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Abstract Book
Poster Presentations

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The Ki-1 antigen (CD30), a novel target in neoplastic canine mast cells, is downregulated by interleukin-4

Karin Bauer et al.

Presenter: Karin Bauer

The Ki-1 antigen (CD30) is a novel target in human mast cell (MC) neoplasms. However, so far, CD30 has not been investigated in the context of canine MC tumors. Here, we examined the expression, regulation and function of CD30 in neoplastic canine MCs. The canine mastocytoma cell lines C2 and NI-1 expressed CD30 as determined by immunocytochemistry. Flow cytometry confirmed cell surface expression of CD30 on canine MCs with higher levels found in NI-1 cells compared to C2 cells. To study the regulation of CD30 expression, we applied a panel of immunomodulatory cytokines. Interestingly, of all cytokines tested, interleukin-4 was found to downregulate expression of CD30 on canine MCs. Next, we asked whether CD30 can serve as a therapeutic target in mastocytomas. We found that the CD30-targeting antibody brentuximab vedotin induces growth inhibition in canine MCs, with higher IC50 values obtained in NI-1 cells compared to C2 cells. These growth inhibitory effects of brentuximab vedotin were associated with induction of apoptosis. Additionally, incubation of canine mastocytoma cell lines with brentuximab vedotin as well as with the tyrosine kinase inhibitors masitinib and midostaurin resulted in a significant and dose-dependent decrease in CD30 surface expression on NI-1 cells and C2 cells.

Furthermore, brentuximab vedotin was found to synergize with masitinib and midostaurin in inhibiting the proliferation of NI-1 cells and C2 cells. Together, we have identified CD30 as a target of neoplastic canine MCs. Whether CD30-targeting drugs are effective in canine mastocytoma patients, remains to be determined.
CAR, a novel marker of erythroid differentiation and migration, is downregulated in erythropoietic progenitor cells in MDS

Karin Bauer et al.

Presenter: Karin Bauer

Myelodysplastic syndromes (MDS) are myeloid neoplasms characterized by peripheral cytopenia and by an accumulation of dysplastic erythroid progenitor cells (EryPC) in the bone marrow (BM). In most MDS patients, anemia develops. To examine abnormally regulated genes expressed in EryPC, we have established a screening program involving mRNA expression profiling studies of EryPC in patients with low-risk MDS (IPSS-R score<7) and control BM samples obtained from patients with other BM neoplasms as well as patients with unexplained cytopenia, normal BM or reactive/deficiency cytopenias. EryPC were defined as CD45\textsuperscript{low}/CD105\textsuperscript{+} cells and purified from BM mononuclear cells by multicolor flow cytometry (MFC) and cell sorting. mRNA expression profiles were analyzed by Affymetrix array technology and selected mRNA species were confirmed by qPCR. In mRNA- and MFC-validation experiments, we found that the major Coxsackie-Adenovirus Receptor (CAR) is specifically downregulated in CD45\textsuperscript{low}/CD105\textsuperscript{+} EryPC in MDS patients when compared to EryPC in normal BM or other control cohorts. In line with this observation, the immature erythroblast cell lines HEL, K562 and KU812 stained negative for CAR. Lentiviral transduction of the full-length CAR gene into these cells resulted in a significantly increased expression of various erythroid differentiation antigens, including CD36, CD71 and Glycophorin-A as determined by flow cytometry and qPCR.

Furthermore, CAR transduction resulted in an increased migration of HEL cells against a serum protein-gradient in a transwell assay. Transfection with truncated variants of CAR did not result in an increased expression of erythroid antigens or an increased directed migration. In conclusion, our data show that CAR is a functionally relevant molecule that is specifically downregulated on EryPC in patients with MDS. We hypothesize that CAR-deficiency is pathogenetically relevant as it may not only contribute to the maturation-defect of EryPC in MDS but also to the related accumulation of erythroid cells in the BM that is accompanying the peripheral anemia in these patients.
First discovered by Pan and Johnstone in 1983, exosomes have become the focus of exponentially growing interest. Exosomes represent tiny, by a membrane-protein containing lipid bilayer surrounded vesicles (30-150nm), which are constantly secreted by all healthy and abnormal cells and found in abundance in all body fluids. There is emerging evidence that exosome-mediated cell-to-cell communication is of importance in both health and disease. Together with the finding that e.g. tumor-derived exosomes contain a specific RNA and protein cargo this holds tremendous potential for exosomes as biomarkers and is anticipated to lead to the development of exosome-based minimally invasive diagnostics and next generation therapies within the next few years. We report here on the evaluation of different strategies for isolation of exosomes from human serum and saliva including the subsequent isolation of exosomal microRNA. We further present data from genome-wide microRNA profiling in salivary- and serum-derived exosomes from healthy individuals applying microarray technology.

Our studies reveal a significant overlap between serum and saliva-derived exosomes with respect to microRNA profiles suggesting saliva to be a suitable and most easy to access sample matrix for future non-invasive exosome diagnostics using microRNA biomarkers.
The pan-BCL-2-blocker obatoclax and the PI3-Kinase/mTOR-inhibitor BEZ235 produce synergistic growth-inhibitory effects in ALL cells

Gabriele Stefanzl et al.

Presenter: Gabriele Stefanzl

Acute lymphoblastic leukemia (ALL) is a life-threatening neoplasm characterized by abnormal expansion of lymphoblasts in hematopoietic tissues. Despite improved therapy only a subset of patients can be cured. Therefore, current research is attempting to identify new drug targets and novel targeted drugs to improve therapy in these patients. A number of previous and more recent data have shown that members of the BCL-2 family and components of the PI3-kinase/mTOR pathway are critically involved in the regulation of growth and survival of ALL cells. We examined the effects of the pan-BCL-2-targeting drug obatoclax (GX15-070) and the dual PI3-kinase/mTOR blocker BEZ235 on growth and survival of ALL cells. As assessed by 3H-thymidine uptake, both drugs suppressed the in vitro proliferation of leukemic cells obtained from patients with Ph+ ALL (n=6) and Ph- ALL (n=10) in a dose-dependent manner with variable IC50 values (obatoclax IC50: 0.01-5 µM; BEZ235, IC50: 0.01-5 µM). Both drugs were also found to block proliferation in the Ph+ ALL cell lines BV-173, NALM-1, TOM-1, and Z119 as well as in all Ph- lymphoid cell lines Raji, RAMOS, REH, and BL-41 (obatoclax IC50: <1 µM; BEZ235, IC50: <1 µM).

Moreover, obatoclax and BEZ235 induced apoptosis in all lymphoid cell lines examined. Drug-induced apoptosis was confirmed by Western blotting using an antibody against active Caspase 3 as well as by flow cytometry. Since obatoclax and BEZ235 interact with a different and limited number of targets, we also tested drug-combination effects. Indeed, obatoclax was found to cooperate with BEZ235 in inhibiting the growth and survival of Ph+ and Ph- ALL cells. In BV-173, NALM-1, BL-41, Z119, and RAMOS, synergistic growth-inhibitory effects were obtained. Finally, primary ALL cells, including CD34+/CD38- stem cells and CD34+/CD38+ progenitor cells and all cell lines tested were found to express major targets, including PI3K, mTOR, BCL-2, MCL-1, and BCL-xL. Together, our data suggest that combined targeting of the PI3K/mTOR pathway and anti-apoptotic BCL-2 family members is a potent approach to counteract growth and survival of neoplastic (stem) cells in Ph+ and Ph-ALL.
Targeting STAT5 Oligomerisation in Hematopoietic Cancer

Anna Orlova et al.

Presenter: Anna Orlova

Targeted inhibition of hematopoietic malignances is today predominantly achieved through small molecular weight tyrosine kinases (TK) inhibitors (TKI). However, TKI treatment is often disturbed by development of resistance and severe side-effects. Importantly, these kinases are mutated in cancer and they are hyperactivated. One of their critical substrates that make these driver kinases so important is their downstream activation of the STAT family. Particularly, STAT5 was shown to be critical for disease initiation and progression in hematopoietic cancer. Therefore, strategies are required to target STAT5. Interestingly, STAT5 oligomers generated via interaction of STAT5 N-terminal dimerization, contribute to neoplastic cell growth and survival. STAT5 promotes direct oncogene mRNA up-regulation of MYC, BCL2-family members and D type cyclins in cells. Studies using transgenic mice expressing a STAT5 variant lacking the N-terminus provided evidence of the importance of it to drive oncogene expression in myeloid neoplasm.

Moreover, STAT5 mutants engineered to form only dimers failed to induce neoplasm. Here, we specifically try to target the N-terminus of STAT5. Aim of the study: Establish a screening system that allows for search of STAT5 inhibitors to disrupt STAT5 oligomers to find novel drugs targets against the STAT5 N-domain dimer interface. We developed a unique screening system with engineered hematopoietic cell lines that are driven for growth and survival through the STAT5 N-domain dimerization. This allows for high-throughput and high content compound library screen. Specifically, we fused the N-terminus of STAT5A/B to an active TK domain. We will identify molecules disrupting STAT5 oligomers. Fusion constructs with respective controls displayed that the STAT5-TK fusions convert cytokine-dependent hematopoietic cells into cytokine independent cells, which are transformed. Once potential lead compounds are identified and characterized by cellular studies and NGS profiling, combinatorial effects of selected STAT5 inhibitors with FDA approved drugs, will be evaluated to overcome drug resistance.

Our study should also lead to a better molecular understanding of the function of the N-terminus of STAT5 in respect to cancer development and progression.
Chromosomal translocations in cancer can result in the production of pathogenic fusion proteins. In leukemia, a particular high number of fusion oncogenes have been identified. Fusion proteins involving the Nucleoporin 98 (NUP98) gene are found in ~2% of acute myeloid leukemia (AML) patients. The NUP98 multi-partner translocation family (MPTF) features >20 different fusion proteins, all harbouring the N-terminal part of NUP98 fused to distinct C-terminal partners. Previous studies showed that different NUP98-fusions cause similar AML phenotypes in humans as well as in mouse models. We postulate that NUP98 fusion proteins share molecular mechanisms depending on conserved protein-protein interactions (PPIs) to modulate important oncogenic pathways.

Therefore, we aim to identify critical effectors of the NUP98 MPTF among the protein interactomes of five representative, yet distinct NUP98-fusion proteins using affinity purification-coupled to mass spectrometry (AP-MS). Doxycycline (Dox)-inducible, Strep-HA-tagged variants of selected NUP98 fusion proteins (NUP98-HOXA9, -JARID1A, -DDX10, -NSD1 and -PSIP1) were cloned into retroviral vectors. Human AML cells were transduced with the constructs and selected for stable transgene integration. Dox-mediated transgene induction was reported by expression of GFP. Routinely, 70%-90% GFP+ cells could be detected 24 hrs after Dox treatment. We are using these cell line models to characterize PPIs of wild-type NUP98 vs. NUP98-fusion proteins. While protein complexes around exogenously expressed NUP98-fusion proteins will be purified through affinity reagents targeting the Strep/HA tag, endogenous NUP98 complexes will be purified by antibodies directed against the NUP98 N- or C-terminus. Parental AML cells and cells expressing an N-terminal NUP98 breakpoint control construct will serve as controls. Mass Spectrometry data will be analysed using standard bioinformatic tools to generate the interactomes of each fusion protein. The overlap of different interactomes will reveal a list of common interactors among different NUP98-fusion proteins studied.

In the future we aim to validate the list of common interactors for functional contribution to leukemogenesis to identify targets for in-depth in vitro and in vivo validation studies.
Melanoma invasion and proliferation is regulated by activation or genetic loss of STAT3 controlling the SOX10-MITF pathway

Alexander Swoboda et al.

Presenter: Alexander Swoboda

Melanoma is a very aggressive form of skin cancer, with >76,000 new cases diagnosed annually in the USA. Stage I and stage II lesions can be successfully removed by surgery, but metastasizing melanomas are still challenging to treat, leading to an estimated 10,000 deaths in the USA annually. Activation of signal transducer and activator of transcription 3 (STAT3) and amplification of the 17q locus that encodes STAT3 is frequently seen in malignant melanoma.

Here we investigated how the deletion of the Stat3 gene locus influences the progression of melanoma in a genetic mouse model driven by active NRasQ61K transgene in melanocytes and deletion of the INK4a gene locus. Mice developed metastasizing melanoma after 6 month with a significant earlier disease onset in the Stat3 deleted group, but no change in metastasis. Stat3 deletion resulted in compensatory upregulation of genes regulated by the SOX10-MITF pathway, essential for melanocyte development, homeostasis and melanoma development and changes in the expression of surface receptor molecules related to the STAT3 and SOX10-MITF signaling pathways. Reintroduction of STAT3 silenced MITF transcription. Results from transgenic mice were confirmed with knockdown in five human melanoma cell lines and clinical validation was performed on a cohort of primary melanomas and metastases thereof.

Thus, STAT3 and SOX10-MITF signaling act antagonistic and stratify patients into subgroups driving different oncogenic signaling mechanisms in melanoma patients.
The gene encoding the transcription factor CCAAT-enhancer-binding protein (CEBPA) is mutated in 9% of Acute Myeloid Leukemia (AML) cases. These mutations include N-terminal frameshift mutations, resulting in expression of a smaller variant of C/EBPα, termed p30. In addition, mutations in the C-terminal basic leucine zipper domain (bZip) disrupt the DNA-binding ability of C/EBPα. It has recently been shown that AML genomes with mutated CEBPA share other genetic lesions, including mutations in NPM1 and the tyrosine kinase FLT3 (FLT3-ITD).

In addition, mutations in transcription factors (GATA2, RUNX1, STAT5A) and chromatin modifiers (ASXL1, TET2) were also identified. It is known that individual combinations of mutations can influence the outcome of AML, although the mechanisms for this are still unknown. We hypothesise that the N-terminally truncated p30 variant of C/EBPα differs from the longer, wild type p42 C/EBPα protein in its potential to interact with components of the chromatin modifying machinery, leading to alteration of the epigenetic landscape. Resulting global changes in gene expression might thus depend on combinatorial effects of CEBPA mutations together with additional genetic lesions that are present in the same leukemic clone. Using a Doxycycline (Dox)-inducible RNAi system in murine Cebpa(p30/p30) cells we have analysed global genomic and transcriptomic changes upon Cebpa knockdown with ChIP-Seq and RNA-Seq, focusing specifically on enhancer and super-enhancer regions.

In addition, we are using the CRISPR-Cas9 technology to establish novel murine and human cell lines harbouring combinations of mutations found together with mutant CEBPA in AML patients. To elucidate molecular mechanisms underlying changes that depend on CEBPA mutations in combination with other relevant AML mutations, we will profile the genomic and transcriptomic landscapes of the established cell lines to identify common and differentially regulated pathways. Understanding and deciphering global epigenetic and transcriptomic changes that depend on CEBPA mutations will help to identify pathways that are significantly altered in the CEBPA-mutated AML and provide starting points for the development of novel treatments.
Gene hunting: powerful next-generation sequencing (NGS) approaches aimed at identifying new gene defects in patients with primary immunodeficiency disorders

Ana Krolo et al.

Presenter: Ana Krolo

Primary immunodeficiency (PID) disorders comprise a heterogeneous group of rare diseases that mainly manifest in early childhood and frequently present with severe, persistent and recurrent infections that can be life-threatening. According to current estimations, 50-60% of PID cases remain undiagnosed either because their genetic etiology is still unknown or due to lack of NGS-based screening centers.

In both cases, the collaborative effort of expert physicians and geneticists is crucial to elucidate the underlying genetic cause of the clinically heterogeneous manifestations. In recent years, NGS technologies have significantly enhanced the efficacy of genetic diagnosis and boosted the identification of novel monogenic PID-causing defects. Here, we describe our experience with two different strategies which facilitate the identification of both known and novel causative variants. We have developed a customized panel that simultaneously screens for more than 500 genes including all known PID-causative genes according to the latest IUIS classification and additional suspected candidates. Using our PID-targeted panel we analyzed a representative cohort of 150 PID samples comprising of 47 clinically diagnosed SCIDs, 99 heterogeneous PIDs and 4 controls with previously identified variants. All 4 control variants were detected, confirming the validity of the method. Notably, we successfully identified disease related variants in 40% of SCID and 31% of remaining PID samples.

Intriguingly, we also identified variants in several seldom-reported genes that are typically not screened for. Recently, we have established a hematology (HM) panel that was used to screen 20 patients with the success rate of approx. 40%. While this approach proves to be a valuable tool in genetic diagnostics, whole-exome sequencing (WES) allows for the discovery of yet undescribed pathogenic variants. Employing a WES approach, we have successfully identified novel PID-causing genes such as PRKCD, IL21, ITK, NIK and DOCK2 whose causality was proved experimentally. Our experience shows that NGS-based targeted enrichment methods have all potential to become a standard tool in genetic diagnostics and that whole-exome sequencing is still a gold standard in identifying novel gene defects.
The corepressor NCOR1 regulates the development of conventional and innate-like T
cell lineages

Lena Müller et al.
Presenter: Lena Müller

Nuclear receptor corepressor 1 (NCOR1) is a transcriptional regulator that bridges repressive
chromatin modifying enzymes with transcription factors. NCOR1 has been implicated in
many biological processes, however the role of NCOR1 in T cells is not known. Here we
show that Vav-iCre-mediated deletion of NCOR1 resulted in the conversion of conventional
CD8-lineage thymocytes into IL-4-induced innate memory CD8+ T cells. This was due to an
increase in promyelocytic leukaemia zinc finger (PLZF) expressing and IL-4-producing NKT2
cells and Vγ1.1+ γδ T cells. While IL-4 producing PLZFhi NKT2 cell subsets were enhanced,
PLZFlo NKT1 cells were reduced in the absence of NCOR1, indicating a crucial function for
NCOR1 in iNKT sublineage differentiation. Late-stage deletion of NCOR1 using Cd4-Cre
resulted in reduced SP thymocyte numbers due to impaired survival of signaled DP
thymocytes. These results define NCOR1 as a key regulator of innate-like T cell lineages and
further demonstrate that NCOR1 is required for the survival of positively selected
conventional T cell lineages.

Keywords: NCOR1 / PLZF/ innate memory CD8+ T cells / iNKT cells / γδ T cells
Loss of c-Jun enhances tumor formation in a mouse model of prostate cancer

Astrid Aufinger et al.

Presenter: Astrid Aufinger

Aims: Prostate cancer (PCa) is the second most common cancer in men worldwide. Current diagnostic tools in PCa such as PSA are insufficient to distinguish whether tumors remain non-aggressive or develop into metastasizing disease. The lack of PCa markers stratifying low and high risk groups results in frequent overtreatment with severe side effects. The contribution of c-Jun, an important member of the AP-1 transcription factor family, to PCa progression is controversial. We aim to investigate whether c-Jun acts as tumor suppressor or oncogene in the Pten-deficient PCa mouse model.

