significant difference in copeptin plasma concentrations between T1MI and T2MI patients (7.95 pmol/l [IQR 13.53] vs 20.45 pmol/l [IQR 85.74]; p=0.002) (Figure). Univariable logistic regression model for copeptin as a predictor for T2MI was statistically significant (OR 1.007 [95% CI 1.001-1.013]; p=0.023). After adjustment for the significant confounder (heart rate) elevated copeptin levels remained significantly associated with the diagnosis of T2MI (OR 1.017 [95% CI 1.004-1.029]; p=0.008). Conclusion: Compared to T1MI patients copeptin levels were significantly higher in patients with T2MI. This association persisted after correction for significant confounders. A more pronounced elevation of copeptin levels might help in differentiating between patients with T1MI and T2MI in combination with clinical judgement.

IL-33/ST2 in patients with chronic obstructive pulmonary disease
Authors: Stojkovic, Stefan; Urban, Matthias; Brekalo, Mira; Valipour, Arschang; Wojta, Johann; Burghuber, Otto
Presenter: Stefan Stojkovic

Background and Aim: The IL-33/ST2-system is implicated in pathophysiology of COPD. Cigarette smoke induces IL-33 expression in human bronchial epithelial cells, and upregulates ST2 expression on macrophages (M0), natural killer cells and dendritic cells. We investigated here the potential role of IL-33 and soluble ST2 (sST2) as biomarkers in patients with COPD. Methods and Results: Circulating IL-33 and soluble sST2 was assessed using ELISA in 60 patients with stable COPD, 30 patients with exacerbated COPD, and 40 controls (20 smokers and 20 non-smokers). IL-33 plasma levels were similar in all groups of patients. In contrast, highest sST2 levels were observed in patients with COPD exacerbation (35.7 ng/ml), followed by stable COPD (25.2 ng/ml), smokers (22.4 ng/ml) and non-smokers (17.1 ng/ml, p for comparison between groups < 0.001). sST2 was associated with COPD exacerbation with an odds ratio (OR) 3.8 (95% CI 1.1 - 13.1, p=0.031) after adjustment for age, sex, pack-years and FEV1. Furthermore, after median follow-up time of 8.9 years 34 COPD patients died. sST2 was strong, independent predictor for all-cause mortality in patients with COPD with a hazard ratio (HR) 4.1 (95% CI 1.4 - 11.8, p=0.009) after adjustment for age, sex, packyears and FEV1. Conclusion: Circulating sST2 is associated with severity of disease and outcome in COPD patients. Thus, sST2 might be a novel, easily accessible biomarker for improved risk stratification of patients with COPD.

Long-term mortality in TakoTsubo patients treated with different antiaggregation therapy
Authors: Piackova, Edita; Weihs, Valerie; El-Razek, Abd; Geppert, Alexander; Nürnberg, Michael; Wesely, Emil; Smetana, Peter; Huber, Kurt
Presenter: Edita Piackova

Background: TakoTsubo syndrome (TTS) is an acute and usually reversible heart failure syndrome, which initially presents similarly as an acute coronary syndrome (ACS). Although the underlying pathophysiology of TTS is different from myocardial infarction, patients are occasionally discharged on antiplatelet therapy due to the lack of clear recommendations. Aim: This study aimed to investigate if antiplatelet treatment improves the outcome of patients with TTS syndrome after discharge compared to no such strategy. Patients and Methods: Data from 117 consecutive TTS patients, who were admitted to our department between 2006 and 2016, were analyzed. Patients on oral anticoagulation were excluded. The study population was stratified into patients with no antiplatelet therapy, patients on aspirin only life-long and patients on dual antiplatelet therapy for 12 months (DAPT) followed by ASA monotherapy, respectively. The different secondary prevention strategies were based on the discretion of the treating cardiologist. Differences in patient characteristics, as well as all-cause and cardiovascular long-term mortality, were investigated. Multivariable regression analysis was performed to adjust for confounders. Results: In total 99 patients were included into the study (no antiplatelet therapy, n=11; aspirin only, n=44; DAPT, N=44). Mean follow-up time of all patients was 5,9 years. There were no differences in patient´s characteristics between the three groups. Neither the long-term cardiovascular or all-cause mortality was significantly different in the crude (cardiovascular mortality $\chi 2(2) = 0{,}835$, p=0,659; all cause mortality $\chi 2(2) = 0{,}387$, p=0,824, respectively) and multivariable regression analysis (long-term cardiovascular mortality HR= 0,611; CI= 0,209-1,788, p=0,368; long-term all-cause mortality HR= 0,811; CI= 0,449-1,464, p=0,488, respectively). Conclusion: Despite some limitations, the study was neither randomized nor blinded, single center and retrospect in design, the data obtained show that long-term antiplatelet treatment in patients after an index TTS has no impact on hard clinical outcome and has, therefore, no indication.

Effects of cardiac rehabilitation on advanced glycation endproducts (AGEs)
Authors: Thauerer, Bettina; Stritzinger, Barbara; Kullich, Werner
Presenter: Bettina Thauerer

Less is known about the effects of inpatient cardiac rehabilitation on advanced glycation end products (AGEs) and sRAGE. AGEs are generated through non-enzymatic glycation and oxidation of proteins, lipids and nucleic acids. These heterogeneous molecules affect nearly every cell in the body and are thought to play a key role in developing age-related chronic diseases, such as neurodegeneration, arteriosclerosis and cardiovascular disease.Therefore AGEs represent a novel marker of vascular complications in high-risk patients for cardiovascular disease (Yamagishi S et al. Mol Med 2015). Different forms of AGE-receptors are known. The soluble form of the receptor (sRAGE) can bind the ligand, but works, because of lacking the cytoplasmic domain, as protective decoy receptor. The aim of this study was to examine the impact of a 3-week multidisciplinary rehabilitation stay (physical exercise, dietary nutrition, medical supervision) on AGEs and oxidative stress. Blood samples at the beginning (baseline) and the end of the rehabilitation stay (discharge) from 62 patients with coronary heart disease (CHD), aged from 33 -75 years, were investigated for different parameters by ELISA technique: Myeloperoxidase (MPO) as a marker for oxidative stress, AGEs and also the soluble receptor (sRAGE). Additionally AGEs were measured with a special non-invasive method by an AGE-reader using autofluorescence properties on the skin. For getting a more reliable marker the quotient AGE/sRAGE was formed (Prasad K et al. Curr pharmac design 2017). The study showed that a multidisciplinary rehabilitation (including passive and active physical therapy, exercise training, special diet) can decrease oxidative stress significantly by reducing MPO levels ($p<0.05$). Furthermore the AGE-level was slightly reduced in serum and the protective receptor increased simultaneously. Therefore we can observe a significant decline in the calculated quotient AGE/sRAGE ($p<0.01$). Inpatient rehabilitation is a powerful instrument to improve situation of patients by reducing oxidative stress and the AGE/sRAGE quotient. The multidisciplinary rehabilitation combines active and passive exercises, dietary nutrition and medical supervision and is important for recovery, to keep well and fit and resumption of work.