Methods: We generated a transgenic PCa mouse model by crossing Pb-Cre4 mice with mice carrying floxed alleles of Pten and/or c-Jun (Pten c-JunPC-/−). We analyzed and compared PCa development of Pten c-JunPC-/− double knockout mice to PtenPC-/− and wild type mice aged 19 and 38 weeks. We characterized tumors macroscopically and histopathologically and performed gene and protein expression analyses to study the effects of loss of c-Jun during PCa development. We also analyzed large cohorts of patient samples for c-Jun mRNA and protein expression levels.

Results: Concomitant loss of Pten and c-Jun leads to significantly increased PCa tumor growth and early lethality compared to PtenPC-/− mice. Pten c-JunPC-/− deficient tumors show increased proliferation and decreased apoptosis rates. Pten c-JunPC-/− tumors revealed an unexpected bypass of senescence response and accordingly showed reduced levels of tumor suppressors p16, p19, p53. These data indicate that cell cycle regulation and senescence are disrupted in PCa when c-Jun is absent. We found reduced expression levels of the histone demethylase JMJD3 implicated in the activation of the INK4a locus leading to senescence induction via p16 and p19. Finally, we show that loss of c-Jun mRNA and protein expression correlate with poor prognosis in a large cohort of PCa patients.

Conclusion: We identified c-Jun as a tumor suppressor in mouse PCa. Furthermore, loss of c-Jun mRNA expression correlates with poor outcome in large cohorts of PCa patients. Loss of c-Jun mRNA may thus be a novel marker to stratify high and low risk PCa patients, which may be exploited therapeutically.
Oncogenic role of Kmt2c in prostate cancer

Tanja Limberger et al.

Presenter: Tanja Limberger

Aims: Prostate cancer (PCa) is the second most prevalent type of cancer in men worldwide and ranks among the leading causes of cancer related death. A lack of diagnostic tools stratifying aggressive from slow progressing tumours still leads to frequent overtreatment with severe side effects with greatly reduced quality of life for the patients. Our objective is to investigate the contribution of the epigenetic regulator Kmt2c to PCa initiation and progression using a Pten-deficient mouse model of PCa.

Methods: We started to generate a mouse model with a double deletion of both Pten and Kmt2c specifically in the prostate epithelium (PtenPC-/- Kmt2cPC-/-). Prostate tissue of PtenPC-/- Kmt2cPC-/- mice will be compared to PtenPC-/- samples at 19 and 38 weeks of age. To establish a human relevance of this study we analysed a cohort of patient samples and human PCa cell lines on their Kmt2c expression. To support our findings we exploited publicly available databases to gain insight on mutational frequency of Kmt2c in PCa and expression levels at different stages of disease. Furthermore we investigated the impact of a Kmt2c depletion on cellular proliferation in various human PCa cell lines.

Results: Analysis of tumour tissue from PtenPC-/- mice showed increased expression of Kmt2c at late stage PCa. PtenPC+/- Kmt2cPC+/- mice are born at Mendelian ratios, are viable and fertile and therefore suitable for our ongoing breeding purposes. Analysis of a patient cohort showed high levels of Kmt2c especially in therapy-resistant and metastasizing samples. Consistently, datasets of gene expression in prostate tumours mirror those results. A Kmt2c depleted human PCa cell line showed reduced proliferation further supporting our data.

Conclusion: Aberrantly increased expression levels of Kmt2c correlate with prostate cancer progression both in the murine and human situation. Additionally, depletion of Kmt2c in human PCa cells in vitro leads to reduced cellular proliferation. We therefore hypothesize that Kmt2c plays an oncogenic role in human PCa. Kmt2c might be an important factor in PCa progression and potentially represents a novel target for diagnostic or therapeutic approaches.
Comparative analysis of antineoplastic activities from statins and bisphosphonates

Heidrun Karlic et al.

Presenter: Karlic

Inhibitors of the mevalonate pathway are increasingly recognized as anti-cancer drugs. Thus, the aim of this study was to identify the molecular mechanisms and biological pathways associated with the anticancer effects of statins and bisphosphonates, which are known to downregulate the farnesylation and geranyl-geranylation of essential membrane-associated signal-transducers such as RAS and RHO proteins. Transcriptomic, proteomic and methylomic analyses were done from the neoplastic cell lines MDA-MB-231 breast cancer, PC-3 prostate carcinoma, MG-63 and U2-OS osteosarcoma and HMC-1 mast cell leukemia being treated for 6 days with pharmacologic doses with a representative statin (simvastatin) and a bisphosphonate (ibandronate). Bioinformatic analyses involved the gene set enrichment analysis (GSEA) and Pathvisio software as pathway recognition algorithms. Statin-treatment regulated more than double as much genes as bisphosphonate. With the statin, there was an up to 90% reduction in gene expression of 3 genes namely topoisomerase (TOP2A), thymidilate synthase (TYMS) and anillin (ANLN). A stimulation of sestrin2 (SESN2) was observed both with ibandronate and statin. All these 4 genes are known for their association with a regulation of cell cycle. The downregulations of TOP2A, TYMS, and ANLN and the upregulation of SESN2 were most significant in epithelial-like cancer cell lines (MDA-MB-231 and PC-3), which also showed a significant statin-associated increase in the oxidation of NADPH to NADP and associated metabolic and epigenetic consequences. In addition, treatment with simvastatin or ibandronate downregulated the epigenetic enzymes DNMT1 and the histone deacetylases (HDACs) and regulated some micro RNAs (MIR21, MIR520E and MIR612).

This provides some explanation for clinical observations, by indicating both shared and differential mechanisms for the anti-cancer and bone-preserving activities of statins and bisphosphonates in different types of malignancies.
Do leukemic cells interact with the osteoblastic niche?

Franz Varga et al.

Presenter: Heidrun Karlic

Osteoblasts and bone lining cells in the endosteal zone of trabecular bone play a critical role for regulating the long term maintenance of haematopoietic stem cells (HSC) as well as leukemic stem and progenitor cells (LSC). Whereas bone-degradation and a weak reservoir of mesenchymal stem cells may support the homing of neoplastic cells from solid tumors, healthy and well growing bone cells (and their progenitors) were discussed for their potential to act as tumor suppressors and to attenuate the proliferation of leukemic cells.

Thus, the aim of the study was to investigate the mutual impact of cells from a leukemic cell line with (pre)osteoblastic cells in a co-culture model. Adherent mesenchymal C3H10T1/2 as well as preosteoblastic MC-3T3-E1 cells, the D1 bone stromal cell line and the MLO-A5 osteocyte cell line were co-cultured with the HL-60 acute promyelocytic leukemia cell line for up to 2 weeks. Gene expression was measured on gene-chips (Affymetrix) and by quantitative PCR. After one week of co-culture, proliferation of HL-60 cells was reduced by MC3T3-E1 (-20%) and with C3H10T1/2 cells (-40%), whereas proliferation of the (pre)osteoblastic cell lines increased by +10% in MC3T3-E1 and +50% in C3H10T1/2 cells. By contrast to these two cell lines, HL-60 cells closely interacted with the D1 and MLO-A5 cells, thus indicating a “homing-like” status. The co-culture induced a trend towards quiescence in HL-60 as evidenced by a 2-fold increase in the expression of the cell cycle inhibitor CDKN2B. Morphology and gene-expression did not indicate any co-culture-associated trend towards differentiation or apoptosis in HL-60. In MC3T3-E1 cells we observed a highly significant downregulation of inflammation markers such as SAA3 (serum amyloid), MMP13 (matrix metalloproteinase) and interleukin 6 in MC3T3-E1 cells, but no significant regulation of differentiation markers (such as alkaline phosphatase or osteocalcin). This was associated with an upregulation of anti-inflammatory (MIR21) and tumorsuppressive (MIR27a) as well as MIR199B microRNAs by HL-60.

In conclusion, the results of this study emphasize the impact of mesenchymal cells on the maintenance of leukemic cells as well as a possible interaction of leukemic cells with osteoblastic cells.
**Crosstalk between heme oxygenase, nitric oxide synthase and NADPH oxidase in macrophages**

Andrea Müllebner et al.

Presenter: Andrea Müllebner

It has been shown in mice that HO-1 knockout in macrophages leads to resistance against metabolic disease associated inflammation, by downregulating the inflammatory response. HO-1 deletion additionally leads to an increased mitochondrial respiration rate of these macrophages. Our aim was to clarify whether modulation of mitochondrial function by HO is linked to the building up of an effective inflammatory response in macrophages. Nitric oxide synthase (NOS) and NADPH-oxidase (NOX) are both essential for mounting an inflammatory response and antibactericidal activity of macrophages. We hypothesized that HO in macrophages promote the inflammatory response via modulation of NOS and NOX in a mitochondrial ROS (mtROS) dependent manner.

We determined mitochondrial oxygen consumption rate by mitochondria in macrophages (J774.A1 cells) and showed that the addition of heme, a substrate of HO, reduces mitochondrial respiration (OxPhos). This effect was likely due to both, a decreased capacity of respiratory chain and a decreased ATP-synthase activity. Zinc protoporphyrin, a HO inhibitor, partially decreased HO activity and diminished effects of heme on OxPhos. Simultaneously, the inhibition of HO activated mitochondrial ROS (mtROS) production but did not influence ROS generated by NOX (NOXROS). Since NOS generates nitric oxide (NO), a molecule acting in a similar way as carbon monoxide, which is formed by HO, we additionally inhibited NOS expecting similarity with HO inhibition. The NOS-inhibitor L-NAME reduced both, formation of mtROS and NOXROS levels. Thus, our data show fundamental differences of HO and NOS on mitochondrial function in macrophages: While HO reduces both OxPhos and mtROS, NOS reduces OxPhos but simultaneously activates both mtROS and NOXROS release. Considering that inhibition of HO causes an additional mtROS formation we assume that such synergistic effect of both enzymes causes sufficient release of mtROS to fuel NOX resulting in increased bactericidal activity. In fact, the mitochondria targeted antioxidant, mitoTEMPO reduced both mtROS and NOXROS levels.

We conclude that in macrophages HO crosstalks with NOS and NOX, the enzymes required for bactericidal activity, via mtROS; the latter appears as an essential regulator of macrophage function.
PDGFRB function in Anaplastic Large Cell Lymphoma

Ines Garces de los Fayos Alonso et al.

Presenter: Ines Garces de los Fayos Alonso

PDGFRB function in Anaplastic Large Cell Lymphoma

Anaplastic large-cell lymphoma (ALCL) is a malignant non-Hodgkin lymphoma (NHL) most commonly diagnosed in children and young adults. A majority of the tumours carry the translocation t(2;5)(p23;q35), resulting in the fusion of the Nucleophosmin (NPM) gene to the Anaplastic lymphoma kinase (ALK) gene. The oncogenic fusion protein (NPM-ALK), consequently contributes to the pathogenesis and progression of over 50% of ALCL cases.

Recent studies from our lab identified AP-1 transcription factors JUNB and cJUN as downstream effectors of NPM-ALK, which directly up-regulate platelet derived growth factor receptor beta (PDGFRB) expression in lymphoma cells. We demonstrated that besides increased receptor expression its ligand, PDGFB, levels are also elevated in both mouse ALCL tumours and human ALCL patient plasma. Furthermore, therapeutic inhibition of PDGFRB with the kinase inhibitor imatinib resulted in rapid, complete, and sustained remission in a late-stage therapy-resistant ALCL patient. Despite the discovery of these critical findings, the underlying mechanisms and the nature of PDGFRB in lymphoma still remain unclear. To investigate the mechanisms of PDGFRB signalling in ALCL, we have crossed a murine ALCL model, which expresses NPM-ALK under the CD4 promoter, to PDGFRB floxed mice and a CD4 promoter driven Cre recombinase, to yield specific deletion of PDGFRB in T cells (CD4-NPM-ALK-CD4 ΔPDGFRB). Intriguingly, CD4-NPM-ALK-CD4 ΔPDGFRB mice have significantly prolonged survival rates due to reduced tumour growth and size, dictated by an increase in apoptosis. Supporting this data, RNA-sequencing data of 23 ALCL patients (5 ALK+ and 18 ALK-) revealed a negative correlation between PDGFRB transcripts and expression levels of DNA-damage repair (DDR) and cell cycle genes. Furthermore, CD4-NPM-ALK-CD4 PDGFRB mouse tumours expressed increased levels of y-H2AX, alongside the deregulation of several DNA damage response markers p53, ATM and CHK2.

Altogether our data further supports the significance of PDGFRB in ALCL as its absence leads to decreased tumour development in NPM-ALK mice via modulation of DDR pathways.
STAT5 transcription factors are essential regulators of differentiation, survival and proliferation of hematopoietic cells. STAT5 signaling requires tyrosine phosphorylation (pYSTAT5), which is frequently elevated in hematopoietic cancers and associated with negative prognosis. Importantly, recurrent gain-of-function STAT5 variants have been reported in Peripheral T Cell Lymphoma (PTCL), a heterogeneous and aggressive disease of mature T cells.

We used cS5F, a hyperactive point mutant of STAT5A, to generate mouse models expressing the transgene under the vav-promoter from hematopoietic stem cells. Low pYSTAT5 levels resulting from one transgene copy integration did not lead to a disease phenotype (vav-cS5lo). High pYSTAT5 levels in vav-cS5hi mice led to a drastic expansion of CD8+ T cells being lethal between 25 and 45 weeks of age. The PTCL-like disease was associated with lymphadenopathy, splenomegaly and T cell infiltrations into various organs. The CD8+ T cells expressed pan-T cell and T cell activation markers, and were transplantable. The expression profile determined by RNA-seq revealed a deregulation of STAT5 target genes and correlated closely with human PTCL.

Our results support the concept that enhanced STAT5 signaling drives T cell lymphoma. Treatment with the JAK inhibitors Ruxolitinib and Tofacitinib as well as with a novel STAT5 SH2-domain inhibitor decreased murine and human PTCL cell line viability in conformity with a dose-dependent pYSTAT5 decline. Therefore, STAT5 represents a target in these life-threatening malignancies and we aim to test the effects of new STAT5 and tyrosine kinase inhibitors in our mouse model.
Lung cancer represents the leading cause for cancer related deaths worldwide. Non-small cell lung cancer (NSCLC), which is the most common NSCLC, is associated with a very bad prognosis. A better understanding of molecular mechanisms responsible for the initiation and progression of NSCLC is critical in order to identify novel drug targets. Somatic Janus kinase 2 (JAK2) aberrations are found in approximately 7% of the NSCLC patients, however their relevance to tumor development and progression is poorly understood. Nevertheless, the JAK2 inhibitors Ruxolitinib (Jakavi®) and Tofacitinib (Xeljanz®) are currently in phase I/II clinical trials for the treatment of NSCLC. We have previously shown that STAT3 (a downstream effector of JAK2) suppresses mutated KRAS NSCLC. Therefore, the role of JAK2 in mutated KRAS NSCLC needs to be clarified.

Patient data from smokers, which are known to be more prone to harbor KRAS mutations, indicate that low JAK2 mRNA expression levels in tumors significantly correlate with reduced overall survival. On the other hand, pharmacological inhibition of JAK2 with Ruxolitinib (Jakavi®) and Tofacitinib (Xeljanz®) in a panel of human and mouse KRAS-mutated NSCLC cell lines (A549, A427, 368T1) attenuates tumor cell proliferation and triggers apoptosis. These data might indicate different functions of stromal and tumor cell intrinsic JAK2 signaling. To follow up on these findings, we are currently investigating the impact of JAK2 deletion specifically in KRAS-mutated tumor cells by taking advantage of a genetically engineered mouse model of KRAS-dependent lung cancer (KrasLSL-G12D/+ mice). These studies will be complemented by using xenograft models of human mutated KRAS NSCLC lacking JAK2.

Altogether, we aim to clarify the potential benefits of inhibiting JAK2 signalling as a therapeutic approach to treat patients harbouring mutated KRAS NSCLC.
The role of STAT3β in myeloproliferative neoplasms

Petra Aigner et al.

Presenter: Petra Aigner

STAT3, the main mediator of IL-6-type cytokine signaling, plays a central role in cell growth, apoptosis and multiple oncogenic pathways. STAT3 is known to be expressed as two isoforms, STAT3α and its truncated form STAT3β, which is lacking the C-terminal transactivation domain. STAT3β was formerly postulated as the dominant negative form of STAT3α, however it has been shown that it has various STAT3α-independent regulatory functions and recently STAT3β gained attention as a powerful anti-tumorigenic molecule.

The aim of this study is to gain better understanding of STAT3β and its anti-tumorigenic function in acute myeloid leukemia (AML) and myeloproliferative neoplasms. This study is using Stat3β-transgenic and knock-out mouse models and cell culture-based methods to investigate the role of STAT3β. In our first approach we combine a Pten-dependent mouse model for myeloproliferative neoplasms with our inducible Stat3β-transgenic mice. We observed that Stat3β significantly delayed the disease progression in Pten-deficient mice and impaired hematopoietic stem cell mobilization from the bone marrow to the spleen. We aim to further examine this STAT3β-specific function and its underlying mechanisms using a similar approach with Stat3β knock-out mice and bone marrow transplantations.

We therefore hypothesize that STAT3β impairs the mobilization of hematopoietic stem cells from the bone marrow into the periphery. Thus, it delays disease progression in myeloproliferative diseases resulting in a more favorable disease outcome.
An interdisciplinary approach to identify novel causal genes in rare diseases

Julia Pazmandi et al.

Presenter: Julia Pazmandi

High throughput screening of genetic defects by whole exome sequencing (WES) accelerated the pace of disease gene discovery, and emerged as a popular method for investigating causal gene defects in rare monogenetic diseases. WES usually results in a long list of potentially disease causing variants that have to be investigated and validated, making disease gene identification rather time and labor intensive. Therefore, an intelligent way is needed to prioritize variants in WES variant lists to pinpoint causal genetic variants. As a proof of concept, we are hereby proposing a bioinformatics-based variant prioritization method for early-onset inflammatory bowel disease (EOIBD), a heterogeneous group of gut inflammatory disorders as a model for for rare Mendelian diseases.