Relation between ventricular assist device position and pump thrombosis
Authors: Aigner, Philipp; Schlöglhofer, Thomas; Plunger, Lea Carmen; Beitzke, Dietrich; Wielandner, Alice; Schima, Heinrich; Wiedemann, Dominik; Zimpfer, Daniel; Moscato, Francesco
Presenter: Philipp Aigner

Introduction: Malpositioning of Left Ventricular Assist Devices (LVADs) is an important risk factor for pump thrombosis caused by disturbed inflow conditions and intraventricular flow patterns. Routinely acquired computer tomography (CT) and X-Ray data can be used to define pump position parameters and to find correlations to pump thrombosis. Methods: Retrospectively patients implanted with a Heartmate II or HVAD (n=115) were analyzed. A pump thrombosis group (PT) and a propensity-score-matched control group (NT) assigned. The position of the inflow cannula was analyzed in X-Ray and CT-scan data sets. Parameters defining the inflow cannula (IC) were defined in respect to cardiac landmarks. In CT data a short axis and three-chamber view was reconstructed and the IC direction deviation to the apex-to-mitral axis measured. Results: In the patient cohort 15 patients (age: 60.3±8.1 y, male n=13, HMII/HVAD n=7/8, BMI: 26.6 kg/m2) experiences a pump thrombus. In the CT short-axis view a shorter distance of the IC to the ventricular wall was measured in the PT-group for both pump types. (0.8±0.8 vs. 1.2±0.5cm; p=0.03). Further a larger deviation of the inflow orientation from the mitral valve was found in the PT group was compared to NT patients (angle α: PT -22.0±4.7° vs. NT -1.2±7.5°; p= 0.006). For the HVAD in frontal X-Rays the projected pump area correlated well with this angle (ρ =-0.922; p=0.003) and the short pump diameter was significantly different in the groups (PT 41.3±4.8 mm vs NT 34.9±6.0 mm, p=0.026). In the lateral view for the PT group the areas of the pump body were smaller (PT 2006±77 mm² vs NT 2138±132 mm², p=0.042). Conclusion: Risk parameters from both X-Rays and CT data were identified that contain information about a possible malposition of the pump that could lead to pump thrombosis. A more frontal posterior inclination of the HVAD pump might imposes some risk of pump thrombosis.

Impact of major bleeding on long-term mortality in patients undergoing Transcatheter aortic valve implantation (TAVI)
Authors: Vujasin, Irena; Tscharre, Maximilian Leopold; Egger, Florian; Rohla, Miklos; Geppert, Alexander; Huber, Kurt; Wojta, Johann
Presenter: Irena Vujasin

BACKGROUND: Transcatheter aortic valve implantation (TAVI) has become the standard therapy for high-risk and inoperable patients with severe symptomatic aortic valve stenosis. In the recent years, there has been also a trend towards the use of TAVI in patients with intermediate risk for surgery. In order to offer this technique safely it is crucial to identify and reduce modifiable risk factors for death such as bleeding hazards. Accordingly, we were interested to investigate the impact of bleeding complications in our TAVI cohort on outcomes. METHODS: We analyzed variables potentially associated with long-term all-cause death in patients undergoing TAVI and their relationship with major bleeding complications during TAVI, as well as after hospital discharge and their impact on outcome. Major bleedings were defined as Bleeding Academic Research Consortiom (BARC) type 3 or greater. Multivariate analysis was performed using a Cox proportional hazards model with backward elimination of insignificant variables at a p-value ≥0.20. RESULTS: The mean age of 149 consecutive patients included into this analysis (patients who had died peri-procedural were excluded) was 83±6 years, the mean STS score was 6.1±3.5% and 59% were female. During a mean follow-up of 24±20 months 62 (42%) patients died. The incidence of major in-hospital bleedings as well as major bleedings during follow-up was 22%, respectively. After multivariate adjustment, factors significantly associated with all-cause death were chronic obstructive pulmonary disease (COPD, HR 3.99, 95%CI 2.24; 7.10, p<0.01), the occurrence of a major bleeding episode (HR 2.99, 95% CI1.66; 5.41, p<0.01), presence of coronary artery disease (CAD, HR 2.28, 95%CI 1.25; 4.18, p<0.01), and baseline hemoglobin (HR 1.16, 95%CI 1.03;1.30, p=0.02 per g/dL decrease), respectively. CONCLUSION: In descending order for relative the risk of death, COPD, the occurrence of major bleedings during follow-up, significant CAD, and anemia were associated with outcomes of patients undergoing TAVI, whereby the age of the patient was the driving factor.

Investigation of CFD and PIV experimental validation for intraventricular flow fields and the prediction of intraventricular thrombosis
Author: Khienwad, Thananya
Presenter: Thananya Khienwad

Currently, Computational Fluid Dynamics (CFD) is widely used to investigate the intraventricular flow patterns during Left Ventricular Assist Device (LVAD) support. Based on such simulations, specific parameters for thrombus formation risk analysis have been developed. However CFD simulations of complex flow configurations require a proper validation by in-vitro experiments. To achieve this requirement, Particle Image Velocimetry (PIV) measurement and CFD of ventricular model has been performed. A ventricular model with a well-defined inflow section was analyzed by PIV and replicated by CFD simulations with a tetrahedral mesh of 5 million elements. Four different CFD method including the Laminar, standard k-omega (SKO), shear stress transport (SST) and renormalized group k-epsilon (RNG) were applied and compared to the experimental results from PIV measurement. The relative errors of velocity were calculated for medial coronal and sagittal planes. For Indicators of thrombogenic regions, low velocity areas (v<0.01m/s) and recirculation (Q-value>0.25) were considered.The velocities of the laminar and k-omega methods had low relative errors compared to the experiment in the coronal (laminar: 0.17, SKO: 0.19, SST: 0.18, RNG: 0.59) and sagittal plane (laminar: 0.23, SKO: 0.26, SST: 0.29, RNG: 0.48), however, all numerical methods under predicted the areas of stagnation and recirculation (PIV: 4.2 cm², laminar: 3.3 cm², SKO: 2.9 cm², SST: 2.4 cm², RNG: 1.6 cm²). Nevertheless, laminar model showed the same positions as PIV, likely indicating the critical areas for intraventricular thrombosis. Although several CFD methods are available for the analysis of left ventricular flow patterns, most of them (SKO, SST, RNG) failed to identify the critical areas for flow stagnation and recirculation when compared to an in-vitro model. However, this study shows that the laminar method seems to be most appropriate to simulate realistic intraventricular flow fields and predict risk areas for thrombosis.

REGENERATIVE MEDICINE RESEARCH

"Tell us!" about accidental injuries: Crowdsourcing Clinical Knowledge to Spark Innovation in Traumatology Research
Authors: Missbach, Benjamin; Hruschka, Veronika; Malfent, Lucia; Redl, Heinz
Presenter: Benjamin Missbach

Background: What if patients and clinical experts could spark and define the direction of multidisciplinary science projects without knowing the scientific literature? The LBG Open Innovation in Science Center and the LBI for Experimental and Clinical Traumatology set up a project in order to fill the gap between clinical knowledge and scientific research. This novel approach in the field of Traumatology aims to bridge the gap between the conventional bench-to-bedside approach within this discipline. Methodology: "Tell us!" — about accidental injuries leverages crowdsourcing methodology to spark new lines of research.Over a period of 4 months, a broad community of international stakeholders and participants was established and motivated to submit research questions in the area of the diagnosis, treatment and rehabilitation of patients with major traumatic accidents. Results: 227 participants registered to the tell-us.online platform. In total, 118 users contributed a total of 190 research questions within 4 months of crowdsourcing (Mai — August 2018). Overall, 60% of all contributions came from experts coming from physiotherapy (n = 13), MDs (n = 13) and occupational therapy (n = 11). Most contributions came from the field of nursery with research questions ranging of of great detail to more general questions. Conclusion: Applying Open Innovation in Science is a major quest in order to open up scientific workflows. In particular, using crowdsourcing to identify research questions from bedside-to-bench can be a method of great potential to spark innovation in the field of Traumatology.

Evaluation of functional transfer of extracellular vesicle cargo between endothelial and adipose-derived stem cells during vascular network formation
Authors: Schneider, Jaana; Pultar, Marianne; Reddy Bobbili, Madhusudhan; Mühleder, Severin; Priglinger, Eleni; Redl, Heinz; Spittler, Andreas; Grillari, Johannes; Holnthoner, Wolfgang
Presenter: Jaana Schneider, Marianne Pultar

Extracellular vesicles (EV) play an important role in cell communication due to the transfer of biomolecules between cells. Recent data suggest vascular network formation by co-cultures of adipose derived stromal cells (ASC) and endothelial cells (EC). There are conflicting data if the proangiogenic effects are due to direct cell-to-cell contact or to EVs released from the ASC. In this study we address this question by discriminating cells which have received functional EV cargo from cells which have not. Therefore, we employ the Cre-LoxP system, which is based on reporter cells and cells expressing the Cre recombinase, whose mRNA is packaged into EVs. Reporter cells contain a construct of floxed dsRed upstream of the eGFP coding sequence. After EV uptake and translation of Cre mRNA, they switch from dsRed to eGFP expression due to site specific recombination resulting in deletion of the floxed dsRed. In order to test functional transfer of EV cargo, the reporter and the Cre construct were subcloned into a retroviral backbone. To study communication between EC and ASC, human umbilical vein endothelial cells (HUVEC), human induced pluripotent stem cells-endothelial colony forming cells (hiPSC-ECFC) as well as ASC were retrovirally transduced with either the reporter or the Cre construct. HUVEC and ASC reporter cells were incubated with the supernatants from Cre-expressing ASC and HUVEC. Furthermore, co-cultures containing HUVEC reporter cells and Cre-expressing HUVEC or ASC in different ratios (1:10 and 1:50, respectively) were investigated for color switch for up to three weeks by fluorescence microscopy and flow cytometry. We detected single cells switching from dsRed to eGFP expression after four days in all experiment set ups, except in co-cultures with a ratio of 1:50 (sorted reporter cells:Cre cells). Additionally, as expected, HUVEC formed tube like structures during cultivation with ASC. Due to background recombination eGFP positive cells were also detected in the negative controls. Nevertheless, the detected change in expression indicates Cre-dependent recombination. This interpretation is supported by superinfection of reporter cells with the Cre construct, thereby confirming the suitability of this system to analyze cell-cell communication by EVs.

A non-invasive one-step reporter system for live detection of miRNA influence on cellular migration
Author: Sperger, Simon; Brenner, Teresa Kristina; Mayr-Harting, Lucia; Redl, Heinz; Hacobian, Ara
Presenter: Simon Sperger

Without cell migration, there would be no wound healing or immune response. Unfortunately, understanding of migratory processes is still in its early stages. Further advancing scientific research in this area would not only be tremendously beneficial for regenerative medicine, it could also contribute greatly to a better understanding of cancer metastasis and other pathologies that are dependent on the migration of cells. By specifically silencing target mRNAs and thus influencing gene expression patterns, endogenous micro RNAs (miRNAs) are an efficient regulator of cellular metabolism and are known to highly modulate the process of cell migration. The majority of miRNA-specific gene regulation takes place in the untranslated regions of genes. This holds true for the guanine nucleotide exchange factor Tiam1, a key regulator protein in the process of cell migration. In this study, we developed a novel one-step non-viral reporter system to efficiently evaluate miRNA influence on cell migration by fusing different miRNA binding sites derived from Tiam1's 3' untranslated region to a secretory embryonic alkaline phosphatase (SEAP) reporter. SEAP expression can easily be quantified in cell culture supernatants via alkaline phosphatase (ALP) assay. Following transfection of different cell lines and subsequent detection of ALP activity at several timepoints, we were able to identify specific miRNAs that influence cell migration via the Tiam1 pathway very potently due to silenced SEAP expression after reaching confluency of cells and arrest of cellular migration, respectively. Furthermore, culturing transfected cells in a pro-migratory environment – e.g. serum starvation, addition of pro-migratory growth factors – resulted in lack of silencing of reporter gene expression. In conclusion, we were able to construct a minimally invasive one-step reporter system that enables direct evaluation of specific miRNA-mediated silencing of cell migration. In addition, the blueprint of this reporter system is applicable for investigating basically all miRNA-mediated processes in vitro.

Vascular Morphogenesis in the Context of Inflammation: Self-Organization in a Fibrin-Based 3D Culture System
Authors: Rüger, Beate; Buchacher, Tanja; Giurea, Alexander; Kubista, Bernd; Fischer, Michael; Breuss, Johannes
Presenter: Beate Rüger

Key cellular players of the neovascularization process in an inflammatory setting are immune cells recruited to perivascular niches together with endothelial progenitor cells, where they interact within complex networks with resident endothelial- and stromal cells directly or indirectly through the secretion of paracrine factors. In an attempt to mimic the complexity of an in vivo remodeling process and in order to study vascular morphogenesis in the context of inflammation, we established a 3D fibrin matrix system for the culture of inflamed synovial tissue fragments. To specifically investigate the contribution of perivascular cells to neo-vessel formation, mesenchymal stromal cells (MSC) were co-cultured with peripheral blood mononuclear cells (PBMC) in the fibrin matrix. Cellular and structural re-arrangement were characterized by confocal laser-scanning microscopy of topographically intact 3D cultures, cytokine levels were evaluated by Bio-Plex assay and ELISA. Neo-vessels originating from both the embedded synovial tissues and from clusters locally formed by emigrated mononuclear cells were closely associated with CD45+ leukocytes and Collagen (Col)-IV+ stromal cells. MSC-PBMC co-cultures formed vasculogenic clusters consisting of and surrounded by CD45+ leukocytes and Col-IV+ MSC and matrix structures with emerging cells of endothelial phenotype further developing into complex vascular sprouts. No vascular structures were observed in control 3D monocultures of PBMC or MSC. Both cultured synovial tissue fragments and MSC-PBMC co-cultures secreted high levels of VEGF, GCSF and IL-6. Cross-talk and cluster formation of MSC with PBMC support neo-vessel growth within the 3D fibrin environment via secretion of pro-angiogenic factors and through self-organization can lead to the emergence of complex vascular structures.