First, WES variants will be subjected to a filtering algorithm to get rid of common, synonymous and low quality variants. Next, variants will undergo pathogenicity prediction based on the variant type and the evolutionary conservation of the residing gene. In order to take advantage of network biology methods, a disease specific interactome will be constructed using IBD specific seed genes: genes that have been identified to be causal or associated with the disease, pathways that are vital in IBD pathogenesis, and will be further enriched for immune specific biological functions, and disease specific gene expression data. Network exploration techniques will be used to gain insight into the possible disease causing role of each variant, measuring their potential effect on the IBD interactome. Phenotypic data form our in-house database will be used to identify patient subgroups. As a final step, the different attributes will be combined in a meaningful manner using machine learning classification algorithms, to receive a score of potential causality.

Here we propose a WES variant prioritization method that will combine data from various disciplines, including genetics, bioinformatics, and network biology in a meaningful manner using machine learning algorithms. We hypothesize that our method will exceed current means of disease gene identification and will be successful to define new disease drivers in IBD. Subsequently, our approach may be feasible in other types of rare diseases as well.
Anti-proliferative, cytotoxic and pro-apoptotic effects of novel resveratrol-salicylate hybrid molecules on Jurkat leukemia CD4+ T cells

Jenny Breitenbach et al.

Presenter: Jenny Breitenbach

Introduction: Resveratrol (Res) is a naturally occurring polyphenol produced by many plant species (red grapes, raspberries, blueberries, etc.). It can regulate many biological processes including hemostasis, glucose metabolism, cell division, free radical scavenging and inflammation. Moreover, many studies demonstrated that Res also induces apoptotic cell death in various human cancer cell lines. Recently, we reported on the anti-inflammatory and antioxidant properties of resveratrol-salicylate hybrid molecules in vitro and in vivo, revealing an enhanced anti-inflammatory potential of these compounds compared to the parent drug Res. In the present study, we compared the anti-proliferative, cytotoxic and pro-apoptotic potential of Res and two Res-salicylate hybrid molecules (C7 and C10) on Jurkat leukemia CD4+ T cells.

Materials and Methods: Jurkat T cells were treated for 48 h with different concentrations of Res, C7 and C10. Proliferation rate was monitored by WST-1 assay. Cell viability and cytotoxicity were evaluated with MultiTox-Fluor Multiplex Cytotoxicity Assay (Promega, Madison, WI, USA). Ongoing apoptotic processes were analyzed by Annexin-V/7-AAD staining and Western blotting.

Results: Res, C7 and C10 reduced proliferation and viability of Jurkat T cells in a dose-dependent manner. In WST-1 assay, C7 and C10 inhibited proliferation of Jurkat T cells two times more potently than Res. Consistently, in cell viability assays and Annexin-V/7-AAD staining, the effects of C7 and C10 were considerably more pronounced compared to Res.

Conclusion: Data show that the resveratrol-salicylate hybrid molecules C7 and C10 have potent anti-proliferative, cytotoxic and pro-apoptotic activities against Jurkat leukemia CD4+ T cells. Both compounds reduced cell viability and induced apoptosis considerably more effectively than Res itself. Therefore, we suggest that these Res-salicylate hybrid molecules might be promising chemopreventive substances for the treatment of acute T cell leukemia in the future.
Oncogenic lipogenesis and signaling - how do they interact in ovarian cancer?

Thomas W. Grunt et al.

Presenter: Thomas W. Grunt

Fatty acid synthase (FASN), a regulator of lipogenesis, is overexpressed in ovarian cancer and indicative of poor prognosis. FASN-inhibition abolishes proliferation and survival. Control of FASN by PI3K-mTORC1 signaling is well documented, whereas reverse interaction from FASN towards PI3K-mTORC1 is poorly understood and underlying molecular mechanisms are still elusive. However, since malignancy is driven by PI3K-mTORC1, control of this pathway by FASN is relevant for therapy prompting in-depth analyses.

Here, SKOV3, OVCAR-3 or HOC-7 cells were exposed to FASN-blockers (C75, G28UCM) and analyzed by thin-layer chromatography, MALDI-TOF-MS/MS, ELISA, Western blotting, quantitative micropatterning and growth assays.

We demonstrate that FASN-blockade normalizes cell membranes by preferential incorporation of polyunsaturated fatty acids such as arachidonic acid and diminishes diacylglycerols. Inhibition of FASN affects PI- and lipid raft-composition, which impairs upstream EGF-receptor/ERBB/HER complex function/expression and Grb2 recruitment causing PI3K-silencing represented by depletion of its product PIP3 and inactivation of downstream AKT. Moreover, FASN-blockers rapidly induce cell stress responses characterized by up-regulation of HIF-1α and its target-gene REDD1(RTP801/DIG2/DDIT4), followed by activation of the energy-sensor AMPK. REDD1 and AMPK in turn act as mTORC1-repressors blocking ribosomal S6 protein. Thus, blockade of malignant lipogenesis efficaciously impairs receptor-PI3K-mTORC1 at multiple molecular levels. Interestingly, concurrent targeting of PI3K/mTOR with dactolisib/NVP-BEZ235 does not augment FASN-inhibitor efficacy indicating that FASN-blockade fully silenced PI3K-mTORC1 already on its own. In contrast, FASN-inhibitor-mediated silencing of receptor-PI3K-mTORC1 releases cross-repression of MEK-ERK thereby causing its concurrent trans-activation. Accordingly, co-treatment with MEK-inhibitor selumetinib/AZD6244 significantly enhances FASN-inhibitor action.

This is to the best of our knowledge the first characterization of PI3K-mTORC1 cross-silencing by FASN-inhibitors, which may be crucial for the anticancer effects of these drugs. Moreover, our data encourage therapeutic approaches using FASN-antagonists together with MEK-ERK-inhibitors.
**Targeting BRD4 as a potential therapeutic approach in JAK2 V617F+ myeloproliferative neoplasms**

Alexandra Keller et al.

Presenter: Alexandra Keller

Classical myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) and frequently harbor the JAK2 V617F mutation. In most patients, MPN are chronic and indolent, however, some progress into acute myeloid leukemia (AML). So far, the only curative approach for MPN is stem cell transplantation.

Therefore, current research is evaluating new targets in MPN. Recently, the epigenetic reader bromodomain-containing protein 4 (BRD4) was identified as a promising target in AML. In this study, we examined the value of BRD4 as a target in MPN. We employed two JAK2 V617F+ cell lines, HEL and SET-2, primary bone marrow (BM) cells obtained from 19 patients with JAK2 V617F+ MPN (PV, n=8; ET, n=5; PMF, n=6) and three targeted drugs (JQ1, BI2536, BI6727). Using qPCR and immunocytochemistry, we found that HEL, SET-2, and primary MPN cells express BRD4 and its downstream target MYC. BRD4-targeting shRNA and the applied drugs suppressed the proliferation of HEL and SET-2 cells (IC50: 0.02-0.5 µM). Moreover, JQ1, BI2536, and BI6727 inhibited the proliferation of primary MPN cells in 8/8 patients tested (IC50: 0.5-1.0 µM). We also analyzed drug effects on (neoplastic) stem cells (CD34+/CD38-) in three PV patients, one ET patient, and one PMF patient. Drug-exposure was followed by a decrease in CD34+/CD38- cells in all donors. Furthermore, we examined drug effects on cell cycle progression and apoptosis by flow cytometry. BI2536 and BI6727 induced a G2/M phase arrest in both cell lines, whereas JQ1 induced a G1-arrest in HEL cells. These differences may be explained by additional targets of BI2536 and BI6727, like PLK1.

In addition, all tested drugs induced apoptosis in HEL and SET-2 cells. Finally, we examined whether BRD4 inhibitors modulate MYC expression. In qPCR and Western blot experiments, JQ1, BI2536, and BI6727 were found to downregulate MYC mRNA as well as MYC protein levels in HEL and SET-2 cells. In conclusion, our data show that BRD4 and MYC are expressed in JAK2 V617F+ MPN cells and that BRD4 inhibition is associated with decreased proliferation and survival of neoplastic cells. The value of BRD4 as a therapeutic target in MPN remains to be determined in clinical trials.
Lung cancer is still the leading cause of cancer deaths worldwide. While it is well documented that patients harboring mutated EGFR non-small cell lung cancer (NSCLC) benefit from epidermal growth factor receptor (EGFR) inhibition, the use of EGFR blockers in V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutated NSCLC is unclear. Clinical studies addressing this issue show controversial results and hence it is not yet clear whether patients harboring KRAS mutations could benefit from therapy with EGFR inhibitors. To investigate the clinical potential of EGFR tyrosine kinase inhibitors (TKI) in preclinical models, we implemented a KRAS G12D driven mouse model of lung cancer which is inducible via inhalation of a Cre recombinase expressing adenovirus.

Intriguingly, mice harboring KRAS mutated tumors with concomitant EGFR deletion had a significantly increased survival advantage compared to their EGFR wildtype controls. This correlated with reduced tumor number and area in lungs of mice harboring EGFR deficient tumors accompanied by significantly decreased proliferation in these tumors. Using the human lung adenocarcinoma (AC) cell line A549 which harbors a KRAS G12V mutation, we found that EGFR knockout via Cas9 technology significantly inhibited proliferation of these cells compared to controls, both in vitro and when xenografted into NOD scid gamma (NSG) mice. Strikingly, administration of the pan-EGFR TKI afatinib ameliorated KRAS driven lung tumorigenesis in the KRAS inducible in vivo model as well as after transplantation of AC cell lines into NSG mice. Mechanistically, we found that upstream EGFR expression was required for full blown (mutated) KRAS activity, as evidenced by gene set enrichment analysis of RNAseq data comparing KRAS G12D mutated primary mouse alveolar type II cells with and without EGFR deletion. This latter result was further verified in A549 cells, where EGFR deficient cells exhibited decreased activated (i.e. GTP bound) KRAS.

Taken together, we propose that KRAS mutated lung AC depend on upstream EGFR expression and activation, and therefore represents a therapeutic target in this subset of patients, with FDA approved TKI already available.
Identification of a novel STAT5 inhibitor to interfere with the oncogenic activities of STAT5 in AML

Bettina Wingelhofer et al.

Presenter: Bettina Wingelhofer

STAT5 is frequently hyper-activated in a variety of hematopoietic cancers and solid tumors as a result of deregulated tyrosine kinase (TK) signaling. To date, various TK inhibitors (TKIs) are used in the clinic or in clinical trials for treatment of hematopoietic diseases. However, TKI treatment is often accompanied by resistance development and cytotoxicity as a result of poor kinase selectivity. Bypassing TKs through direct inhibition of STAT5 phosphorylation could be advantageous for therapy development.

Although STAT5 is an attractive molecular target for the development of novel cancer therapeutics, only first generation pharmacologic STAT5 inhibitors are currently available for clinical development. We therefore aim to identify a selective and specific inhibitory compound to interfere with STAT5 signaling in hematopoietic cancers to contribute to the development of a new generation of targeted drugs.

We identified a small inhibitory molecule, called AC-4-130, which binds to the SH2 domain of STAT5, subsequently resulting in the disruption of the reciprocal STAT5-phosphopeptide interactions. It efficiently blocked kinase-mediated phosphorylation, dimer formation, nuclear translocation, DNA binding and STAT5 mediated target gene expression. Furthermore, AC-4-130 led to a cell cycle blockade in G0/G1 and the induction of apoptosis. Studies with human AML patient-derived samples similarly showed the induction of apoptotic cell death and decreased colony forming capabilities. A combinatorial drug screen revealed synergistic effects of AC-4-130 with TKIs, as well as with drugs standardly used in the treatment of AML patients. Finally, AC-4-130 significantly suppressed xenograft tumor growth in vivo without general toxicity in healthy organs.

In summary, our findings indicate that AC-4-130 is a potent and selective inhibitor of STAT5. This compound provides a lead structure for further chemical modifications and clinical development, especially in combination with standard treatment, to improve existing therapies and overcome resistance development in hematopoietic malignancies.
Simultaneous inhibition of STAT3 and STAT5: a novel approach to overcome drug resistance in chronic myeloid leukemia

Karoline Gleixner et al.

Presenter: Karoline Gleixner

In chronic myeloid leukemia (CML) resistance against BCR-ABL1 tyrosine kinase inhibitors (TKI) can develop because of BCR-ABL1 mutations or activation of additional pro-oncogenic pathways. Drug combinations covering a broad range of targets may overcome resistance. Bardoxolone methyl (CDDO-Me) is an oleanane triterpenoid that suppresses a number of survival-related molecules, including AKT, mTOR, and STAT3. In this study, we were able to show that CDDO-Me inhibits the proliferation of human CML cell lines (IC50: 0.1-0.5 µM) and Ba/F3 cells harbouring BCR-ABL1 mutations (T315I, E255K, G250E, H396P, or F359V) or T315I-including compound mutations (IC50: 0.1-0.25 µM). These effects were accompanied by induction of apoptosis. CDDO-Me was also found to inhibit the growth of primary cells isolated from 14 patients with CML (TKI-resistant: n=4; BCR-ABL1 mutations detected: n=3; blast phase: n=1) and from one patient suffering from ponatinib-resistant Ph+ ALL harbouring BCR-ABL1T315I/E255K (IC50: 0.1-0.5 µM in all samples).

Furthermore, CDDO-Me was found to synergize with BCR-ABL1 TKI in producing growth-inhibition. The combination ‘CDDO-Me+ponatinib’ was highly effective in Ba/F3 cells expressing point-mutations or T315I-including compound mutations of BCR-ABL1. We next performed experiments with shRNA directed against STAT3 or STAT5 and the specific STAT5-inhibitor AC-3-019. Knockdown of STAT3 was found to produce synergistic effects with TKI and with AC-3-019 in K562 cells and KCL22-cells, whereas STAT5-knockdown sensitized CML cells against CDDO-Me, pointing to a new effective concept of dual STAT3+STAT5 inhibition. However, CDDO-Me was found to increase the expression of heme-oxygenase-1 (HO-1), a heat-shock-protein that triggers drug resistance and cell survival in CML. We therefore combined CDDO-Me with the HO-1 inhibitor SMA-ZnPP, which also resulted in synergistic growth-inhibitory effects. Moreover, SMA-ZnPP was found to sensitize KU812 cells and Ba/F3 cells expressing BCR-ABL1T315I/F311L against the combination ‘CDDO-Me+TKI’. Together, combined targeting of STAT3, STAT5, and HO-1 overcomes multiple forms of TKI resistance in highly resistant CML clones.

Whether such drug combinations are effective in vivo in TKI-resistant patients remains to be elucidated.
Establishing high-content imaging based drug screening for rare disease zebrafish models

Caterina Sturtzel et al.

Presenter: Caterina Sturtzel

Zebrafish has proven to be a cost effective vertebrate model organism for drug screening with great potential to bridge the gap between two-dimensional in vitro drug screens and expensive and laborious mammalian model based screens. Especially the development of therapeutic strategies for rare diseases is likely to be boosted by this alternative animal model system.

Costello Syndrome is a rare disease belonging to a group of developmental disorders termed RASopathies. The underlying genetic alteration is a mutation in HRAS rendering RAS constitutively active. We generated a tissue specific HRASG12V overexpression system in zebrafish, which induces formation of characteristic phenotypes mimicking Costello Syndrome.

Here we present a screening setup we established in collaboration with the platform Austria for Chemical Biology (PLACEBO), which allows us to test potential therapeutic compounds on the Costello-like zebrafish model in semi-automated medium-throughput. Compounds are transferred to 96-well-plates by a robot and dissolved in fish water, wherein fish larvae are incubated until 5 days post fertilization. The readout based on morphology and fluorescence is performed on an Operetta high content imaging system.

We successfully identified two small compounds, which can effectively prevent the formation of the morphological abnormalities resembling Costello Syndrome in our zebrafish model. Our medium-throughput drug screening setup promises to be applicable to a broader range of zebrafish disease models.
Uncoupling INK proteins from CDK4/CDK6 HSCs - uncoupling proliferation from senescence

Michaela Prchal-Murphy et al.

Presenter: Michaela Prchal-Murphy

Hematopoietic stem cells (HSCs) give rise to all lineages of the blood and persist for life time. The balance between proliferation and quiescence is a delicate matter in HSCs and carefully regulated. Cell cycle regulation plays a critical role maintaining this balance. Cdk4 and Cdk6 are highly related cell-cycle kinases that regulate progression from G1 to S-phase. D-type cyclin-binding leads to Cdk4/6 activation and retinoblastoma protein (Rb) phosphorylation. Whereas binding of INK4 proteins (p16INK4a, p15INK4b, p18INK4c and p19INK4d) inhibit Cdk4 and Cdk6. To test whether this inhibitory mechanisms play a role in hematopoiesis we used double knock-in mice harboring a mutation in Cdk6 (Cdk6-R31C) or Cdk4 (Cdk4-R24C) that prevents binding of INK4 proteins.

Mice harboring Cdk4-R24C and Cdk6-R31C mutations have comparable numbers of cells as well as of hematopoietic lineages in the bone marrow. Exposing the animals to stress uncovered a rapid recovery of hematopoiesis with enhanced cell cycle activity. Serial transplantations studies were performed to test HSC long term self-renewal. Importantly, CDK4R24CCDK6R31C HSCs self-renewal activity was significantly improved compared to WT HSCs. CDK4R24CCDK6R31C HSCs displayed no signs of exhaustion during serial BM transplantation and maintain the ability to give rise to both, myeloid and lymphoid lineages. In a long-term competitive BM transplantation setting, CDK4R24CCDK6R31C HSCs outcompete WT HSCs multi-lineage reconstitution. Of note, loss of p16Ink4ap19ARF does not phenocopy CDK4R24CCDK6R31C HSCs self-renewal activity.

Taken together these observations shed new light on the regulation of cell proliferation and HSC renewal. We show that CDK4R24CCDK6R31C expression results in enhanced phosphorylation of Rb and accelerated proliferation of hematopoietic precursors. Against the expectations the enhanced proliferation is not accompanied by a more rapid exhaustion of the stem cell compartment.
The role of the protocadherin CDHR5 in colorectal cancer

Monira Awad et al.

Presenter: Monira Awad

Protocadherins constitute the largest subgroup of the cadherin protein superfamily and are frequently downregulated in human cancers suggesting a negative role in oncogenesis. The protocadherin CDHR5 is a transmembrane protein that is located in the microvillar brush border of enterocytes, cholangiocytes and kidney epithelial cells. CDHR5 crosslinks microvilli and has been implicated in regulation of beta-Catenin activity. We are interested in CDHR5 functions in colorectal cancer. We found that CDHR5 expression is downregulated in altered crypt foci, adenomas, carcinomas and colorectal liver metastasis.

We further demonstrate a tumor-suppressive role of CDHR5 in colorectal cancer using transplantation experiments of cell lines with gain or loss of CDHR5 function. We generated CDHR5 knock-out mice to further investigate CDHR5 functions in autochthonous colorectal tumors. Knock-outs were viable and did not show an overt intestinal phenotype but displayed shortening of microvillus length. Formation of colorectal cancer, induced with the chemical Azoxy methane/Dextran sulfate protocol, was not affected in CDHR5 knock-out mice but the number of aggressive carcinomas invading the muscularis mucosa was substantially increased. These data suggest that CDHR5 is a metastasis suppressor gene in colorectal cancer.

We are currently using intestinal organoid cultures, cotransfection experiments and RNASeq of RNA, isolated from intestinal epithelial cells, to unravel molecular function of CDHR5 in colon cancer metastasis.
**Identification, characterization and targeting of putative leukemic stem cells in human mast cell leukemia**

Gregor Eisenwort et al.

Presenter: Gregor Eisenwort

Systemic mastocytosis (SM) is a rare hematopoietic neoplasm characterized by an abnormal expansion of mast cells (MCs) in the bone marrow (BM) and other organs. Whereas patients with indolent SM (ISM) have a normal life-expectancy, patients with advanced forms of SM have a grave prognosis. MC leukemia (MCL), the rare leukemic variant of advanced SM, is defined by a rapid expansion of immature MCs in various hematopoietic organs and a poor prognosis with short survival.

Although MCL is considered a stem cell disease, little is known about the origin and phenotype of MCL-initiating leukemic stem cells (LSCs). We examined the phenotypic and functional characteristics of putative LSCs in patients with aggressive SM and MCL. Highly enriched, sorted, BM cell fractions were injected into NOD-SCID-IL-2Rγ-/- mice exhibiting human membrane-bound SCF (NSG-SCF). Whereas cell fractions containing CD34+CD38- cells engrafted in NSG-SCF mice with a MCL-like disease, no substantial engraftment was produced by MC-rich but stem cell-depleted KIT+/CD34- cell fractions or CD34+/CD38+ progenitor cells obtained from the same patients. As assessed by flow cytometry, the CD34+/CD38-MCL LSCs were found to co-express several stem cell markers, as well as various cell surface targets, including CD33 and CD52. Subsequently, we examined the effects of target-specific drugs. As assessed by flow cytometry, treatment of primary MCL LSCs or the human MCL-like cell line ROSA KIT D816V with the CD117-targeting kinase inhibitor PKC412 or the CD33-targeting antibody conjugate gemtuzumab-ozogamicin induced dose-dependent apoptosis. The CD52-targeting antibody alemtuzumab was found to induce lysis of ROSA KIT D816V cells and of CD34+/CD38—cells in all MCL samples analysed. Furthermore, pre-incubation of MCL cells with alemtuzumab prior to injection into NSG-SCF mice resulted in a significantly reduced engraftment. In conclusion, our data show that the MCL clone originates from a primitive hematopoietic stem cell that resides in a CD34+/CD38—fraction.

In addition, our data indicate that MCL LSC express several clinically relevant surface targets. These observations may lead to the development of novel LSC-eradicating treatment concepts in this highly aggressive and drug-resistant type of leukemia.
Human DOCK2 mutations underlie a pleiotropic immunodeficiency syndrome with early onset, invasive infections

Cecilia Dominguez Conde et al.

Presenter: Cecilia Dominguez Conde

Congenital defects of immunity can manifest with increased susceptibility to life-threatening infections and/or immune dysregulation. Defining the molecular cause of these diseases is a fundamental step towards understanding their pathogenesis and rationalizing therapeutic strategies. Using homozygosity mapping and exome sequencing, we identified bi-allelic deleterious mutations in DOCK2, encoding the end effector of cytokinesis 2, as the molecular etiology of a novel immunodeficiency syndrome in five unrelated patients. DOCK2 is a guanine exchange factor for the small GTPase RAC1 and is involved in the control of actin dynamics. Analysis of patients’ lymphocytes showed impaired Rac1 activation and decreased levels of F-actin as well as abrogated lymphocyte motility and NK-cell degranulation. Furthermore, antiviral immunity, including type I and III interferon responses, was defective in patient cells.

These results pinpoint a central role for DOCK2-dependent pathways in human immunity, emphasizing the importance of actin dynamics and other Rac1-dependent pathways in immune homeostasis. Ongoing investigations employing pull-down proteomics aim to define the cellular context of DOCK2.
Characterization and target expression profiles of CD34+/CD38− and CD34+/CD38+ stem- and progenitor cells in acute lymphoblastic leukemia

Katharina Blatt et al.

Presenter: Katharina Blatt

Acute lymphoblastic leukemia (ALL) is a life-threatening malignancy defined by leukemic expansion of lymphoblasts in hematopoietic tissues, including the bone marrow (BM). The clinical course and prognosis vary among patients, depending on the variant of ALL, age, cytogenetic and molecular abnormalities, and response to initial therapy. An emerging new target of therapy in clinical hematology is the leukemic stem cell (LSC). The LSC concept has been established with the intention to explain cellular hierarchies and the biology of various leukemias, and to improve drug therapy through the elimination of disease-initiating cells.

In ALL LSC supposedly express CD34 and often lack CD38. However, little is known about markers and targets expressed in ALL LSC. We examined marker- and target expression profiles in CD34+/CD38− LSC in patients with Ph+ ALL (n=21) and Ph− ALL (n=19) by multi-color flow cytometry and qPCR. ALL LSC expressed CD19 (B4), CD44 (Pgp-1), CD123 (IL-3RA), and CD184 (CXCR4) in all patients tested. Moreover, in a sub-group of patients, LSC also displayed CD20 (MS4A1) (10/32=31%), CD33 (Siglec-3) (17/39=44%), CD52 (CAMPATH-1) (15/31=48%), IL-1RAP (13/21=62%), and/or CD135 (FLT3) (4/12=33%). CD25 (IL-2RA) and CD26 (DPPIV) were expressed on LSC in Ph+ ALL exhibiting BCR/ABL1p210, whereas in Ph+ ALL with BCR/ABL1p190, CD34+/CD38− LSC variably expressed CD25 but did not express CD26. In Ph− ALL, CD34+/CD38− LSCs expressed IL-1RAP in about half of the patients, but did not express CD25 or CD26. Normal stem cells stained negative for CD25, CD26, and IL-1RAP, and expressed only low amounts of CD33 and CD52. In functional studies, ALL LSC engrafted NSG mice after 20 weeks, and targeting of LSC with antibodies against CD33 (gemtuzumab-ozogamicin) and CD52 (alemtuzumab) resulted in reduced engraftment. Together, LSC in Ph+ and Ph− ALL express unique marker- and target expression profiles. In Ph+ ALL with BCR/ABL1p210, the LSC phenotype closely resembles the marker-profile of LSC in chronic myeloid leukemia, confirming the close biologic relationship of these neoplasms. Targeting of LSC with specific antibodies may facilitate LSC-eradication in patients with ALL.
Multiple myeloma (MM) is a malignancy characterized by monoclonal paraproteinemia and tissue plasmacytosis. In many patients, cytopenia and osteopathy develop. Although several effective treatment strategies have been developed in recent years, there is still a need to identify new drug targets and new agents effective in patients with advanced MM. In fact, in many patients, drug resistance develops and no curative drug-based treatment concepts have been developed in MM to date. A new emerging target relevant to the development of curative treatment strategies are neoplastic stem cells.

However, in MM only little is known about the phenotype, target expression profile, and drug responses in disease-initiating and propagating neoplastic stem cells. In the present study, we examined the effects of 15 targeted drugs on growth and survival of primary MM cells and 5 MM cell lines (MM.1S, NCI-H929, OPM-2, RPMI-8226, U-266). The PI3-kinase blocker BEZ235, the pan-BCL-2 inhibitor obatoclax, the Hsp90-targeting drug 17AAG, and the Polo-like kinase-1 inhibitor BI2536, were found to exert major growth-inhibitory effects in all 5 MM cell lines. Moreover, these drugs were found to inhibit in vitro proliferation of primary MM cells and to induce apoptosis at pharmacologic drug concentrations (EC50 <1 µM). Apoptosis-inducing effects were not only seen in the bulk of neoplastic cells but also in the putative CD138−/CD20+/CD27+ MM stem cells. Synergistic growth-inhibitory effects were observed in MM cells using various drug combinations, including 17AAG+BI2536 in MM.1S, OPM-2, RPMI-8226, and U-266 cells, 17AAG+BEZ235 in MM.1S, OPM-2, RPMI-8226, and U-266 cells, 17AAG+obatoclax in MM.1S, NCI-H929, OPM-2, and RPMI-8226 cells, BI2536+BEZ235 in MM.1S, NCI-H929, OPM-2, and RPMI-8226 cells, BI2536+obatoclax in MM.1S, OPM-2 and RPMI-8226 cells, and BEZ235+obatoclax in MM.1S and RPMI-8226 cells.

Together, our data show that various targeted drugs induce profound and often synergistic anti-neoplastic effects in MM cells which may have clinical implications and may contribute to the development of novel treatment strategies in advanced MM.
Functional Dissection of the Transcriptional Network Regulated by NUP98 Translocations in Acute Myeloid Leukemia

Johannes Schmoellerl et al.

Presenter: Johannes Schmoellerl

Chromosomal rearrangements involving the Nucleoporin 98 (NUP98) gene are recurrently found in patients suffering from acute myeloid leukemia (AML) and are often associated with poor prognosis. In these patients, the N-terminal part of the NUP98 gene is fused to the C-terminal portion of a partner gene, resulting in expression of an oncogenic fusion protein. More than 25 distinct NUP98 fusion proteins have been identified in patients so far. To date, personalized targeted therapy of patients with NUP98 rearrangements 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 a common oncogenic mechanism that is 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 aim to elucidate global effects of distinct NUP98 fusion oncoproteins on the transcriptome in a systematic manner.

We have developed genetically engineered mouse models for AML driven by tetracycline (tet)-regulated expression of five common NUP98 translocation proteins. Despite expressing molecularly distinct NUP98 oncoproteins, mice developed phenotypically similar AML-like symptoms, including splenomegaly and >80% GR-1/Mac-1 positive cells in bone marrow and spleen. Different NUP98 fusion proteins led to overlapping, yet distinct changes in global transcriptomic patterns, pointing towards shared oncogenic mechanisms.

Analysis of changes in global gene expression of leukemic cells upon tet-mediated oncogene withdrawal identified a conserved core of genes whose expression is induced by distinct NUP98 oncoproteins in vivo. To identify leukemia-specific vulnerabilities within this comprehensive dataset of common core target genes of NUP98 fusion proteins, we adopted a CRISPR/Cas9-based negative selection screening methodology in cultured cells from NUP98 fusion AML models. Thus, this study might reveal novel critical effectors in NUP98-fusion-protein-dependent leukemia that could impact on the development of future therapies.
**Early onset protein losing Enteropathy, Bowel Inflammation, and Thrombosis in patients with complement regulator deficiency**

Rico Chandra Ardy et al.

Presenter: Rico Chandra Ardy

Background: The study of monogenic gastrointestinal diseases has revealed distinct molecular pathways crucial for gut homeostasis and enabled the development of targeted therapies. A disease caused by complement dysregulation primarily affecting the gastrointestinal tract is heretofore unknown.

Methods: We studied 10 patients suffering from early-onset abdominal pain and diarrhea caused by early-onset protein-losing enteropathy (PLE) with lymphangiectasia, edema, malabsorption, and, less frequently, bowel inflammation, recurrent infections, and angiopathic thromboembolic disease. DNA sequencing and homozygosity mapping was performed to identify gene variants. Complement regulatory function, including complement deposition, costimulation, and anaphylatoxin responses, was assessed by shRNA and CRISPR gene suppression and rescue of protein expression using lentiviruses.

Results: We identified deleterious autosomal recessive mutations leading to loss of protein expression in the gene encoding blood group antigen with a complement regulatory function. Patient T lymphocytes and protein-deficient cell lines displayed abnormal deposition of complement factor C3d. Reconstitution of wild type expression prevented excessive C3d deposition. Stimulation of anaphylatoxin receptors on patient T lymphocytes produced increased tumor necrosis factor alpha (TNFα), which caused a pro-coagulatory state in HUVECs. Costimulation of TCR and the contingent production of interleukin-10 (IL-10), was defective in patient T lymphocytes.

Conclusion: We here describe a novel syndrome of complement regulator deficiency with hyperactivation of complement, angiopathic thrombosis, and PLE (CHAPLE) disease characterized by abnormal complement deposition due to recessive loss-of-function (LOF) mutations.

R.C. Ardy, A.Ozen, and W.A.Comrie are shared first authors, and K. Boztug and M.J. Lenardo are shared senior and corresponding authors.
The Normalization Visualization Tool: a novel bioinformatic resource to identify optimal normalization strategies for RNA-Seq experiments

Thomas Eder et al.

Presenter: Thomas Eder

RNA-Seq experiments play a major role in experimental and clinical cancer research. A common task in the analysis of RNA-Seq data is the identification of differentially expressed genes between different states. However, beforehand it is crucial to normalize the raw count data derived from the aligned sequence reads for differences in RNA/cDNA abundance, amplification efficiency and sequencing performance. As all subsequent steps in differential gene expression analysis will be based on normalized read counts, the outcome and the interpretation of the RNA-seq experiment critically depends on the choice of an adequate normalization strategy.

Multiple normalization methods have been described, and all of them are based on certain, different assumptions. Yet, these may or may not be suitable for the type of data they are applied on. For instance, if gene expression levels of healthy vs. rapidly growing tumor cells are compared, the assumptions that the majority of genes are not differentially expressed or that the amount of sequenced mRNA was equal, might not apply. Therefore, researchers need to select the optimal data normalization strategy depending on the specific design of the RNA-Seq experiment and its general setup. This selection includes exploration of different normalization methods as well as their comparison.

We developed the Normalization Visualization Tool (NVT), which provides a fast and simple way to analyze and evaluate multiple normalization methods via visualization and representation of correlation values, based on a user-defined set of uniformly expressed genes. NVT enables researchers to visualize and compare effects of 11 different normalization methods and thus assists in choosing the optimal normalization method for their actual RNA-seq experiment. NVT is implemented as R library and is freely available.

In summary, NVT can ensure that all subsequent steps in the analysis of differential gene expression are based on a solid foundation of correctly normalized datasets. Therefore, NVT will contribute to increased quality of RNA-seq data analysis through enabling and improving the detection of differentially expressed genes. This might have a strong impact on both experimental as well as clinical research involving RNA-seq experiments.
Ewing sarcoma is an aggressive and lethal pediatric malignancy driven by the EWS/FLI1 fusion protein and it affects predominantly bone and soft tissues. Despite advances in genetic strategies, the reliable and genetically defined preclinical mouse model remains unresolved. This prevents detailed genetic and molecular understanding of Ewing sarcoma on a firm biological basis and therapy. As functional approach we took advantage of mesenchymal Prx1-CreErt2-directed conditional EWS/FLI1 expression in mice. Notably, targeted Prx1-CreErt2 mediated EWS-FLI1 expression in mesenchymal stem cells led to formation of metastatic Ewing sarcomas. The histopathological analysis revealed an Ewing’s sarcoma phenotype with expression of Ewing Sarcoma-like markers. Importantly, mouse Ewing sarcomas showed preferentially RNA-Seq expression signature and characteristics seen in a patient-derived Ewing sarcoma. The Ewing sarcoma mouse model predominantly showed metastasis in distant organs like liver, bone, muscle or adrenal gland reminiscing patient situation.

We propose that this is the first transgenic mouse model that could resemble a faithful model for solid EWS/FLI1 induced tumors for comparative and preclinical human Ewing sarcoma studies.
CD44 is a RAS-regulated invasion molecule that is overexpressed in neoplastic mast cells and triggers disease expansion in advanced mastocytosis

Niklas Mueller et al.

Presenter: Niklas Mueller

CD44 is a multifunctional adhesion molecule that mediates homing and expansion of normal and neoplastic stem- (SC) and progenitor cells (PC). Although mast cells (MC) are known to express CD44, little is known about the regulation and functional role of this receptor on neoplastic cells in systemic mastocytosis (SM). We found that CD34+/CD38- SC, CD34+/CD38+ PC, and KIT+/CD34- MC express CD44 in all SM variants, including indolent SM (ISM), SM with associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL).

Furthermore, soluble CD44 was detectable in the sera of SM patients. CD44 expression on SC, PC, and MC as well as soluble CD44 levels increased significantly with the aggressiveness of SM. All human MC lines examined, including HMC-1.1, HMC-1.2, ROSA-KIT WT, ROSA-KIT D816V, MCPV-1.1, MCPV-1.2, MCPV-1.3, and MCPV-1.4 expressed CD44. Incubation with the demethylating agents decitabine or 5-azacytidine (0.1-5 µM) for 96 hours resulted in significant upregulation of CD44 expression in all MC lines. In contrast, incubation with the MEK-inhibitor refametinib (RDEA119) (0.1-5 µM) or the STAT5 blocker pimozide (2.5-10 µM) for 48 hours resulted in a significant downregulation of CD44 in all MC lines. These data suggest that the RAS/MEK- and the STAT5 pathway are involved in expression of CD44 in neoplastic MC. We next transduced HMC-1.2 and ROSA-KIT WT cells with KRAS-WT or oncogenic KRAS-G12V. RAS overexpression resulted in a significant upregulation of CD44 expression compared to empty vector. To study the functional role of CD44 in neoplastic MC, we employed a mouse xenotransplantation model using severe combined immunodeficient (SCID) mice, HMC-1.2 cells, and shRNA against CD44. In this model, the shRNA-induced knockdown of CD44 in HMC-1.2 cells resulted in reduced MC expansion, reduced tumor formation, and significantly prolonged survival compared to cells transduced with control shRNA. In conclusion, CD44 is expressed in neoplastic MC as well as in neoplastic SC and PC in patients with advanced SM. Our data also suggest that CD44 is an important mediator of evolution and malignant spread of neoplastic MC into various organs in SM.

Future studies will show whether CD44 can serve as a therapeutic target in patients with advanced SM.
Identification of BRD4 as a Novel Drug Target in Ph+ CML: The BRD4/MYC blocker JQ1 overrides TKI resistance in CML cells

Barbara Peter et al.

Presenter: Barbara Peter

Chronic myeloid leukemia (CML) is a hematopoietic neoplasm in which BCR-ABL1 acts as a major driver of proliferation and survival of leukemic cells. In most CML patients, the disease can be kept under control by BCR-ABL1 tyrosine kinase inhibitors (TKI) such as imatinib, dasatinib, nilotinib, bosutinib, or ponatinib. However, resistance against TKI may develop. Therefore, research is searching for novel promising drug targets in CML. The epigenetic reader BRD4 has recently been introduced as a new drug target in acute myeloid leukemia. The aims of our study were to determine the expression of BRD4 and its downstream target MYC in CML cells and to explore whether BRD4 may serve as a drug target in CML. As determined by qPCR, primary CML cells as well as the CML cell lines KU812 and K562 expressed BRD4 and MYC mRNA. Moreover, both CML cell lines and primary CML cells were stained positive for BRD4 and MYC by immunocytochemistry.