Estrogen depletion alters mineralization regulation mechanisms in an ovariectomised monkey animal model
Authors: Paschalis, Eleftherios; Gamsjaeger, Sonja; Condon, Keith; Klaushofer, Klaus; Burr, David
Presenter: Eleftherios Paschalis

Ovariectomized animal models have been extensively used in osteoporosis research due to the resulting loss of bone mass. The purpose of the present study was to test the hypothesis that estrogen depletion alters mineralization regulation mechanisms in an ovariectomized monkey animal model. To achieve this we used Raman microspectroscopy to analyze humeri from monkeys that were either SHAM-operated or ovariectomized (N=10 for each group). Measurements were made as a function of tissue age and cortical surface (periosteal, osteonal, endosteal) based on the presence of calcein fluorescent double labels. In the present work we focused on osteoid seams (defined as a surface with evident calcein labels, 1µm distance away from the mineralizing front, and for which the Raman spectra showed the presence of organic matrix but not mineral), as well as the youngest mineralized tissue between the second fluorescent label and the mineralizing front, 1µm inwards from the front with the phosphate mineral peak evident in the Raman spectra (TA1). The spectroscopically determined parameters of interest were the relative glycosaminoglycan (GAG) and pyridinoline (Pyd) contents in the osteoid, and the mineral content in TA1. At all three cortical surfaces, significant correlations were evident in the SHAM-operated animals between osteoid GAG (negative) and Pyd content, and mineral content, unlike the OVX animals. These results suggest that in addition to the well-established effects on turnover rates and bone mass, estrogen depletion alters the regulation of mineralization by GAGs and Pyd.

NADPH oxidase-derived reactive oxygen species – a requirement for the proliferation of human amniotic mesenchymal stromal cells
Authors: Dumitrescu, Sergiu; Weidinger, Adelheid; Banerjee, Asmita; Wolbank, Susanne; Redl, Heinz; Kozlov, Andrey
Presenter: Sergiu Dumitrescu

The transition from a quiescent to an active stem cell (SC) is an obligatory event for the maintenance of tissue homeostasis and regeneration. It has been suggested that mitochondria and reactive oxygen species (ROS) play an essential role in this process which is still not completely understood. This study aims to determine the impact of functional mitochondria and mitochondrial ROS on the vitality and proliferation of human neonatal SCs. Adjusting specific components of the incubation medium and passage numbers we have established two different culture conditions, facilitating either the active (proliferating) or quiescent state of hAMSCs; both states manifested healthy cellular morphology. In both, active and quiescent cells we modulated the mitochondrial respiration, as well as generation of ROS by mitochondria and NADPH-oxidase. We have observed that FCCP, a mitochondrial uncoupler, completely inhibited cell proliferation under activating conditions and even exerted a slight cytotoxic effect, while oligomycin, an inhibitor of ATP-synthase, and antimycin A, an inhibitor of mitochondrial complex III and simultaneously an inducer of mitochondrial ROS release, did not exert any effect on proliferation rate. Similarly, the mitochondria targeted antioxidant mitoTEMPO did not change the rate of proliferation, suggesting that mitochondrial ROS do not contribute to the activation of hAMSCs. This was in line with the data obtained by laser scanning microscopy, which revealed that hAMSCs have a much lower mitochondrial ROS levels compared to parenchymal cells such as hepatocytes. In contrast to these results, inhibitors of NADPH oxidase (apocynin and DPI) as well as extracellular ROS scavengers (SOD and catalase) nearly completely inhibited proliferation. Our results indicate that the activation of hAMSCs require coupled mitochondria and ROS derived from the NADPH oxidase; NADPH-oxidase derived ROS activate hAMSCs in an autocrine and/or paracrine manner.

Human induced pluripotent stem cells-based strategies for bone tissue engineering and regeneration during aging
Authors: Marolt, Darja; Hanetseder, Dominik; Ogrin, Laura; Stetco, Alexandra-Larissa; Redl, Heinz
Presenter: Darja Marolt

Background and aim: Compromised bone tissue physiology and the decreased function of bone forming cells during aging limit the native tissue capacity for healing. Thus, regeneration of bone defects in the elderly patients presents significant challenges. We have previously reported on engineering of bone tissue substitutes from human induced pluripotent stem cells (hiPSCs) (1), an autologous source of potentially rejuvenated osteogenic progenitors available for any patient in unlimited quantities. The aim of our current project is to explore hiPSCs as a source of secreted factors and extracellular matrix (ECM) for cell-free bone tissue engineering and to test the effects of specific components on primary bone marrow stromal cells from traumatology patients of different ages. Methods: hiPSCs were expanded and differentiated into embryonic-like mesenchymal progenitors (hiPSC-MPs) with high proliferation and osteogenic differentiation potential, as in our previous studies. hiPSC-MPs were expanded in culture and used for the preparation of conditioned media (CM) as well as for in vitro engineering of ECM layers. Major cytokine and growth factor components of the CM were determined using a Luminex array. ECM structure and composition were identified and compared before and after decellularization procedure by immunofluorescent staining (collagens type I and IV, fibronectin, laminin). Using a library of primary BMSCs exhibiting diverse proliferation and differentiation potentials, we found that hiPSC-MP-derived CM and ECM modulate the primary BMSC responses in a patient-specific manner. In particular, some aged BMSC lines only exhibited increased proliferation and osteogenic differentiation (alkaline phosphatase activity, osteogenic gene expression) when cultured in the presence of hiPSC-MP secreted components. Conclusions: Our studies suggest that hiPSC-MP-derived microenvironment components can modulate the regenerative potential of adult/aged BMSCs. Further investigation of patient-specific responses is expected to offer insight into the underlying mechanisms and guide the therapeutic approaches to enhance bone regeneration in elderly patients. References: 1. de Peppo GM et al. Proc Natl Acad Sci USA, 2013

Tuning mitochondria to set the course for human amniotic cell activation
Authors: Weidinger, Adelheid; Lindenmair, Andrea; Hennerbichler, Simone; Steinborn, Ralf; Kozlov, Andrey V.; Redl, Heinz; Wolbank, Susanne; Banerjee, Asmita
Presenter: Mirjam Dellinger

Recently, vital human amniotic membrane (hAM), containing cells with distinct stem cell properties, has come into focus for clinical applications in regenerative medicine. Most stem cells reside in low oxygen niches (1-3%), and generate energy predominantly via glycolysis. Common cell culture laboratories, usually set up at 20% oxygen, pose a challenge for stem cell cultivation, since a possible metabolic switch from glycolysis to oxidative phosphorylation could impact stem cell fate and cell function. We tested the impact of short term cultivation (4 days) at either 3% or 20% oxygen on human amniotic mesenchymal stromal cells (hAMSCs), regarding mitochondrial and inflammatory parameters, in order to clarify whether the energy metabolism is substantially different at these two oxygen levels. Human amniotic mesenchymal stromal cells were isolated from two sub-regions of hAM, placental and reflected. Measurement of mitochondrial activity was performed with high-resolution respirometry (Oxygraph-2k, Oroboros Instruments, Austria). Nitric oxide (NO) was detected with Sievers 280i-NO Analyzer (General Electrics), and levels of reactive oxygen species (ROS) were analysed with electron paramagnetic resonance spectroscopy (EPR). We found 20% oxygen to increase mitochondrial oxidative phosphorylation, and the release of IL-6. This increase was more pronounced in placental hAMSCs, suggesting placental hAMSCs to be more sensitive to changes in surrounding oxygen levels. Interestingly, the respiration rate negatively correlated with intracellular reactive oxygen species levels. The release of NO was elevated at 20% oxygen but independent of the amniotic region. Our data show that exposure of hAMSCs to atmospheric oxygen strongly impacts cellular physiology. We hypothesize that the choice of oxygen tension can be used to tune mitochondria to set the course for cellular differentiation and proliferation.

Human placenta extracellular matrix hydrogels for surface coating to improve cell seeding efficiency on vascular grafts
Authors: Rohringer, Sabrina; Schneider, Karl Heinrich; Eder, Gabriela; Lieber, Roman; Windberger, Ursula; Bergmeister, Helga
Presenter: Sabrina Rohringer

Introduction: Efficient re-endothelialization of acellular vascular grafts is crucial for graft patency since endothelial cells have a barrier function and inhibit thrombosis. To improve cell seeding efficiency, a hydrogel for graft coating was developed and tested to create additional anchoring-points for the cells and to support cell adhesion and migration. Human placental tissue has major advantages to be used as tissue source, due to its protein composition and its wide availability as clinical waste product. Therefore, decelluarized human placenta arteries were used as raw materials to create placental extracellular matrix hydrogels (pECM-HG). Material and Methods: pECM-HG were produced by tissue decellularization followed by an enzymatic digestion using acidic pepsin solution. pECM-HG were biochemically analyzed for DNA residuals and collagen and the proteoglycan content was quantified. Rheological tests with different hydrogel concentrations were performed to examine the polymerization behavior of the material. Scanning electron microscopy (SEM) was used to visualize fiber structure of pECM-HG coated plane surfaces. Cytotoxicity, cell adherence and migration assays were carried out using HUVECs on pECM-HG coated culture plates. Gelatin coatings were used as controls. PECM-HG coated and non-coated acellular vascular grafts were further analyzed in a perfusion bioreactor system with regard to endothelial cell attachment during reseeding. Results: pECM-HG showed low amount of DNA residuals and preservation of extracellular matrix proteins. SEM analysis revealed formation of specific fibrous structures dependent on the respective hydrogel concentration. Cell binding and viability assays showed significantly enhanced cell adherence and increased proliferation rates when seeded on surfaces of culture dishes coated with pECM-HG ($p<0.001$). Furthermore, pECM**-**HG showed beneficial properties for cell reseeding on acellular vascular grafts in a flow bioreactor system. Cell attachment was increased on grafts coated with pECM-HG compared to uncoated controls. Conclusion: Improved seeding efficiency on vascular grafts by surface coatings will support future experiments in the field of tissue remodeling using a pulsatile flow bioreactor test system.

Evaluation of different BMP combinations to improve osteoinduction in mouse C2C12 and human iPSC-derived mesenchymal progenitor cells

Author: Frischer, Amelie; Hercher, David; Marolt, Darja; Redl, Heinz; Hacobian, Ara
Presenter: Amelie Frischer

In the field of bone regeneration, the TGFβ-superfamily of growth factors contains candidate proteins offering the possibility to improve the healing process. Belonging to this family of proteins, bone morphogenic proteins (BMPs) have been studied intensively in respect to their involvement in bone development. Several BMPs are known for their osteoinductive activity - BMP-2, -4, -6, -7, and -9 constituting the most promising candidates. In clinics, BMPs are applied as recombinant proteins. A major bottleneck is the need for relatively high doses and repeated administration. Gene therapy constitutes an attractive alternative, not only because of lower production costs when compared to recombinant proteins, but also in terms of efficiency by enabling locally restricted and prolonged production of the respective proteins. In their active form, BMPs can act as either homodimers or heterodimers. It has been reported that heterodimers exhibit a higher potential to induce osteogenic differentiation. In this study, sequence optimized versions of BMP-2, -4, -6, -7 and -9 were cloned into gene expression vectors under the control of a constitutive EF1α-promoter. Subsequently, different BMP combinations were analyzed regarding their capability to induce osteogenesis in transfected mouse myoblast C2C12 and human iPSC derived mesenchymal progenitor cells via alkaline phosphatase activity assay (ALP) and realtime-PCR. Results showed significantly improved osteoinduction compared to transfection with single BMPs. In addition to the already known strong osteoinductive capacity of BMP-2 and -7 heterodimers we identified several equally potent combinations of BMPs. Interestingly, single transfection with BMP-9 showed similar osteoinduction. Keywords: Gene therapy; Bone morphogenic proteins; Osteogenic induction

Human amniotic membrane as vital carrier for human articular chondrocytes

Authors: Lindenmair, Andrea; Wolbank, Susanne; Fürsatz, Marian; Hennerbichler, Simone; Redl, Heinz; Nürnberger, Sylvia
Presenter: Andrea Lindenmair

Introduction/Aim: Human amniotic membrane (hAM) has been successfully applied in the clinic for many years and is worth to be further established as native biomaterial for tissue engineering strategies and regenerative medicine. As hAM is a thin, highly flexible, but strong membrane, it can be used as carrier material for easier application of therapeutic cells. Being composed of cells with proven stem cell characteristics including anti-inflammatory and differentiation capacities, the chondrogenic differentiation potential of hAM and isolated cells thereof has already been demonstrated. Therefore, we aim to combine hAM with human articular chondrocytes (hAC) to provide a vital anti-inflammatory carrier that supports and potentially improves the chondrogenic conversion of both hAM and cultured hAC for tissue engineering of cartilaginous constructs. Methods: To evaluate the interaction between hAM and hAC different settings were tested. hAC were cultured with hAM-punch biopsies or in hAM-conditioned media. Furthermore, different differentiation media with or without supplementation of additional growth factors (TGFbeta, BMP-6) were compared to proliferation media. Samples for histology (e.g. collagen type II), mRNA expression of markers relevant for chondrogenic conversion (e.g. COL2 versus COL1, Aggrecan versus Versican, Sox-9, MIA) and glycosaminoglycan quantification were analyzed after three weeks of (co-) culture. Results: hAC could adhere to the epithelial as well as to the mesenchymal layer of hAM, even after hAC-pellet preculture for three weeks. hAC pellets in coculture stained positive for collagen type II and showed a trend to be bigger than the controls. Conditioned media of hAM only showed sparse effects upon cultivation of hAC-pellets for three weeks. mRNA expression of chondrogenic markers confirmed chondrogenic differentiation, the degree of which was influenced by TGFbeta concentration supplemented to the media. Conclusion: Differentiation of hAC in combination with hAM is at least as effective as hAC cultured alone. Hence, hAM seems to be a suitable viable carrier for therapeutic cells intended to be used for cartilage tissue engineering and regeneration.