Furthermore, shRNA-induced knockdown of BRD4 in KU812 and K562 cells resulted in reduced growth compared to control shRNA. The BRD4-targeting drugs JQ1 and OTX-015 were found to inhibit 3H-thymidine uptake and thus proliferation in KU812 cells (IC50: 0.25-1 µM) and in primary CML cells (IC50: 0.1-5 µM). However, no substantial growth-inhibitory effects were seen in K562 cells (IC50: >5 µM). As determined by Annexin V/PI staining, JQ1 and OTX-015 induced apoptosis in KU812 cells whereas no effects were seen in K562 cells. Moreover, JQ1 decreased expression of MYC mRNA and MYC protein in CML cell lines and primary CML cells. We also found that JQ1 and OTX-015 cooperates with BCR-ABL1 TKI in inhibiting proliferation of KU812 and K562 cells. In co-culture experiments using CAL-72 cells, we were able to show that JQ1 partially overcomes osteoblastic-induced resistance against TKI in K562 cells. Moreover, JQ1 inhibited INF-G-induced upregulation of PD-L1 in primary CML stem cells. Together, our data show that BRD4 is a new target in CML cells, that BRD4 blockers cooperate with BCR-ABL1 TKI in inducing growth-inhibition, and that BRD4/MYC blockers overcome niche-mediated and checkpoint-related TKI-resistance in CML cells.

Whether these concepts are applicable in vivo in patients with TKI-resistant CML remains at present unknown.
(A-)typical cannabinoid receptors GPR55 and CB1 have differential roles in colon cancer

Carina Hasenoehrl et al.

Presenter: Carina Hasenoehrl

Background: Cannabinoid receptor 1 (CB1) has recently been shown to play a tumor-suppressing role in colon cancer (Wang 2008). This led us to investigate whether G protein-coupled receptor 55 (GPR55), recently termed the "third CB" and shown to be involved in various cancers, plays a role in colon carcinogenesis.

Methods: Colitis-associated colon cancer was chemically induced in GPR55 and CB1 knock out (KO) mice, GPR55/CB1 double knock out mice and wild type littermates (WTs). Tumor burden was evaluated macroscopically after 12 weeks and tumor biomarkers, receptor expression levels, cytokines and infiltrated leukocytes were analyzed from the collected tissue. A model of spontaneous colon cancer, i.e. without the induction of colitis, was also carried out in GPR55 and CB1 KO mice. Patient data were obtained from a publicly available data base (http://r2.amc.nl) and from the OncoTrack EU project.

Results: GPR55 appeared to play a tumor-promoting role in colon cancer since GPR55 KO mice showed reduced tumor numbers and areas compared to WTs in both models. Also, in human patients, high GPR55 expression correlated with a reduced relapse-free survival. The mechanism by which GPR55 exerts its effects was found to involve the modulation of the tumor microenvironment. This was evident through the reduced expression of e.g. COX-2 and STAT3 and altered recruitment of CD8+ T cells (increased) and myeloid-derived suppressor cells (decreased) in tumors of GPR55 KO mice.

The tumor-promoting role of CB1 was confirmed since CB1 KO mice showed increased tumor numbers and areas. Additionally, we found that GPR55/CB1 double KO mice had a tumor burden equal to WTs.

qRT-PCR revealed that, in tumors of WT mice, GPR55 mRNA was up-regulated whereas CB1 mRNA was down-regulated compared to healthy control colon. Differential regulation of receptor expression was also found in patients where expression levels of both receptors correlated with disease severity, albeit in a differential manner.

Conclusion: (A-)typical cannabinoid receptors GPR55 and CB1 play differential roles in colon carcinogenesis. Since they share certain ligands, this is of importance when targeting the endocannabinoid system for future therapy of colon cancer.
The methyltransferase SETD2 is required for MLL-rearranged Acute Myeloid Leukemia

Anna Skucha et al.

Presenter: Anna Skucha

Acute Myeloid Leukemia (AML) frequently harbors chromosomal rearrangements involving the Mixed Lineage Leukemia (MLL) gene. MLL-fusions involving over 65 different partner genes were found in AML patients, and many of them can act as strong cancer drivers. While critical effectors of several distinct MLL fusion proteins were identified, it is not clear if transforming mechanisms are conserved across the entire family of MLL fusions.

We hypothesized that common oncogenic mechanisms are encoded in stable physical and genetic MLL-fusion-specific interaction networks. Thus, we aimed to identify common critical effectors of different MLL fusion proteins that are presumed to employ different mechanisms of oncogenic transformation.

We purified protein complexes around 7 MLL fusion proteins and characterized their composition by affinity purification coupled to mass spectrometry (AP-MS). Data analysis revealed a densely interconnected protein-protein interaction network of >950 proteins, comprising previously known MLL-interacting protein complexes (such as PRC2 or SWI/SNF), as well as a high number of new interaction partners of MLL. 128 proteins were found to interact with ≥5 of all 7 MLL-fusions. This subset of conserved MLL-interaction partners is highly enriched for proteins with functions in chromatin metabolism and transcriptional control. By systematic functional investigation of the conserved MLL-fusion interactome using shRNA screens we identified the methyltransferase SETD2 as a critical effector of MLL fusion proteins. RNAi- and CRISPR/Cas9-mediated suppression of SETD2 induced myeloid differentiation and apoptosis in human and mouse MLL-rearranged cell lines. In addition, depletion of SETD2 in MLL-AF9-transduced cells resulted in loss of serial re-plating capacity in vitro and prolonged disease onset in vivo. Lastly, knockdown of SETD2 caused proliferative disadvantage in primary cells from AML patients with MLL-rearrangements without affecting MLL-wild-type AML cells.

In summary, our data highlight the functional relevance of combined proteomic-genomic cellular screening to identify critical effector of MLL fusion proteins and show that SETD2-dependent mechanisms are specifically required for initiation and maintenance of MLL-rearranged AML.
Acute myeloid leukemia (AML) is a heterogeneous class of leukemia with prognosis predicted by a number of cytogenetic and molecular abnormalities. Patients suffering from AML have poor prognosis and high mortality rate despite considerable advances in chemotherapy and hematopoietic stem cell transplantations.

Mutations of the Fms-like tyrosine kinase 3 (FLT3) gene is a frequent event in AML and usually involves internal tandem duplications of the juxtamembrane domain (FLT3-ITD) or point mutations of the tyrosine kinase domain (FLT3-D835). Up to 30% of patients suffering from AML express constitutively activating FLT3-mutations on initial diagnosis and additional patients may acquire them on relapse. Such mutations are associated with poor prognosis and a shortened overall survival. A number of FLT3 tyrosine kinase inhibitors (FLT3-TKI) are being developed as targeted therapy for FLT3-driven AML; however, their use is complicated: they provide short-term disease control but relapse invariably occurs within months, highlighting the urgent need for additional therapeutic targets. PIM protein kinases are oncogenic targets that are expressed in AML cells.

We show that the US Food and Drug Administration-approved CDK4/6 kinase inhibitor palbociclib induces apoptosis of FLT3-mutant leukemic cells. The effect is specific for FLT3-mutated cells and is ascribed to the transcriptional activity of CDK6: CDK6 but not its homolog CDK4 is found at the promoters of the FLT3 and PIM1 genes, another important leukemogenic driver. There CDK6 regulates transcription in a kinase-dependent manner. Of potential clinical relevance, combined treatment with palbociclib and FLT3 inhibitors results in synergistic cytotoxicity. Simultaneously targeting two critical signaling nodes in leukemogenesis could represent a therapeutic breakthrough, leading to complete remission and overcoming resistance to FLT3 inhibitors.

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Does Notch-signaling induced by cocultured leukemia-cells modulate differentiation of osteoblasts?

Thomas Heugl et al.

Presenter: Thomas Heugl

Mineralization of bone tissue is an important process for bone growth and remodelling. Bone-mineralizing cells differentiate from mesenchymal stem cells to osteoblasts and osteocytes. During differentiation cells increase the expression of genes involved in mineralization like alkaline phosphatase (Alpl) and Phospho1. It has been shown that acute lymphoblastic leukemia cells inhibit the differentiation of co-cultured bone mesenchymal stem cells (BMSCs) in-vitro by activating the notch intracellular signalling pathway. The focus of this study was to find out, whether this inhibitory effect could also be achieved by acute myelogenous leukemia (AML) cells.

We used two adherent murine cell lines, preosteocytic MLO-A5 and D1 cells, the latter cloned from a multipotent bone marrow stromal precursor. After a culturing time of one week, HL-60 cells derived from an AML (acute promyelocytic leukemia), were added and co-cultured for a further week in presence and absence of 5 mM β-glycerophosphate. Finally, proteins and mRNA were isolated. The extent of mineralization was determined by Alizarin-Red staining. Gene expression was evaluated by real-time quantitative PCR.

Interestingly, the normally in suspension growing HL-60 cells, adhered very strongly to both mesenchymal cell lines, although the morphology of the HL-60 cells was not changed. Furthermore, the presence of HL-60 cells significantly inhibited the mineralization compared to the murine cell lines cultured in absence of HL-60. Not surprisingly, this inhibition was accompanied by down-regulation of Alpl and Phospho1. A strong up-regulation of the expression of the Notch1, Notch2 and Jagged1 (Jag1) in both murine cell lines, which were co-cultured with HL-60 cells, suggested the involvement of the notch-pathway in the differentiation process of D1 and MLO-A5 cell lines. From our results we conclude that AML cells not only modulate osteoblastogenesis but also regulate the expression of genes involved in the mineralization in preosteocytes.
The TYK2-STAT1 pathway in aggressive T-cell lymphoma - a novel therapeutic intervention site?

Nicole Prutsch et al.

Presenter: Nicole Prutsch

Whole genome sequencing of T-cell lymphomas has revealed mutations in Januskinase family genes (JAK1, JAK2, JAK3, TYK2), known to have essential functions in cytokine signalling, motility and proliferation. Targeting Januskinases has opened new therapeutic intervention sites in cancer and autoimmune diseases. Tyrosinekinase 2 (TYK2) the first JAK kinase to be identified has important roles in inflammation and anti tumor immunity. Here we report pathway dependence on TYK2 in anaplastic large cell lymphoma (ALCL), a CD30 positive, aggressive Non-Hodgkin T-cell lymphoma. The importance of TYK2 is underlined by the recent description of TYK2-fusions driving oncogenic signalling in ALCL. Interestingly, using published RNAseq data of 23 primary ALCL cases, TYK2 expression was generally high as compared to PBMC independent of the presence of TYK2-fusions, suggesting active TYK2 signalling in large fraction of patients. Ablating TYK2 in ALCL cell lines, using lentivirally transduced shRNAs as well as CRISPR/CAS9 technology, led to rapid cell death induction. To test the therapeutic relevance of our findings, ALCL cell lines and PBMCs were treated with small molecule inhibitors of TYK2 (TYK2#2,Bayer-18) leading to apoptosis induction exclusively in tumor cells. Immunoblotting and shRNA mediated knockdown of potential downstream targets of TYK2 revealed an essential function for STAT1 in ALCL cell survival.

Flow cytometric analyses revealed active regulation of PD-L1 in TYK2 depleted cells. Tumor intrinsic and immune-checkpoint relevant consequences of TYK2 knockout are currently being assessed using an ALCL mouse model with T-cell specific TYK2 knock-out and experiments employing T-cell activation reporter cell lines with and without PD-1 expression.
DNA methylation is an epigenetic process, by which methyl groups are added to the C5 position of Cytosines on DNA. Methylation modifies the function of DNA by altering gene expression and is essential for normal gene regulation and development. Hypomethylation of gene promoters is usually associated with transcriptionally active DNA, while hypermethylation causes gene repression. Aberrant DNA methylation patterns are widely observed in a variety of tumors. This includes hypermethylations of CpG-Islands in promoters of tumor suppressor genes leading to epigenetic silencing.

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

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

Future experiments will include invasion assays with organotypic spheroid models and more prostate cancer cell lines, in order to validate these results.
STAT5BN642H is a driver mutation for leukaemia

Ha T. T. Pham et al.
Presenter: Ha T. T. Pham

Signal transducer and activator of transcription (STAT) 5B is part of the JAK-STAT pathway and it has been found to be frequently mutated in hematopoietic cancer. The most frequent mutated variant, STAT5B N642H has been discovered in more than 90 leukemic patients, however, the direct causal effect of STAT5B N642H has not been determined and the development of novel therapeutic concepts against the mutation has been hampered by the unavailability of a suitable mouse model. We herein show that leukemias can be driven by the given mutation in a transgenic mouse model. Expression of the STAT5B N642H in the hematopoietic compartment is sufficient to rapidly cause leukaemia that results in terminally diseased state as early as 40 days of age. The disease is fully transplantable and characterised by the expansion of the CD8+ T-cell population. The expression of STAT5B N642H displays persistent tyrosine phosphorylation in vivo, which leads to a significant up-regulation of genes that promote cell-cycle progression and survival such as Cdk1, Cdk6 and Bcl2. Importantly, efficient activation of STAT5B N642H still requires cytokine stimulation, which makes it sensitive to inhibitors of its upstream kinases. Thus, upon treatment with JAK kinase inhibitors, Ruxolitinib and Tofacitinib, the level of STAT5B N642H tyrosine phosphorylation is dramatically reduced.

In conclusion, we demonstrate that STAT5B N642H is a driver mutation and sufficient to cause leukemia in vivo and inhibitors of JAK kinases could be a potential therapeutic strategy for leukemic patients harbouring the STAT5B N642H mutation.
The multi-kinase inhibitor DCC-2618 counteracts growth and survival of neoplastic cells in systemic mastocytosis

Mathias Schneeweiß et al.

Presenter: Mathias Schneeweiß

Systemic mastocytosis (SM) is a myeloid neoplasm defined by abnormal growth and accumulation of neoplastic mast cells (MC). Most patients with SM express a D816V-mutated variant of KIT that confers resistance against several tyrosine kinase inhibitors (TKI). DCC-2618 is a novel multi-kinase inhibitor that has been described to block the kinase activity of KIT D816V.

The aims of this study were to evaluate the effects of DCC-2618 on proliferation and survival of neoplastic MC and other cell types that may play a role in SM. As assessed by 3H-thymidine-uptake, DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC50 values measured in KIT D816V- HMC-1.1 cells (11.2±4.3 nM) and ROSAKIT WT cells (61±11 nM) than in KIT D816V+ HMC-1.2 cells (147±68 nM), ROSAKIT D816V cells (133±43 nM), and the multi-resistant MC line MCPV-1. The DCC-2618 metabolite DP-5439 showed comparable growth-inhibitory effects in all cell lines. DCC-2618 was also found to inhibit proliferation of primary neoplastic MC obtained from patients with indolent SM (ISM) and MC leukemia. Furthermore, DCC-2618 induced apoptosis and blocked phosphorylation of KIT in all MC lines tested. Moreover, we were able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC50 2±0.6 nM) and in the FLT3 ITD-mutated AML cell lines MV4-11 (IC50 130±18 nM) and MOLM-13 (IC50 110±26 nM). In addition, DCC-2618 was found to block proliferation in primary leukemic cells in patients with monoblastic AML and chronic myelomonocytic leukemia (CMML) which may have clinical implications as CMML and AML are the most prevalent types of AHN in advanced SM. Finally, DCC-2618 was found to inhibit growth of cultured human vascular endothelial cells, suggesting that the drug may also counteract SM-related angiogenesis.

Together, our data show that DCC-2618 is a new potent multi-targeted TKI that counteracts growth and survival of neoplastic MC, leukemic monocytes, AML blasts, eosinophils, and endothelial cells in vitro. Whether DCC-2618 is also able to inhibit the growth of neoplastic MC and other leukemic (AHN) cells in vivo in patients with advanced SM remains to be determined in clinical trials.
NPM-ALK positive anaplastic large cell lymphoma is a rare and aggressive Non-Hodgkin’s lymphoma of T cell origin, which is driven by constitutive activation of the oncogenic anaplastic lymphoma kinase ALK through deregulation of several signaling pathways promoting cell proliferation and survival.

Recent work suggested that ALK signaling could directly impact on epigenetic alterations, such as DNA methylation in tumor cells. There is evidence that STAT3, the key downstream mediator of ALK signaling, regulates and targets the DNA methyltransferase DNMT1 to promoters of tumor suppressor genes inducing their silencing. Furthermore, in cell lines chemical inhibition of DNMT1 by 5-Aza-2'-Deoxycytidine impairs ALK signaling and causes loss of STAT3 activity, resulting in cell cycle arrest and apoptosis.

We show that T cell specific deletion of the maintenance methyltransferase gene Dnmt1 abolishes tumor formation in a transgenic NPM-ALK lymphoma mouse model (NPM-ALK;Dnmt1-/-). Intriguingly, we do not observe altered proliferation or apoptosis in Dnmt1 deficient NPM-ALK thymocytes. Further, the oncogene NPM-ALK and its downstream target STAT3 are highly active in the targeted T cells, indicating that downstream ALK signaling via STAT3 is maintained upon Dnmt1 deletion in NPM-ALK expressing T cells in vivo.

We are currently performing gene expression profiling using RNA sequencing as well as reduced representation bisulfite sequencing (RRBS) to identify genome wide changes in gene expression and aberrant DNA methylation patterns in NPM-ALK and NPM-ALK;Dnmt1-/- mice. Integration of DNA methylation (RRBS) and gene expression (RNA-Seq) data will allow us to infer the relation of oncogenic signaling and epigenomic aberrations and will give us important information about the role of DNMT1 in lymphomagenesis and in T cell development.

Together, our data suggest that aberrant DNA methylation is critically involved in ALK dependent lymphomagenesis and DNMT1 might be essential for the fusion kinase to exert its oncogenic potential.
Immunodeficiencies are a significant clinical challenge due to their heterogeneity and prevalence. Strategies for pharmacological intervention are often unsuccessful, in part because of a lack of mechanistic understanding of disease pathologies. The study of rare monogenic disorders causing immune dysregulations (primary immunodeficiencies, PIDs) may provide clearer, causal genotype-phenotype relationships. Even more so, combining such approaches with additional layers of defined perturbations, e.g. by applying chemical drugs with known target profiles, may enable novel insights into underlying disease-causing mechanisms and suggest novel treatment routes. As chemical therapeutics have rarely been explored on inborn errors of the human immune system, this represents an almost untouched area of research with unique potential.