Adipose Tissue-derived Therapeutic Cells in their Natural Environment as Autologous Cell Therapy Strategy: The Microtissue-Stromal Vascular Fraction

Authors: Maier, Julia; Nürnberger, Sylvia; Lindner, Carolin; Strohmeier, Karin; Wurzer, Christoph; Slezak, Paul; Suessner, Susanne; Holnthoner, Wolfgang; Redl, Heinz; Wolbank, Susanne; Priglinger, Eleni
Presenter: Julia Maier

Human adipose tissue is an attractive and abundantly available source for autologous tissue rich in adult stem cells applicable in regenerative medicine and tissue engineering. Current isolation methods depend on enzymes, lysis buffer, long incubation steps and mechanical stress resulting in single cell dissociation from their natural microenvironment. The aim of this study was to limit cell manipulation and obtain a derivative comprising therapeutic cells (Microtissue-SVF) without dissociation from their natural extracellular matrix by employing a gentle GMP-grade isolation with low enzyme concentration. Microtissue-SVF yielded higher numbers of viable cells compared to standard isolation and a minimal content of dead cells. Microtissue-SVF comprised stromal tissue compounds (collagen, glycosaminoglycans, fibroblasts), capillaries and vessel structures (CD31+, smooth muscle actin+). A broad range of cell types were identified by surface marker characterization including mesenchymal, hematopoietic, pericytic, blood and lymphatic vascular and epithelial cells. Subpopulations such as supra-adventitial adipose-derived stromal/stem cells and endothelial progenitor cells were significantly higher in Microtissue-SVF corroborated by significantly higher potency for angiogenic tube-like structure formation in vitro. Microtissue-SVF showed the characteristic phenotype and tri-lineage mesenchymal differentiation potential in vitro according to the ISCT/IFATS, as well as an immunomodulatory and pro-angiogenic secretome. In vivo implantation of Microtissue-SVF combined with fat demonstrated successful graft integration in nude mice. In this study, we present a fast and gentle isolation by minor manipulation of liposuction material, achieving a therapeutically relevant cell population with high vascularization potential and immunomodulatory properties still embedded in a fraction of its original matrix.

Effects of photobiomodulation in a diabetic mouse wound healing model

Authors: Slezak, Paul; Sutalo, Sanja; Keibl, Claudia; Karner, Lisa; Meixner, Barbara; Chaudary, Sidrah; Dungel, Peter
Presenter: Paul Slezak

Poor wound healing is one of the most important chronic complications in diabetic patients. Light therapy is a promising tool to enhance healing. This study aimed to investigate the effects of photobiomodulation (PBM) by LED of different wavelengths in a wound healing model in diabetic mice. A circular full-thickness skin wound was excised in db/db mice on the mid-dorsum and covered with transparent dressing. PBM (Repuls) at 632nm, 530nm or 470nm was applied immediately after surgery and on every second day at 80mW/cm2. Light absorption, body and surface temperature and wounds size were recorded. Perfusion was analysed by Laser Doppler Imaging. Excised samples were analysed histologically. Light showed a wavelength-dependent enhancement of wound healing, with red light being most effective. Light therapy also increased perfusion in the wound area. The positive trend on reduction of wound size by red light starting at day 8 reached statistical significance on day 12. Healing rate was significantly increased in the red and green light group during the first week and wound volume was smaller in all light groups. These data correlate with increased angiogenesis quantified as an increased number of CD31-postive vessels in histologic analyses. Light therapy by red and green light positively affected wound healing in a diabetic wound healing model in mice while blue light in this model was ineffective. Thus, light therapy might influence wound healing as well as angiogenesis. Further studies have to focus on intracellular signaling and possible synergistic effects by different wavelengths in order to optimize this promising, alternative application in tissue regeneration and wound therapy.

Tracking therapeutic shockwaves and their impact on regeneration
Authors: Slezak, Paul; Weihs, Anna; Fuchs, Christiane; Rose, Roland; Slezak, Cyrill; Mittermayr, Rainer; Redl, Heinz
Presenter: Paul Slezak

Extracorporeal shockwave treatments have been shown to accelerate tissue regeneration in diverse clinical situations, ranging from bone to soft tissue. The underlying mechanisms of these mechanically induced beneficial effects are not yet fully understood. A statistical analysis of single shot parameters over the duration of a treatment yielded insight into the tissue transmission of shockwaves providing clinically relevant data as our findings show a significant dependence on the type of shockwave generating technology. Additionally, cell culture tests carried out in a waterbath setup identyfied extracellular ATP as a trigger of the biological effects of shock wave treatment and an increase in cell proliferation was observed. Purinergic signaling-induced Erk1/2 activation was found to be essential for this proliferative effect, which was further confirmed in an ischemic flap wound healing model in rats where shock wave treatment induced proliferation and increased wound healing in an Erk1/2-dependent fashion.

The effect of low-energy extracorporeal shockwave treatment in sub-acute and chronic phases of traumatic spinal cord injury
Authors: Ashmwe, Mohamed; Posa, Katja; Hercher, David; Heimel, Patrick; Heinzel, Johannes; Mittermayr, Rainer; Redl, Heinz
Presenter: Mohamed Ashmwe

Extracorporeal shockwave therapy (ESWT) was first clinically introduced for treating concrements in the urinary tract more than 30 years ago. Since then, ESWT has also proven to be effective for treating various pathologies of different tissues (eg non-union fractures, tendinopathies), including the peripheral nervous system. Recent basic research studies have enabled a better understanding of underlying mechanisms of shockwave therapy, including the release of various growth factors, enhanced cell proliferation, improvement of local tissue perfusion by vasculogenesis and modulation of inflammation. The clinically most relevant time point for therapeutic intervention in spinal cord injury (SCI) is believed to be the sub-acute and chronic phase after SCI, in which secondary pathophysiological processes take place. Therefore, we investigate effects of ESWT in a sub-acute (2 weeks after injury) as well as chronic (5 weeks after injury) setting in a rat contusion model at the 11th thoracic vertebra. The functional outcome was assessed using BBB-Score and Catwalk®-analysis at different timepoints. Histology as well as µCT imaging provided insights into morphological changes after ESWT. Also, a large number of systemic microRNAs were screened for changes in expression by comparing tratment and control groups, as well as various timepoints to explore effects and underlying mechanisms of this therapeutic approach. In the chronic setup, ESWT treated animals (n=15) showed significant improvement in their functional outcomes in contrast to untreated rats (n=21) after SCI. The subactute setup trial is currently still ongoing. Furthermore, establishing improved µCT images using Lugol´s iodine as contrast agent, allowed visualisation of various structures within the spinal cord. Based on our preliminary successful outcomes, we are highly optimistic that exploring effects of ESWT in these therapeutically relevant time windows could offer a powerful and non-invasive tool in the treatment of SCI.

Hernia Surgery as Door Opener for Regenerative medicine in large Scale Applications
Authors: Petter-Puchner, Alexander; Gruber-Blum, Simone; Fortelny, René; Redl, Heinz
Presenter: Alexander Petter-Puchner

Introduction: Hernia repairs the most frequent procedures in general surgery. More than 3 million meshes and scaffolds are implanted worldwide per year. They shall not only reinforce the repair of abdominal wall defects, but improve regeneration, functionality and viability of the surrounding tissue. Methods: Implants will be discerned and explained as part of the following categories: -Classical 2D polymer meshes (Polypropylene and Polyester) -Biomeshes derived from animal collagen (bovine, porcine, equine) -Synthetic absorbable 3D scaffolds (eg PLA compounds) Results: Pros and cons, as well as relevant parameters (e.g. foreign body reaction, tissue integration and cell attraction, silent infections) will be discussed.

Mature collagen-link formation is inversely associated with osteomalacia in X-linked hypophosphatemia
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Presenter: Nadja Fratzl-Zelman

X-linked hypophosphatemia (XLH) caused by activating mutations in the PHEX gene is the most common form of heritable rickets. Patients have high renal phosphate wasting and low 1,25-dihydroxyvitamin D levels. Osteopontin, a potent inhibitor of mineralization normally degraded by PHEX, was reported to accumulate within the unmineralized XLH osteoid. However, it is not clear whether bone matrix is generally modified or less mineralized. We present here four adult patients with prescribed oral phosphate and calcitriol supplementation. Two of them had a discontinuous treatment adherence (DTA). Transiliac bone biopsies were obtained and evaluated by histomorphometry, Fourier-transform infrared imaging (FTIRI) and quantitative backscattered electron imaging (qBEI) to assess matrix composition and material properties. DTA patients had increased ALP and PTH levels, severe osteomalacia and twice the amount of mineralized trabecular bone volume as the compliant ones. Compliant patients had very low indices of bone formation and osteoid thickness mostly within normal range. FTIRI analysis of osteoid showed that while divalent collagen cross-links were evident in all patients, the mature trivalent cross-link pyridinoline (Pyd) was present only in the compliant ones. Interestingly, also in the compliant patients, the few osteoid seams that were broader than 5µm did not have any measurable Pyd. However, Pyd was evident in the mineralized tissue in all patients. Unexpectedly, osteoid in all samples exhibited considerable acidic lipid content. qBEI analysis revealed that the average degree of matrix mineralization in XLH bone is within normal range, but the percentage of lowly and highly mineralized matrix are both elevated, resulting in a broad heterogeneity in mineralization. In summary, the increased trabecular bone volume in DTA along with the finding that bone mineralization was not decreased in any patient is consistent with densitometric reports of elevated trabecular BMD in XLH. Differentiation of osteoid based on width and therapy compliance indicate altered collagen maturation while the abundant acidic lipids may be contributing to the delayed mineralization. Our data highlight novel organic matrix alterations that may contribute to the impaired mineralization in XLH.

A constellation of Orthopaedic Deformities Are in Connection with Cartilage Oligomeric Matrix Protein Mutation
Author: Al Kaissi, Ali
Presenter: Susanne Kircher

Objective: Trendelenberg´s gait can be observed in Legg-Calvé-Perthes disease, antalgic gait observed in osteo-arthropathy, and waddling gait is usually seen in genu varum and circulduction gait in patients with genu valgum. Disabling pain is a prime manifestation in slipped capital femoral epiphysis. Limited joint range of motion with an inability to bear full weight on an affected extremity with swaying, and wide-based gait are seen in patients with mal-alignment of the lower limbs. All the above mentioned deformities have been labelled as idiopathic. The main objective of this contribution is to approach to the etiology understanding. Methods: Ten children (3 girls and 7 boys with average of 9 years) presented with variable deformities: Perthes like deformity, genu varum / valgum, osteoarthropathy and one patient with slipped capital femoral epiphysis. Clinical and radiological phenotypes were the base line tool of diagnosis. Genotypic characterizations were performed. Results: Diverse clinical presentations of Perthes like disease, osteoarthropathy, genu varum / valgum and slipped capital femoral epiphysis were the most prominent skeletal abnormalities in patients manifested with Cartilage Oligomeric Matrix Protein (COMP) - gene mutation. Conclusion: The value of presenting this contribution is fourfold. Firstly, to signify that mutation study was essential for the increment of knowledge related to the genotype-phenotype relationships. Secondly, to indicate that professional awareness is needed to differentiate between the hidden pathologies in patients with Perthes-like genu varum, genu valgum and early osteoarthritis in correlation with COMP gene mutation. Thridly, it is mandatory to question the validity of the term idiopathic. Fourthly, this contribution is an attempt to sensitize orthopaedic physicians and surgeons that deformities might be stemmed from diverse forms of intrinsic bone disorders.

Antiresorptive treatment either with bisphosphonates or denosumab improve survival in hip fracture patients
Authors: Behanova, Martina; Reichardt, Berthold; Kocijan, Roland; Klaushofer, Klaus; Zwerina, Jochen
Presenter: Martina Behanova

Background: Sustaining a hip fracture in elderly is associated with increased excess mortality risk and with a higher risk of sustaining another hip fracture (HF). The fracture is mainly associated with a bone fragility caused by osteoporosis. We aimed to compare the effectiveness of two therapies – the most commonly used therapy with bisphosphonates (BPs) and a novel approach represented by denosumab (DMAB). We explored differences in incidence for subsequent HF and for all-cause mortality between therapies. Methods: We employed a population-based retrospective cohort study and used national data on all patients in Austria, aged ≥ 50 who sustained a HF between 2012-2016 and were followed for a subsequent HF and all-cause mortality until 2017. Crude and adjusted Cox proportional hazard ratios were calculated for mortality and logistic regression for subsequent HF. Results: A total number of 54 805 patients (mean age 80, SD 10.5; 71.8% of women; mean follow-up 26 months, SD 17.0) sustained a HF. We identified 46152 (84.2%) patients who did not receive any antiosteoporotic treatment after their HF and 7523 (13.7%) patients on BPs therapy and 1130 (2.1%) on DMAB. During the study follow up 2671 (4.9%) of patients sustained a new hip fracture and 18718 (34%) died. Women on DMAB therapy had significantly longer survival time as compared to their counterparts from BPs group (56.9 months, 95% CI 55.5-58.2 and 55 months 95% CI 54.5-55.6, respectively; P = 0.02). With each month of a treatment a risk of mortality significantly decreased by 5% in DMAB group and by 4% in BPs group compared with untreated patients (adjusted HR 0.95, 95%CI 0.94-0.97, p<0.001 and HR 0.96, 95%CI 0.96-0.97, p<0.001). In a direct BPs-DMAB comparison, DMAB reduced a mortality risk by 19% (HR 0.81, 95%CI 0.68-0.97, p<0.05). Treatment with BPs was associated with an incidence rate of a subsequent hip fracture of 26.5 per 1000 person-years, as compared with 24.0 per 1000 person-years in patients treated with DMAB. Compared to untreated patients, a risk for a subsequent HF was 1.26 times higher among patients treated with BPs (OR 1.26, 95%CI 1.12-1.41, p<0.001). Conclusions: Antiresorptive treatment with either BPs or DMAB reduces mortality and should be administered immediately after hip fracture.