We especially aim at exploring druggabilities and drug-gene interactions in autoimmune disorders with genetic etiology and life-threatening outcome. While for instance cancer drugs often times aim at directly inhibiting upregulated signaling cascades, PIDs mostly act through disruption of protein activity or, in case of haploinsufficiency, through reduced gene expression, adding additional layers of complexity for drug targeting approaches. Here, chemical drugs need to aim at altering signaling pathways indirectly, or increasing protein levels and/or –stability. We focus on CTLA-4 deficiency/haploinsufficiency as initial model disease for T-cell driven autoimmunity due to the protein’s central role in initiating T-cell suppression. We screened a library of annotated chemical drugs in order to collect potential drug candidates reconstituting CTLA-4 levels and functional T-cell homeostasis. Our preliminary data suggest several molecules including HDAC inhibitors and lysosomal inhibitors affecting CTLA-4 abundance, as well as novel drug/target combinations with as of yet completely unknown mechanisms for CTLA-4 biology, which we aim to decipher in detail. The expected findings on drug-gene relationships may provide further understanding of the fundamentals of human (auto)immunity, and provide general principles in targeting PIDs with chemical drugs.
Delineation of effects of ponatinib on vascular endothelial cells: a potential basis and explanation for the occurrence of vascular adverse events in CML patients treated with ponatinib

Emir Hadzijusufovic et al.

Presenter: Emir Hadzijusufovic

The potent BCR-ABL1-targeting drug ponatinib is used for treatment of multi-resistant chronic myeloid leukemia (CML). However, an increase in cardiovascular events has been described in ponatinib-treated patients. To uncover the etiology of ponatinib-induced adverse vascular events, we evaluated the effects of ponatinib on endothelial cells in vitro and in a mouse model of angiogenesis. In a chemical proteomics approach using the human microvascular endothelial cell line HMEC-1, ponatinib was found to bind to several proteins known to play a role in angiogenesis, including TEK, MAPKAPK2, PDGFRB, EPHA2, and EPHB4. In a phospho-receptor tyrosine kinase assay performed with human coronary artery endothelial cells (HCAEC), ponatinib inhibited the activation of VEGF2 receptor, MER proto-oncogene, and insulin receptors, which play a role in angiogenesis, vascular homeostasis, and vessel protection.

We also found that ponatinib induces dose-dependent apoptosis in HCAEC in a caspase assay (relative apoptosis: 2.13±0.42 ponatinib 100 nM). In addition, ponatinib inhibited the proliferation of HMEC-1 and of human umbilical vein endothelial cells (HUVEC) as determined by thymidine-uptake assay (IC50: 100-250 nM). In HUVEC, ponatinib (1 μM) also increased adhesion to a plastic-surface (adherent cells: 85% ponatinib vs. 10% DMSO-control). In C57BL/6 mice, ponatinib (5 mg/kg/day for 35 days) inhibited blood flow recovery after femoral artery ligation (perfusion ratios of the ischemic vs. non-ischemic limb: 0.67±0.07 in control group vs. 0.56±0.1 in ponatinib group). Ponatinib-treatment was also associated with toe necrosis (score: 0.3 control vs. 1.3 ponatinib). As examined by wire myography, ponatinib (100 nM) enhanced norepinephrine-induced vasoconstriction (pEC50: 7.76±0.06 M ponatinib-free vs. 7.96±0.05 M ponatinib-incubated) and attenuated acetylcholine-mediated vasodilatation (pIC50: 7.45±0.05 M ponatinib-free vs. 7.06±0.1 M ponatinib-incubated) in aortic rings from C57BL/6 mice.

In conclusion, our data show that ponatinib is a potent inhibitor of multiple endothelial cell functions which may explain the occurrence of vascular events in CML patients treated with ponatinib.
Resveratrol and a resveratrol-salicylate hybrid molecule: a comparative study in CD4+ T-cells

Katrin Goldhahn et al.

Presenter: Katrin Goldhahn

Introduction: Aberrant T-cell responses are involved in the pathogenesis of systemic autoimmune diseases such as rheumatoid arthritis (RA) leading to chronic inflammation and organ damage. Consequently, substances modulating T-cell activation may have therapeutic benefit in RA and related rheumatic diseases. Resveratrol is a natural occurring polyphenol mainly produced in plants. The beneficial effects of resveratrol are due to its anti-inflammatory and anti-carcinogenic activities. The aim of this study was to compare the effects of resveratrol and a novel resveratrol-salicylate hybrid molecule (C10) on human CD4+ T-cells.

Methods: CD4+ T-cells from healthy donors were pre-incubated with different concentrations of resveratrol or C-10 before being stimulated with anti-CD3/anti-CD28 antibodies. After 24h and 72h, respectively, cell culture supernatants were harvested and IL-2, IFN-γ and TNF-α release were quantified by ELISA. Proliferation rate was measured by thymidine incorporation. In addition, the up-regulation of the early activation markers CD25, CD69, CD71 and CD98hc was analyzed and phosphorylation of ERK, AKT, S6RP and STAT5 was determined by westernblot or flow cytometry. Results: Inhibition of IL-2, IFN-γ and TNF-α release was significantly more effective when the cells were treated with C-10. A decrease of cytokines was observed already at 6.25µM C-10 whereas resveratrol inhibited cytokine production only at 25µM or 50µM significantly. Moreover, proliferation rate in CD4+ T-cells was significantly more decreased in the presence of C-10. The expression of CD25, CD69, CD71 and CD98hc was reduced to a similar degree by both compounds. Phosphorylation of ERK, Akt and S6RP was attenuated when the cells were incubated with resveratrol or C-10. For STAT5, a significantly higher inhibition by C10 in comparison to resveratrol was observed.

Conclusion: Our data demonstrate that C-10 suppressed cytokine secretion and proliferation more effectively than resveratrol. Both compounds influence the phosphorylation of important signalling molecules. Thus the resveratrol-salicylate hybrid molecule C-10 significantly amplified the effects of resveratrol in CD4+ T-cells and might be used in the future for treatment of RA and other T-cell driven autoimmune diseases.
Hypermineralization and increased accumulation of Zinc in osteosarcoma tumor matrix
Phaedra Messmer et al.
Presenter: Phaedra Messmer

Osteosarcoma is the most common primary malignant bone tumor with a peak incidence in childhood and adolescence. The tumor is frequently occurring at sites of rapid bone growth. Osteosarcoma cells produce tumor matrix that can mineralize. Based on the radiological appearance, osteosarcoma are divided into sclerotic, osteolytic and mixed pattern. In the case of sclerosing osteosarcoma little is known about the structure and the degree of mineralization of the tumor matrix representing an additional variant of mineralized tissue. Moreover there is some evidence that certain levels of the trace element Zinc (Zn) in the tissue is associated with different types of cancers. It is well known that Zn plays an important role in bone metabolism. Thus the aim of our study was to measure the mineral content of the tumor matrix as well as the relative Zn content and compare the results with that of normal / intact bone.

For this purpose quantitative backscatter electron imaging (qBEI) in the scanning electron microscope and synchrotron radiation induced x-ray fluorescence (SR-XRF) was applied. Tumor bone samples from 8 patients between 10 and 18 years old, with highly-malignant G3 osteosarcoma who underwent wide resection of the tumor were investigated.

qBEI revealed an about 20% increase of mineral content in the mineralized tumor matrix compared to coexisting intact cortical or trabecular bone matrix of the patients. SR-XRF outcomes showed on average a 6 times higher count-rate fraction of Zn (Zn/(Zn+Ca) for tumorous bone areas than for healthy bone areas. In our samples, we found regions with varying portions of mineralized to non-mineralized tumor tissue. Interestingly, we did not found a significant difference in Zn fraction between tumor areas with a low or a high portion of mineralized tissue indicating that this ratio is relatively constant in the mineralized tumor matrix. Remarkably, no Zn accumulation was found in soft tissue.

In conclusion the high radiodensity in sclerosing osteosarcoma is due to both the amount of mineralized tumor matrix and its high degree of mineralization. Our findings of increased Zn levels warrant further studies on the role of Zn on bone cancer.
Histone deacetylases (HDACs) play an important role in cell differentiation because they regulate genes on a chromatin level. Their removal of acetyl groups from histone tails leads to a compact chromatin conformation which represses transcription. While essential for development, HDACs have been found to be dysregulated in numerous types of cancer. Their chemical inhibition has been shown to induce differentiation, apoptosis and growth arrest in malignant cells. Furthermore, FDA approved HDAC inhibitors (HDACi) are already used in cancer therapy, for instance in the treatment of cutaneous T-cell lymphoma.

This project investigates the effect of HDAC inhibition on the development of the NPM-ALK positive Anaplastic Large Cell Lymphoma (ALK+ ALCL), an aggressive T-cell lymphoma mainly found in children and young adults. In contrast to our in vitro experiments, which confirm the expected apoptotic effect of HDACi on human ALCL cell lines, our in vivo data show accelerated lymphoma development in a mouse model with a T-cell specific NPM-ALK transgene and HDAC1 deletion.

Although the predisposition of mice with reduced HDAC1 and HDAC2 activity in thymocytes towards lymphoma development has already been discovered, the underlying molecular mechanisms remain unclear. Our experiments show increased amounts of Bcl-XL and other members of the Bcl2 family on protein as well as mRNA level. We propose that the overexpression of anti-apoptotic proteins as a consequence of HDAC1 deletion causes the advanced tumor formation in the thymuses of NPM-ALK transgenic mice. We will further investigate this hypothesis through mouse treatments with HDAC and Bcl2 family inhibitors. Our data provides insight to the pathways responsible for the beneficial and adverse effects of HDACi therapy in lymphoma treatment.
Identification of CD25 (IL-2RA) and CD26 (DPPIV) as novel markers and targets in CD34+/CD38− LSC in Ph+ CML

Irina Sadovnik et al.

Presenter: Irina Sadovnik

Chronic myeloid leukemia (CML) is a stem cell (SC) neoplasm characterized by BCR-ABL1-dependent expansion of myeloid progenitor cells. Using BCR-ABL1-targeting drugs most patients enter long-term disease-free survival. However, not all patients respond to anti-leukemic therapy which may be due to resistance of leukemic stem cells (LSC). So far, little is known about the phenotype of CML LSC.

We applied a molecular screen-approach combining gene chip, qPCR and surface marker studies. We were able to show that in contrast to normal SC, CD34+/CD38− CML LSC specifically express CD25, CD26, CD56, and IL-1RAP. While CD26 and CD56 were identified as rather specific markers of CML LSC, CD25 and IL-1RAP were also identified on LSC in acute myeloid leukemia. Highly purified CML LSC were found to express BCR-ABL1 and to engraft irradiated NSG mice with BCR-ABL1+ cells, whereas CD25−/CD26− SC from the same patients produced BCR/ABL1-negative engraftment. In functional studies we were able to demonstrate that CD25 is a STAT5-dependent regulator of LSC proliferation, and that CD26 (DPPIV) plays a major role in LSC mobilization from the BM SC niche through enzymatic inactivation of stromal cell-derived factor-1. DPPIV-mediated effects on CML cells were counteracted by DPPIV-inhibitors (gliptins). Transfection of either STAT5A or STAT5B resulted in enhanced expression of CD25 in Lin−/Sca-1+/Kit+ cells in C57/Bl6 mice. In line with this observation, a shRNA against STAT5 was found to downregulate CD25 expression in KU812 cells. In addition, the BCR-ABL1-inhibitors nilotinib and ponatinib decreased STAT5-activity and CD25-expression in KU812 cells and primary CML LSC. We also found that CD25-targeting shRNAs augment the proliferative capacity of KU812 cells and primary CML LSC in vitro as well as KU812 cell engraftment in vivo in NSG mice. In contrast, the PI3K/mTOR-blocker BEZ235 was found to promote pSTAT5- and CD25 expression and to produce synergistic anti-neoplastic effects with nilotinib and ponatinib in CML cells. Together, our data show that CD25 and CD26 are novel markers and potential targets in CML LSC.

Whether these targets can be employed to develop targeted treatment approaches aimed at eliminating CML LSC remains to be determined in clinical trials.
Comparison of extracellular vesicles isolated from human tumor cells

Martha Blank et al.

Presenter: Martha Blank

Extracellular vesicles (EVs) are small bodies involved in cell-cell communication. They differ in size and function and are indispensable for basic biological processes. Their content depends on their function and consists of micro-RNA, proteins, and lipids for the most part. As EVs are specific for cells, especially for tumor cells, they provide new approaches for diagnostics and therapies. Matrix vesicles are a subgroup of EVs with a size of 200-1000 nm. These vesicles may play a role in the initiating steps of the mineralization process. Our aim is to investigate the content of EVs from more or less transformed neoplastic cells of bone tissue and the expression of genes involved in the mineralization process.

Non-mineralizing U-2 OS and mineralizing MG-63 osteosarcoma cells were cultured in presence and absence of 5 mM β-glycerophosphate until MG-63 cultures started to mineralize. Whereas vesicles were separated from supernatant by either high-speed centrifugation or an exosome isolation kit, mRNA and proteins were extracted from cells. Subsequently, mRNA was subjected to genome-wide expression analysis (GWEA) by GeneChip (Affymetrix) while proteins were analyzed by immune-blotting. Mineral content of vesicles was addressed by Fourier-transformed infrared spectroscopy and EDX-raster electron microscopy.

GWEA revealed differential expressions of Tetraspanins, such as C9, CD63 and CD81, which are important marker genes of EVs. Furthermore, expression of members of the heat shock 70 kDa protein family (HSPA2, HSPA4L) was higher in the mineralizing MG-63 cell line. In this cell line the expression of PHOSPHO1, ALPL and PLS3 was also increased; these genes are associated with the mineralization process in osteoblasts. Comparison of EVs isolated by different methods revealed that extraction by the exosome isolation kit yielded more EVs, as suggested by the protein assays. Immune blots from EVs confirmed increased expression of C9, CD63 and CD81, as well as of the Hsp 70 heat-shock proteins of EVs isolated from MG-63 cells. From our results, we conclude that both isolation techniques are useful for extraction of EVs. Comparing the two cell lines we found differential expressions of proteins known to be marker of EVs.
Non-viral CRISPR/Cas9-mediated genome editing and rearrangement: A powerful tool for the generation and functional investigation of large acute myeloid leukemia fusion proteins

Fabio Liberante et al.

Presenter: Fabio Liberante

Acute myeloid leukemia (AML) encompasses a wide diversity of clinically and molecularly-defined diseases, which are associated with very poor prognosis. Expression of oncogenic fusion proteins resulting from chromosomal rearrangements often represent driver events in many AML subtypes. Although the most common AML fusion proteins have been extensively characterized, we still lack functional understanding of the many rare fusions, which make up <5% of AML cases in total. Together, these rare fusions affect a significant number of patients (~1,000 new cases every year in the EU) with limited treatment options. The large size of many rare fusion proteins has precluded studies of their molecular function.

In our proposal, we aim to establish in vitro and in vivo models for clinically-relevant, rare AML gene fusions through CRISPR/Cas9-mediated engineering of chromosomal translocations at the genomic level. The use of transient intracellular delivery of ribonucleoprotein (RNP) complexes of Cas9 and crRNAs minimizes off-target effects from constitutive expression of genome editing components. In preliminary experiments using RNPs and plasmids in K 562 cells we have achieved >20% and >80% knock-out of CD81, respectively. Among the specific fusions, including coding length, we will generate are DEK-NUP/ABL214 (~6kbp) and MYST3 and CREBBP fusions (5-10kbp). We will first characterize the transforming potential of these fusion proteins both in vitro and in vivo by targeting primary human cells derived from cord blood CD34+ cells. Intracellular gene editing will be applied to an isogenic cellular background. This will enable us to perform comprehensive comparative epigenetic and expression profiling of the fusions under study. Furthermore, we will simultaneously affinity-tag the created fusion proteins to perform fusion-specific ChIP-Seq and affinity-purification coupled to mass spectrometry to identify interaction partners. Finally, we will employ CRISPR/Cas9-based loss-of-function screens to identify co-targets of the fusion that are synthetically lethal.

Our project will contribute to improved understanding of these rare fusions. The work will thus provide insight into general mechanisms of AML etiology and unearth novel therapeutic strategies for patients.
BTK-inhibition is able to suppress IgE-mediated activation and histamine release in human basophils and mast cells

Dubravka Smiljkovic et al.

Presenter: Dubravka Smiljkovic

Basophils (BA) and mast cells (MC) are major effector cells of anaphylactic reactions in patients suffering from IgE-dependent allergies. Both cells produce a number of biologically active mediators, including histamine, lipid mediators, and cytokines, and both cell types express high-affinity receptors for immunoglobulin E (IgE). Recent data suggest that Bruton’s tyrosine kinase (BTK) is an emerging therapeutic target in IgE receptor (IgER) cross-linked basophils. We examined the effects of four BTK inhibitors, ibrutinib, dasatinib, AVL-292, and CNX-774, on IgE-dependent activation and histamine release in blood basophils obtained from allergic patients (n=8) and non-allergic donors (n=5).

In addition, we examined the effects of these drugs on growth of various human basophil and mast cell lines, including KU812, HMC-1, and the IgER+ cell line ROSAKIT WT. Results: All four BTK blockers were found to inhibit anti-IgE-induced or allergen-induced secretion of histamine in basophils in a dose-dependent manner, with IC50 values ranging between 0.05 and 0.1 µM, and the following rank-order of potency: dasatinib>ibrutinib>AVL-292>CNX-774. Dasatinib and ibrutinib were also found to counteract anti-IgE-induced and allergen-induced upregulation of CD13, CD63, CD164, and CD203c on basophils, whereas AVL-292 and CNX-774 showed no significant effects in these experiments. We also examined whether BTK inhibition is associated with reduced growth of human basophils (KU812 cells) or mast cells (HMC-1 and ROSAKIT WT). Whereas dasatinib and CNX-774 were found to inhibit growth in all three cell lines examined, no substantial effects were obtained with pharmacologically relevant concentrations of ibrutinib or AVL-292, although ibrutinib suppressed BTK activation and IgE-mediated histamine release in ROSAKIT WT cells (IC50: 0.5-1.0 µM).

Together, BTK-targeting drugs are potent inhibitors of IgE-dependent activation of basophils and mast cells. The clinical value of BTK inhibition remains to be determined in clinical trials.
Prostate cancer (PCa) is the most frequent cancer diagnosed in men in the western world. PCa growth is highly dependent on androgens, therefore androgen ablation therapy through chemical castration using an androgen receptor (AR) blocker is a cornerstone of current therapeutic approaches. γ-Crystalline (CRYM) is the main component of the kangaroo’s eye lens. It binds thyroid hormone (T3) in a NADPH-dependent manner therefore sequestering it from being transcriptionally active in the nucleus. The role of T3 binding CRYM in prostate cancer is largely unexplored. This study identifies low CRYM as a negative prognostic factor in PCa using IHC data for Kaplan-Meier analysis. In PCa CRYM expression was reduced, an effect that is further pronounced in metastases. In contrast, thyroid hormone receptor β (TRβ) showed high expression in PCa that is again increased in metastases. Overexpression of CRYM in PCa cell lines led to increased uptake of T3 and reduced invasive capacity. Genome wide RNA-Seq analysis reveals androgen receptor and dihydrotestosterone induced genes to be specifically suppressed by CRYM suggesting it as an hormone antagonist in PCa. Moreover, high CRYM expression leads to deregulated lipid metabolism.

Finally, using NMR and mass spectroscopy based metabolome analysis we see that high CRYM expression is able to mask T3 effects in the metastatic PC3 PCa cell line but not in normal prostate tissue represented by the RWPE-1 cell line. This study identifies CRYM as a key antagonist to T3 and androgen signaling in PCa.
Glibenclamide: an old anti-diabetic drug with anti-cancer activities?

Silvia Loebsch et al.

Presenter: Silvia Loebsch

Introduction: Glibenclamide is a sulfonylurea drug used in the treatment of type 2 diabetes. It acts through sulfonylurea receptors (SURs) on pancreatic cells. SURs are subunits of ATP-sensitive potassium channels (KATP channels), which are inhibited by glibenclamide. Over the last decade, increasing evidence has supported a regulatory function of KATP channels in cancer cell proliferation and survival. In the present study, we investigated possible cytotoxic and pro-apoptotic effects of glibenclamide on two human cancer cell lines.

Materials and Methods: Jurkat leukemia CD4+ T cells and the colon cancer cell line HCT-116 were treated for 48 h with different concentrations (12.5 – 200 µM) of glibenclamide. Cell viability and cytotoxicity were measured with MultiTox-Fluor Multiplex Cytotoxicity Assay (Promega, Madison, WI, USA). Ongoing apoptotic processes were monitored by Annexin-V/7-AAD staining. Expression and cleavage of specific apoptotic marker proteins (caspase-3/-7/PARP/Bax) were analyzed by Western blotting.

Results: Glibenclamide reduced the viability of both cancer cell lines in a dose-dependent manner. It reduced phosphorylation of Akt1, a kinase which plays a key role in proliferation and survival of many cancer cell lines. Furthermore, glibenclamide induced apoptotic cell death via the caspase-3/PARP signaling pathway. Interestingly, in the human colon cancer cell line HCT-116 higher concentrations of glibenclamide (200 µM) were necessary to induce apoptosis compared to Jurkat T cells (50 µM).

Conclusion: Data demonstrate that the “old” anti-diabetic drug glibenclamide triggered apoptotic cell death in two different human cancer cell lines via blocking KATP channels.
Effects of Histamine Receptor 1 antagonists on growth and survival of canine neoplastic mast cells

Susanne Gamperl et al.

Presenter: Susanne Gamperl

Advanced canine mastocytoma are characterized by uncontrolled growth of neoplastic mast cells (MC), mediator-related symptoms, and poor prognosis. Drugs inhibiting the histamine receptor 1 (HR1) are frequently used to treat mediator-related symptoms in these dogs. Recent studies showed that certain HR1-antagonists such as loratadine and terfenadine are able to inhibit growth of neoplastic MC.

We asked whether additional HR1-antagonists commonly used in veterinary clinics, exert effects on viability of MC and on the release of histamine. In our experiments we used six HR1-antagonists (rupatadine, diphenhydramine, cyproheptadine, dimetindene, desloratadine, and loratadine) and two canine MC lines, NI-1 and C2. Drug effects on proliferation were examined by measuring 3H-thymidine-uptake and apoptosis was analyzed by morphologic examination and TUNEL-assay. In addition, drug effects on IgE-dependent histamine release in NI-1 cells were determined. Several HR1-antagonists were found to decrease proliferation of C2 and NI-1 cells after 48 hours, with following IC50 values (µM): loratadine (5-10), rupatadine (10-20), desloratadine (10-50), and cyproheptadine (10-35) (p<0.05). Furthermore, the combination of midostaurin (PKC412) and loratadine showed additive anti-proliferative effects on C2 and NI-1 cells. Reduced proliferation was associated with apoptosis-induction, with following ED50 values (µM): loratadine (15-25), rupatadine (25-45), desloratadine (25-50), and cyproheptadine (35-50) (p<0.05). Dimetindene and diphenhydramine showed no significant effects on growth and viability in both MC lines. Higher concentrations (50-100 µM) of rupatadine, loratadine, and desloratadine were also found to inhibit histamine release in NI-1 cells.

Together, our data show that several HR1-antagonists exert anti-proliferative and apoptosis-inducing effects in canine neoplastic MC. In addition, at higher concentrations, some drugs may also counteract MC activation. The clinical relevance of these observations remains to be determined.
Red blood cells (RBCs) are the carriers of oxygen throughout the body. Therefore it is necessary that RBCs are highly flexible in order to be able to passage through narrow capillaries and small vessels. The RBC flexibility is controlled by its membrane. In certain genetic or acquired pathologic conditions the morphology and mechanical features of RBCs can be critically affected.

In this study single cell force spectroscopy (SCFS) was used to characterize the local mechanical properties “elasticity” (stiffness as apparent Young’s modulus Ea) and “adhesion” of adult human, equine, camel and poultry RBCs as well as of fetal human RBCs. Different influences such as medium (0.9% NaCl, autologous plasma) and temperature (room temperature, 32°C, species’ native body temperature) were observed on a single-cell level. In a physiologic milieu the results strongly differed from measurements under aberrant conditions.

A low stress Si3N4 cantilever with a spring constant of 0.085 N/m and a non-coated 8 nm tip was used to physically indent the RBCs up to a given setpoint of 1 nN. The obtained force-distance data were processed employing the Hertz model at 500nm indentation depth.

Throughout every species Ea decreased with increasing temperature. In saline, equine and camel RBCs showed the highest Ea followed by human fetal and adult RBCs. In plasma Ea was lower compared to saline. Human fetal RBCs also showed higher Ea in comparison to human adult. In chicken the results depended on whether the nucleus or the peripheral region was indented.

Adhesion of the RBCs could only be observed in NaCl and was highest in horse followed by camel, human fetus and human adult RBCs. There was almost no adhesion visible in chicken RBCs. Temperature affected adhesion without any discernible pattern. In all species adhesion was highest at the native body temperature except for chicken. In plasma, adhesion was not present.

Temperature and medium play an important role for stiffness and adhesion of RBCs. Temperature increased the deformability in each species, regardless of size and shape of the RBC. However, medium has a greater effect than temperature: suspension of RBCs in plasma contributed to deformability by a median factor 2.4 compared to measurements in NaCl at native body temperature.
**Differential expression of the plasminogen receptor Plg-RKT in monocyte and macrophage subsets - possible functional consequences in atherogenesis**

Barbara Thaler et al.

Presenter: Barbara Thaler

Human monocytes can be divided into a classical (CM, CD14++CD16-), a non-classical (NCM, CD14+CD16++), and an intermediate subset (IM, CD14++CD16+). CM are mainly phagocytes, whereas NCM and IM are pro-inflammatory. Similar to monocytes, macrophages also exhibit distinct heterogeneity. M1 macrophages promote inflammation and might be involved in plaque vulnerability. M2 macrophages are anti-inflammatory and linked to plaque stabilization. The plasminogen receptor Plg-RKT might contribute to plaque rupture as it is used together with the receptor of the urokinase plasminogen activator uPAR by cells to activate plasminogen to plasmin which is used to degrade extracellular matrix. Here we aimed to analyse the expression of Plg-RKT on monocyte and macrophage subsets.

PMBCs were isolated from whole blood samples of healthy donors and were stained with fluorochrome-labelled antibodies against CD14, CD16, CD45 and Plg-RKT and uPAR and were analyzed with a flow cytometer. Cells were also incubated with FITC labelled plasminogen and stained and measured as described before. The same experiments were performed with murine blood samples. However, to identify mouse monocyte subsets, CD11b and Ly6-C antibodies were used. Plg-RKT levels were also measured on macrophage subsets via flow cytometry.

IM express the highest levels of Plg-RKT compared to CM (p<0.0005) and NCM (p<0.005). In addition, IM also bind the highest amounts of plasminogen indicating that they have a higher plasminogen activation capacity in comparison to CM and NCM. IM, in addition, have the highest amounts of uPAR compared to CM (p<0.05) and NCM (p<0.05). Interestingly, there seems to be a gender dependent difference in Plg-RKT levels with cells isolated from female donors having higher levels of Plg-RKT as compared to male cells. Ly6-C high expressing mouse monocytes are also able to bind higher amounts of plasminogen in comparison to Ly6-C low expressing monocytes (p< 0.05). M1 macrophages express significantly more Plg-RKT compared to M0 (p<0.00005) and M2 (p<0.005).

Based on our data one might speculate that besides their inflammatory capacity IM as well as M1 macrophages might also be involved in processes requiring matrix degradation such as plaque destabilization in atherosclerosis.
Gender difference in the mouse model of systemic sclerosis

Valentina Biasin et al.

Presenter: Valentina Biasin

Introduction: Systemic sclerosis is a multifactorial disease which shows higher prevalence in female. Lung involvement in systemic sclerosis is characterized by inflammation, fibrosis and development of pulmonary hypertension. Fra-2 over-expressing (fra-2 tg) mice have been described as a model for systemic sclerosis as they show several features of the disease, such as lung inflammation, fibrosis and increase right ventricular systolic pressure (RVSP) accompanied by vascular remodelling. In this study we hypothesized that gender differences can influence progression and disease severity between fra-2 tg males and female.

Methods and Results: Analysis of the cell count in the bronchial alveolar lavage (BAL) and histological assessment revealed that female have higher number of inflammatory cells in comparison to male fra-2 tg mice. The RVSP did not show any difference between female and male fra-2 tg mice however fra-2 tg female mice had a worse lung function parameter (static compliance, Cst), higher amount of fibrosis, tissue density and inflammatory infiltrates than male fra-2 tg mice. The protein expression level of fra-2 was not different between female and male fra-2 tg mice, suggesting that fra-2 protein level is not responsible for the different phenotype.

Conclusion: These results suggest that fra-2 together with sex hormones could influence the phenotype of fra-2 tg female mice. Therefore, understanding of how gender differences influence the molecular mechanism underlying systemic sclerosis can open new avenues for targeted therapies.
Nitric oxide encapsulated poly(ε-caprolactone) small diameter vascular graft

Marjan Enayati et al.

Despite of the intensive research in vascular graft development, an efficient small diameter graft is still unavailable in clinics. This study sought to fabricate nitric oxide loaded (NO) poly (ε-caprolactone) (PCL) vascular grafts via electrospinning technique. NO has crucial roles in cardiovascular system such as: maintaining patency, inhibiting platelet activation, decreasing contractility/heart rate and inhibiting neointimal hyperplasia or vascular smooth muscle cell proliferation/migration at the site of arterial injury. In this study, PCL grafts were fabricated via electrospinning technique using different polymer concentrations (8%, 15%, 22% (w/v)) with and without NO drug. The structural and mechanical characteristics of the grafts were compared via scanning electron microscopy (SEM) and an uniaxial BOSE ElectroForce LM1 testbench system, respectively. The effects of nitric oxide on endothelialization, smooth muscle cells proliferations were assessed via XTT assay using primary HUVECs and human SMCs. The role of NO on expression of adhesion molecules (ICAM-1, VCAM-1), tissue factor (TF) in human endothelial cells were studied.

Furthermore, the biocompatibility of the graft and the role of NO on expression pro- and anti-inflammatory cytokines (IL1α, TNFα, IL10) and M1/M2 macrophage markers (CCR7, CD80, CD163) in human macrophages were studied via PCR. In this study conduits composed of 8% PCL showed the highest NO encapsulation in the PCL grafts. This graft had superior morphological, mechanical compared to 15% and 22% PCL Grafts. NO loaded graft significantly enhanced the endothelialization while preventing the smooth muscle cells proliferation. NO attenuated the expression of ICAM-1, VCAM-1 and TF. Furthermore it up-regulated the anti-inflammatory cytokines (IL10) and M2 macrophage marker (CD163). After a short up-regulation phase of pro-inflammatory genes (CD80, IL1α, TNFα), these genes down-regulated significantly and this was more prominent in NO encapsulated PCL grafts. This study showed NO encapsulated graft supported the endothelialization while inhibiting the SMCs proliferation. They regulated the inflammation and prevented the focal adhesion and accumulation of leukocyte via the inhabitation of ICAM-1 and VCAM-1 expression in endothelial.
Isolation and Characterization of Endothelial Extracellular Vesicles from Cell Culture Supernatants

Carina Hromada et al.

Presenter: Carina Hromada

Once considered as cell debris with no biological function, extracellular vesicles (EVs) that include microparticles and exosomes have recently aroused great interest in the scientific community due to their importance in intercellular communication in both physiological and pathological conditions such as tissue regeneration, cardiovascular diseases and immunity. Here, we aimed to establish a differential centrifugation protocol to separate microparticles and exosomes, and showed that the use of different rotors and centrifugation parameters strongly influences particle yield as assessed by flow cytometry and nanoparticle tracking analysis, thereby highlighting the importance of standardized isolation and characterization protocols to assure reproducibility and study-to-study comparability in the promising and rapidly growing field of EV research.

Moreover, a potential influence of extracellular vesicles on the formation of tube-like structures during co-culture of endothelial cells with adipose-derived stem cells (ASCs) was investigated, and evaluation of changes in the particle profiles revealed a decline in endothelial microparticles over time, the formation of which was presumably hampered by the release of certain factors from ASCs. Since the exact mechanisms of EV-mediated cell-to-cell communication still remain to be investigated, a deeper understanding of the complex and ambivalent, but highly interesting role of these vesicles in the next few years is warranted, which will allow the exploration of the numerous possible clinical applications of EVs.
In-Vitro Measurement of Forces acting on Transvalvular Aortic Cannulas

Martin Stoiber et al.

Presenter: Martin Stoiber

Aim: In transvalvular positioned cardiac support devices the cannula-leaflet interaction is of special interest and importance. Valve damage, backflow and thrombus formation might be improved if the cannula is kept in a central position within the valve orifice.

In a pulsatile in-vitro setup, forces acting on transvalvular cannulas were identified and the influence of cannula diameter and transvalvular pressure were investigated.

Methods: Radial and tangential forces acting on transvalvular cannulas were measured in a pulsatile setup. Fresh native porcine, bioprosthetic and artificial pericardial tissue valves were mounted in a test rig. The cannula position was deflected from a central position to the wall in 10° rotational step for the whole circular range. Further the cannula diameter (4, 6, 8 mm) and transvalvular pressure (40 - 100 mmHg) were varied.

Results: Centering forces of the aortic cusps in the direction of the coaptation point were identified. At the mid of the leaflets and at the largest deflection the forces were highest (up to 0.8 N). In the commissures lower forces (up to 0.2 N) were measured. In symmetric valves with equal cusp sizes (pericardial tissue valve) the position of the commissures and cusps were clearly pronounced by the force distribution. Natural variations in the valve leaflets affected these distributions but lowest forces were always found in the commissures. A change in cannula diameter had only a minor influence. However rising transvalvular pressure linearly increased the forces, but did not alter the distribution patterns.

Conclusion: Centering forces that act on transvalvular cannulas were identified in an in-vitro setup for several valves and valve types. Lowest centering forces were found in the commissures and highest forces were found directly at the cusps. At low pressures low centering forces and an increased cannula movement can be expected.
Small diameter vascular grafts with adjustable mechanical properties

Christian Grasl et al.

Presenter: Christian Grasl

Aim: The need for surgical revascularization therapies in small diameter applications is constantly increasing. Autologous vessels are gold standard but not always available. Vascular grafts which are comparable with those are essential. Electrospinning offers fabrication of fibrous scaffolds imitating the extracellular matrix. During conventional electrospinning fibers are deposited in a chaotic fashion due to various instabilities. Increased control of fiber deposition is essential to manufacture grafts which can mimic the complex layered structure and the biomechanical behavior of the host vessel.

Methods: Polymeric tubular vascular grafts were electrospun from Pellethane® 2363 80A on metal mandrels with a diameter of 2mm. Orientation and fiber alignment was controlled by auxiliary plate-like electrodes using electrodynamic deflection of the electrospinning jet. Prostheses with random, circumferential, longitudinal and 30° fiber direction were fabricated. Grafts were characterized by measuring the wall thickness and gravimetric porosity. Effects of fiber orientation were analyzed in the scanning electron microscope and by measuring the compliance in the physiologic blood pressure range.

Results: The electrospun vascular grafts had a mean wall thickness of 71 ± 6µm. Lowest porosity of 63% was seen in circumferentially electrospun grafts whereas grafts spun with fiber directions in ± 30° showed the highest porosity of 79%. Fiber alignment in the main direction of each selected orientation angle was observed. The prostheses with longitudinal fiber orientation showed a compliance of 18.6 ± 2.8 %/100mmHg, whereas the prostheses with circumferential orientation exhibited the lowest compliance of 7.1 ± 2.6 %/100mmHg.

Conclusion: The developed electrodynamic control method allows to electrospin small diameter vascular prostheses with pre-defined fiber orientations.
The aim of this study is to improve certain risk factors of patients listed for a cardiac transplantation. The treatment group will be compared to an equally sized control group. We will monitor parameters like walking distance, weight, Lean BMI, total body fat, NT pro BNP, HbA1c, ejection fraction (EF), hyperlipidemia, arterial hypertension and physical improvements - done by a special work out training and dietary change. We will also observe the number of days at intensive care unit and survival after transplantation.

The study will be performed as a prospective randomized study. Thirty patients, who are listed for heart transplantation, will be included. All data will be collected and inserted into a data bank. In this study we will use descriptive statistic to show our results. We will divide the cohort in two groups, group 1 will do physical examinations and have diet and group 2 will be used as control. The preoperative risk factors will be compared. Not included will be patients with high urgent status and/or BMI under 25, patients with NYHA IV and/or Angina pectoris and children (<18 years).

The study protocol will be submitted and approved to the Ethics Committee of the Medical University of Vienna / General Hospital Vienna.
**Pulmonary Hypertension in Hypersensitivity Pneumonitis**

Adrienn Tornyos et al.