Bone matrix characterization in diverse mouse models of osteogenesis imperfecta
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Presenter: Ghazal Hedjazi

Brittle bone disease or osteogenesis imperfect (OI) is a heritable genetic disorder of connective tissue characterized by hypermineralized bone matrix. Mutations in COL1A1 and COL1A2 encoding for α1(I) and α2(I) chains of type I collagen cause "classical" autosomal dominant OI type I to IV with high bone fragility. Recessive forms of OI caused by mutations in non-collagen genes, which are important for post-translational modification of collagen have been described more recently.In particular, deficiency of cartilage-associated protein (CRTAP), prolyl 3-hydroxylase1 (P3H1), or Cyclophilin B (CyPB), the components of the prolyl 3-hydroxylase complex in the endoplasmic reticulum cause OI type VII, VIII and IX respectively. Hypermineralization has been demonstrated by quantitative backscattered electron imaging in human biopsy samples and murine models. In general, bone mechanical properties depend on mineral content, but also on the composition and the organization of the collagenous matrix. In order to further elucidate the origin of OI bone fragility, we initiated a combined study of the distribution of mineral density and collagen orientation. As a model for "classical" OI with collagen gene mutation, we use the Brtl/+ mouse with a Gly349Cys substitution in one COL1A1 allele. As an example for recessive OI, we study the cyclophilin B deficient mouse. Cyclophilin B, encoded by PPIB, an ER-resident peptidyl-prolyl cis-trans isomerase functions as a component of the collagen prolyl 3-hydroxylation complex and regulates collagen lysyl hydroxylation, crosslinking and fibrillogenesis. The degree of mineralization of bone matrix was increased in both mutants compared to their respective wild-type (CaPeak + 2.1%, P=0.03, + 3.4%, P=0.003, respectively). Collagen fibril orientation is estimated by second harmonic generation imaging using confocal laser scanning microscopy. The objective of this research is to explore the relationships between OI phenotype, matrix orientation and mineralization.

MENTAL HEALTH, PSYCHOLOGY, PSYCHIATRY

Homeobox protein MOX-2 plays a critical role in nociceptor function
Authors: Lenartowicz, Ewelina; Kokotović, Tomislav; Langeslag, Michiel; Kress, Michaela; Nagy, Vanja; Penninger, Josef
Presenter: Ewelina Lenartowicz

Homeobox protein MOX-2 (MEOX2) is a transcriptional factor that plays a role in development of bones and muscles. MEOX2 mutations are associated with disorders varying from cleft palate to dysfunctional neurovasculature in Alzheimer's disease. Surprisingly, we have identified dysregulation of MEOX2 in a rare sensory nervous system disorder, Congenital Insensitivity to Pain (CIP). CIP is marked by a complete absence of pain perception due to sensory neuron dysfunction or absence. We showed MEOX2 abundance to significantly change in fibroblasts of two unrelated patients suffering from PRDM12-associated CIP, compared to fibroblasts of their respective unaffected, sex-matched parents. Indeed, we reported that knocking down MEOX2 Drosophila melanogaster homologue,btn, specifically from sensory neurons in the fly, resulted in impaired nocifensive behavior. Based on the important role which MEOX plays in the development of dermatomes and their respective nervous system, we hypothesized that MEOX2 is not only a downstream protein regulated by PRDM12, but that it is also essential for the function of post-mitotic nociceptors, cells specialized for detection of painful stimuli as well. We, therefore, sought out to characterize the function of MEOX2 in vertebrates, by analyzing MEOX2-deficient mice. Full body, classic MEOX2 knock-out mice are neonatally lethal, so we performed our analyses on MEOX2 heterozygotes. Here, we report that MEOX2 is expressed in the mouse dorsal root ganglia and spinal cord, and localizes in the nuclei of CGRP-positive sensory neurons, marker for nociceptors. Detailed electrophysiological analysis of cultured nociceptors reviled impaired action potential firing upon depolarization in MEOX2heterozygotes as compared to controls. Nociceptor firing deficiencies result in impaired behavioral responses. In standard behavioral assays for acute and inflammatory pain, we noted that MEOX2 heterozygotes have impaired responses to noxious heat, intraplantar capsaicin injections and intraplantar formalin injections. Based on these observations, we conclude that MEOX2 plays a role not only in the developing mesenchyme, but it is also critical in nociception of the developed sensory nervous system and an important downstream mediator of PRDM12 function.

The effects of endurance training on cognitive function and quality of life in elderly marathon runners
Authors: Batmyagmar, Delgerdalai; Ponocny-Seliger, Elisabeth; Kundi, Michael; Lehrner, Johann; Haslacher, Helmuth; Winker, Robert
Presenter: Delgerdalai Batmyagmar

World Health Organization declared in 2012 that dementia is a Public Health Priority. There is a continuing debate that how physical and mental health can be maintained at an advanced age and which interventions might be cost-effective to prevent or delay age-related physical and mental degradation. It is well known that physical activity is beneficial to overall health and especially in regard to cognitive function. However, the effect of endurance type of exercises including long-distance running on cognitive function and mental health within the elderly population is unknown. Thus, we intended to elucidate if intensive endurance exercise among the elderly population (>60 years) is associated with improved cognitive performance and mental health. Elderly active marathon runners over 60 years who trained more than 2 hours a week were recruited and matched with a physically inactive control individual by age, sex, and years of education in 2008. Overall, 50 athletes and 49 control subjects were included for follow-up in 2012. Cognitive function was assessed using the German version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test. In addition, the short form, quality of life survey (SF-36) was applied to assess self-reported physical, mental and emotional health. Of 15 CERAD subtests, one showed improvements after four years reaching statistical significance. However, while control subjects improved somewhat in 'wordlist recall' and 'wordlist savings' overtime marathon runners performed worse (p for interaction =.005 and .021, respectively). Concerning self-reported health, scores, in all eight domains of the SF-36 marathon runners showed higher self-reported health than controls.

Results suggest that extensive endurance exercise is associated with improved subjective health and well- being in late age but does not unequivocally protect against age-related impairment of cognitive function in elderly persons.

Developing a complex intervention to support early adolescents with and without parents with mental illness during transition from primary to secondary school
Authors: Mitic, Marija; Woodcock, Kate; Schrank, Beate
Presenter: Marija Mitic

Objectives: Social connectedness - a sense of belonging and relatedness with peers - is one of the crucial factors for menta health and wellbeing of young people. A lack of social connectedness is linked to a range of negative social, emotional and educational outcomes. During certain periods of life, such as transition from primary to secondary school, there is a risk of losing social ties and added effort is necessary to build and maintain relationships, this is particulary true for children of parents with mental illness (COPMI). Therefore, transition is a natural target point for prevention strategies to maintain and improve social connectedness. We report on findings from the initial stages of a multi-methods project aiming to foster social connectedness amongst peers in COPMI and non-COPMI adolescents around transition from primary to secondary school. Methods: A set of systematic reviews and qualitative interviews with multiple stakeholders, including children, teachers, parents with and without mental illness, adult COPMI, school psychologists and psychiatric health care staff, sought to understand the determinants and mechanisms of social connectedness and the specific challenges faced by early adolescents during the transition. Results: Social wellbeing of early adolescents around school transition is challenged and added effort is needed to attain or maintain strong social connectedness during this period. Amongst diverse individual, peer, family, school and environmental influences, skills such as emotion regulation and mentalization are particularly important for creating and maintaining strong social ties. A range of interventions exist to foster social-emotional skills in order to improve peer connections. However, they have not yet been systematically applied for preventive purposes in this specific group and in the context of school transition. Conclusion: Theresults of the initial project phase are used to develop a comprehensive dynamic model of social connectedness at school transition. The model will serve as a basis for co- development of a complex interventionthat employs set of school-based workshops and online serious games in order to foster social connectedness amongst children around school transition with a special focus on COPMI.