Presenter: Adrienn Tornyos

Background: Hypersensitivity Pneumonitis (HP) is an interstitial lung disease which may lead to irreversible lung fibrosis and pulmonary hypertension (PH). According to recent data, PH may be a complication of HP but its exact prevalence and prognostic role is not fully explored.

Aims: We aimed to assess the prevalence and clinical relevance of increased pulmonary arterial pressure (PAP) and right heart strain (RHS) in HP patients.

Methods: HP patients undergoing rehabilitation between December 1997 and June 2015 at the Rehabilitation Clinic Bad Gleichenberg were enrolled in this single centre retrospective study. Demographic data, comorbidities and therapy as well as echocardiography data (systolic PAP (SPAP) and signs of RHS) and electrocardiography (ECG) data (right axis deviation (RAD)) were evaluated. The primary endpoint of the study was all-cause mortality. The impacts of baseline characteristics on event-free survival were analysed by using Kaplan- Meier and Cox regression analysis.

Results: 423 patients (283 male, 140 female) were enrolled in this study (mean age: 53.4±10.1). During the follow-up period (median 7.0 years) 5.2 % of the patients died. 165 patients underwent echocardiography and SPAP was assessed in 84 cases. Mean SPAP was 42.1±12.7 mmHg and 50 patients had SPAP>35 mmHg. 94 patients had evidence of RHS by echocardiography. Patients presenting with both SPAP>35 mmHg and RHS (n=42) had worse survival (p=0.02; 5 years: 91% vs. 96%, 15 years: 65% vs. 88%). In patients with ECG (n=419), RAD was present in 32 (7.6%) subjects, which was strongly associated with worse survival (p<0.001; 5 years: 81% vs. 98%, 15 years: 70% vs. 94%).

Conclusion: Based on our retrospective analysis, elevated PAP values and RHS is present in a considerable proportion of HP patients and they are associated with a poor prognosis. Further investigation of the pulmonary hemodynamic in patients with HP is warranted.
Diffusion capacity for nitric oxide in pulmonary hypertension and lung diseases

Balazs Odler et al.

Presenter: Balazs Odler

Background: Diffusion capacity of the lung for carbon monoxide (DLCO) is widely used in the clinical practice. Nitric oxide has much higher affinity for hemoglobin, than CO and the diffusion capacity of the lung for nitric oxide (DLNO) is less influenced by changes in capillary blood volume and may better represent the true membrane diffusing capacity. However, its potential role in the differential diagnosis of pulmonary hypertension (PH) and other pulmonary diseases as well as its relationship with prognostic parameters such as six minute walking distance (6MWD) and hemodynamic variables is unknown.

Methods: Combined DLCO and DLNO, including capillary blood volume (Vc) and alveolar membrane diffusion (Dm) were measured in n=47 patients with PH (idiopathic pulmonary arterial hypertension (IPAH): n=20; chronic thromboembolic pulmonary hypertension (CTEPH): n=27, n=31 patients with chronic obstructive pulmonary disease (COPD), n=38 patients with interstitial lung disease (ILD) and n=20 healthy controls. In addition n=58 patients underwent right heart catheterization (RHC) and DLNO measurement during the study period. Comparisons between groups were evaluated using one-way ANOVA followed by Bonferonni post hoc comparison.

Results: DLNO was significantly lower in all patient groups as compared to healthy controls (control: 34.4±7.3 vs IPAH: 18.2±6.3 vs CTEPH: 19.6±5.8 vs COPD: 17.8±5.6 vs ILD: 16.2±6.7 ml/min/mmHg, p<0.01; respectively). DLNO was 4 to 5 times larger as DLCO in all patient groups and the DLNO/DLCO ratio differed significantly between all patient groups except between IPAH and CTEPH. DLNO was correlated with pulmonary vascular and total pulmonary resistance (r=-0.307 and r=-0.307; p=0.02, respectively) and 6MWD (r=0.71; p<0.01). While DLCO was not significantly correlated with these parameters.

Conclusions: DLNO is decreased in patients with IPAH, CTEPH, COPD and ILD. DLNO may represent a novel marker for pulmonary hemodynamics and exercise capacity.
Piloting patient involvement strategies in European HTA: a focus group approach with cardiac patients

Sabine Ettinger et al.

Presenter: Sabine Ettinger

Background: The involvement of patients in defining the scope of health technology assessments (HTA) is widely recognized as a valuable strategy to ensure that patient-relevant outcomes are considered by researchers. Previous experiences in transnational European HTA showed a need for improved patient involvement methods and processes.

The aim of this project was to pilot the focus group approach with cardiac patients in a European rapid assessment on wearable cardioverter defibrillator (WCD) therapy to explore the potential of this methodology for eliciting the patients’ views on aspects regarding their disease and the WCD therapy, and for identifying neglected outcomes.

Methods: An e-mail was sent to members of the 9 regional associations of the Austrian organization for heart and lung transplant patients to identify eligible participants for the focus group. Guiding questions for discussion were developed based upon a hand search of patient involvement initiatives’ websites and review of appropriate literature. The 4-hour meeting was moderated by a patient support expert and recorded upon approval of participants. The anonymised transcript was analysed using framework analysis.

Results: 10 eligible patients responded of which 5 men, aged between 55 and 73 years (mean 65), from Austria and Germany were able to participate. All respondents experienced heart transplantation, 4 had received an implantable cardioverter defibrillator (ICD) before.

Participants reported that experiencing a sense of security was crucial to them and that they expected to do sports and live a life with few limitations despite receiving a therapy. WCD therapy was not considered a long-term option due to expected restrictions in living a ‘normal’ and secured life.

Challenges included the identification of participants who are representative for this patient group and the complexity of patient histories.

Conclusion: The focus group approach proved useful in scoping the assessment on WCD therapy. Gathered results informed the inclusion of outcome measures relevant to the target group and revealed patients’ views on health-related quality of life. Lessons learned from this pilot project guide us in further improving patient involvement processes for European assessments within the EUnetHTA project.
Increased expression of RGS5 in pulmonary vascular disease

Neha Sharma et al.

Presenter: Neha Sharma

Idiopathic pulmonary fibrosis (IPF) is progressive lung diseases with unknown cause and poor prognosis. Currently, no effective therapy is available to treat IPF, except for Pirfenidone and Nintendanib, which have only limited clinical benefits. The presence of a pulmonary vascular remodelling or a manifest pulmonary hypertension (PH) in IPF significantly reduces the survival of the patients. Although much is known about the pathways that promote proliferative and fibrotic responses, mechanisms that antagonize these pathways have not been as clearly defined. The discovery and functional clarification of reverse remodelling targets are equally important for understanding the molecular mechanisms underlying vascular remodelling in lung fibrosis.

We focus on the role of the endothelial cells and the endothelial dysfunction for the development of pulmonary vascular remodelling in IPF. In this setting we are especially interested in the regulators of G protein couple receptor family (RGS), since RGS5 was shown to be relevant for both the systemic blood pressure regulation as well as for the development of cardiac fibrosis and systemic sclerosis.

Our data revealed a significant higher expression of RGS5 in lung samples obtained from patients suffering from IPF compared to healthy control lungs. Furthermore, analysing the compartmental expression using laser-assisted micro-dissected pulmonary arteries from both IPF and donor lungs showed the upregulation of RGS5 in remodelled pulmonary arteries in IPF. Pro-fibrotic treatment of different lung structural cells with transforming growth factor beta (TGFβ) led to significant upregulation of RGS5 only in epithelial cells. Consistent to our human data, with the progression of the disease, RGS5 was significantly higher expressed in the bleomycin-induced mouse model of IPF compared to control animals.

Our preliminary data show the regulation of RGS5 in PH associated with IPF in human samples as well as in the animal model of the disease. Further experiments are required to prove the implications of RGS5 for the development of the pulmonary vascular remodelling in IPF.
Evaluation of Periodic Pump Speed Variations in Ventricular Assist Device Performance by Using Numerical Simulation

Mojgan Ghodrati et al.

Presenter: Mojgan Ghodrati

Blood stasis assessment is one of the critical issues in evaluating the performance of a Left Ventricle Assisted Device (LVAD), which might lead to blood thrombus formation in left ventricle. For decreasing the area with blood stasis, the flow field in left ventricle has been changed by applying a periodic velocity variation in speed, the so-called the Lavare cycle, of a centrifugal LVAD (HeartWare HVAD). The aim of this study is to distinguish mentioned areas by using Computational Fluid Dynamics (CFD) simulation. Finally the results of the CFD simulation were compared to the particle image velocimetry (PIV) measurement data.

The flow field was simulated with ANSYS software which is based on incompressible finite-volume solver. To evaluate the efficacy of Lavare cycle in reducing areas of potential blood stasis within the left ventricle, we performed CFD simulation for both Lavare cycle off (LOFF) and Lavare cycle on (LON). This simulation was accomplished by applying the actual rotation to the rotor domain utilizing the Sliding Mesh approach. The constant rotational speed for LOFF and transitional rotational speed base on Lavare cycle for LON was exerted to the rotor. We are due to calculate the stagnation index in small local areas e.g. near the ventricular apex and area around inflow cannula which include critical information.

The local mean velocities in the simulated flow field for both cases LOFF and LON were calculated. For the quantitative analysis, to recognize the area with blood stasis in left ventricle and evaluate the effect of Lavare cycle for reducing blood stasis area, the stagnation index was calculated in both LOFF and LON pump performance. With CFD simulation we captured more precise details than PIV measurement. With the LON, there was notable decrease in the stagnation index compared to LOFF.

The results showed a high correlation between the simulated flow field and PIV measurements in both LOFF and LON. With the LON both CFD simulation and PIV measurement demonstrated considerable stagnation index decrease within the ventricle. High correlation between the results of CFD and PIV shall allow the combined use of both methods for research questions both in large scale (easier to be measured) and small local areas (easier to be simulated).
Background: Idiopathic pulmonary fibrosis (IPF) is a progressive disease with bad prognosis. IPF is characterized by lung remodeling including excessive extracellular matrix deposition. Upregulation of cartilage oligomeric matrix protein (COMP) has been observed in IPF lungs. However, the role of COMP in pathophysiology of IPF is unclear. The aim of the study was to reveal molecular mechanisms governing COMP contribution to the disease.

Methods: Lung tissue samples from the explants of the IPF diagnosed patients and unused donor lungs were analyzed by real-time-PCR, western blot (WB) and immunohistochemistry (IHC). Human parenchymal fibroblasts (hPF) were isolated from the donor lung samples. Circulating COMP levels were measured by ELISA kit. Lung fibrosis was induced by intratracheal instillation of bleomycin in the wild type and COMP KO mice. Two weeks after instillation lung function measurements were performed followed by lung sample collection for molecular analysis.

Results: COMP upregulation in the IPF lung samples was detected by real-time-PCR and WB. IHC analysis revealed COMP deposition in the fibrotic regions of IPF lungs. Circulating COMP level was decreased in IPF patients. Bleomycin caused lung fibrosis development in the mouse lungs and was associated with more pronounced lung function deterioration in COMP KO mice. Stimulation of hPFs with TGFβ but not with PDGF-BB induced expression of COMP. Stimulation of the cells with COMP decreased basal as well as PDGF induced proliferation rate of hPF. hPF stimulation with COMP did not influence expression of TGFβ target genes PAI-1 and collagen 1a1.

Conclusion: COMP is abundantly present in fibrotically changed lungs and is regulated by TGFβ. Our results cumulatively showed that COMP is a molecule linking TGFβ and PDGF signaling by inhibiting PDGF induced cell proliferation.
The regulatory role of Tenascin C on matrix metalloproteases expressions induced by hypoxia and reoxygenation in H9C2 cardiomyocytes cell line

Inês Fonseca Gonçalves et al.

Presenter: Inês Fonseca Gonçalves

Background: There is substantial evidence that the upregulation of Tenascin C (TNC) expression play a role in the maladaptive signalling cascade involving left ventricle remodelling following myocardial infarction and hypertension. Of importance, increase in TNC expression is associated with Matrix Metalloproteases (MMPs; 2 and 9 MMP2 and MMP9) upregulation in left ventricle tissue samples. However, the effect of hypoxia and reoxygenation on TNC expression as well as TNC on MMPs formation in cardiomyocytes has not been known.

Aim: This study aims to evaluate the effect of hypoxia and reoxygenation on the expression of TNC, MMP2 and MMP9 as well as whether TNC influences MMPs formation in a rat cardiomyocyte cell line.

Methods: H9C2 rat cardiomyocytes cell line were submitted to 6, 16 and 24 hours of hypoxia in a 95% N2 and 5% CO2 atmosphere, with and without 60 minutes of reoxygenation. Additionally, TNC was added to the same cell line under normoxic conditions in four different concentrations, 1, 3, 5 and 10 µg/mL, for 6 and 24 hours. The mRNA expression of TNC, MMP2 as well as MMP9 expression were determined by RT-qPCR and normalised to β-actin as housekeeping gene.

Results: Under normoxic conditions TNC expression was not detectable. In contrast, hypoxia significantly induced TNC expression in all time points (P<0.05, respectively). The formation of MMP2 and MMP9 were increased by hypoxia. MMP2 expression maximum was obtained after 6 hours of hypoxia and 60 minutes reoxygenation (2.1 fold-change); MMP9 upregulation reached the highest levels following 24 hrs hypoxia (15.8 fold-change). The expression of MMP2 and MMP 9 were markedly increased by the administration of human TNC following 24 hrs (1.5 and 2.8 fold change, respectively).

Conclusion: This study first time demonstrated that hypoxia and reoxygenation markedly increased the expression of TNC, MMP2 and MMP9 in rat cardiomyocytes. Of importance, TNC has a significance effect on MMP2 and MMP9 upregulation. These results might explain the pathological importance of TNC on MMPs following myocardial infarction and hypertension. In addition, the present models may allow to test novel compounds effecting TNC and MMPs expression in cardiomyocytes.
Influence of Tenascin C on Cardiac Reverse-Remodeling - an Aortic Banding - Debanding Model

Philipp Kaiser et al.

Presenter: Philipp Kaiser

Background: Left ventricular hypertrophy is an adaptive mechanism of the heart to those cardiovascular diseases associated with an increased pressure load.

After treatment of these diseases, especially after aortic valve replacement, the pressure load decreases and the heart can regenerate. This regression of the hypertrophy is called reverse-remodeling. The main goal of the project is to evaluate the influence of Tenascin C (Tn-C) on this cardiac reverse-remodeling.

Tn-C is a glycoprotein of the extracellular matrix, which is expressed during embryogenesis, tumorigenesis and in remodeling.

Aim: The aim of the study is to investigate the influence of Tn-C on the reverse-remodeling on the basis of an aortic banding-debanding model and comparing the reverse remodeling at AJ Tn-C knock-out mice to their wild-type littermates.

Methods: Left ventricle hypertrophy is induced by transverse aortic constriction for 10 weeks. After 10 weeks, the opening of the stenotic ligature takes place (Debanding), causing the induction of reverse remodeling.

The mice will be pre-treated with isoflurane gas. After intubation the mice will be and ventilated, following a pre-operative analgesia with buprenorphine. Subsequently, a horizontal skin incision will be performed above the sternum. This will then be followed by a minimal hemi sternotomy.

The aorta will be exposed and then embraced after the junction of the brachiocephalic artery using a 6-0 suture (Prolene®, Ethicon). Then, the suture will be constricted over a 27 G needle. After successful ligation sternum will be closed. Postoperative analgesia will be performed with an oral dose of Piritramid through drinking water.

10 weeks after aortic banding the debanding will be performed. The anesthesia and the pre-treatment of mice will be similar to the aortic banding operation. After the incision, the aorta will be dissected again. After the ligature will been shown, the ligature will be cut through.

Results:

First preliminary MRI data shows a reduction of the Ejection Fraction after 10 Weeks and a increase 2 weeks after the Debanding. This leads to the presumption that this model is a suitable way for the investigation of cardiac reverse remodeling.
SMAD3 contributes to lung vascular remodeling in pulmonary arterial hypertension via MRTF disinhibition: a new pathomechanism

Diana Zabini et al.

Presenter: Diana Zabini

Introduction: Pulmonary arterial hypertension (PAH) is a fatal disease characterized by remodeling of pulmonary arteries, smooth muscle cell hyperplasia and hypertrophy. TGF-β, regulating cell proliferation, migration, and cell death, is elevated in PAH, and has been implicated in its pathogenesis based on clinical and experimental data. TGF-β binding to its receptor activates downstream signalling cascades, such as SMAD proteins. Interestingly, however, recent data suggest that SMAD3 is downregulated in PAH. Since Smad3 can suppress proliferation and was shown to inhibit myocardin-related transcription factor (MRTF), a major myogenic inducer, we hypothesized that the loss of Smad3 may contribute to two major features of PAH: proliferation and hypertrophy of pulmonary artery smooth muscle cells (PASMC). To test this hypothesis, we investigated the regulation of SMAD3 and its interaction with MRTF in human PASMC in vitro, and its potential role in PAH in vivo. Results: TGF-β treatment for 72 h caused a significant downregulation of SMAD3 mRNA and protein levels in PASMC. Loss of SMAD3 was also evident in pulmonary arteries from rats with monocrotaline- as well as hypoxia/Sugen-induced PAH.

Silencing of SMAD3 in PASMCs increased the proliferative response upon stimulation with fetal calf serum as determined by western blotting for proliferating cell nuclear antigen protein, Ki-67 positive cells, and bromodeoxyuridine assay. Co-immunoprecipitation revealed a reduced interaction between SMAD3 and MRTF in TGF-β treated PASMCs compared to control cells, indicative of MRTF liberation. A similar decrease in SMAD3-MRTF interaction was observed in PAH animal models in vivo. In PASMC, lack of SMAD3 led to increased smooth muscle actin expression, a hallmark for hypertrophy, which could be reversed by the MRTF inhibitor CCG1423. Conclusion: The present data suggest that SMAD3 downregulation occurs in PASMC both in vitro and in vivo, and this may represent an important novel pathomechanism contributing to increased proliferation and - through MRTF liberation - hypertrophy of PASMC, key features of lung vascular remodeling in PAH.
S-Nitroso Human Serum Albumin dose-dependently leads to vasodilation and alters reactive hyperaemia in coronary arteries of an isolated mouse heart model

Paul Haller et al.

Presenter: Paul Haller

Background: S-NO-HSA has proven its positive effects in ischemia/reperfusion models to preserve endothelial and cardiac function. This study intends to investigate the vasodilatory potency on coronary arteries. Additionally, the effects of S-NO-HSA are investigated after a short period of ischemia that provokes reactive hyperaemia, a phenomenon that could be modulated using S-NO-HSA.

Materials and Methods: Hearts of male OF-1 mice are crystalloid perfused in a Langendorff-heart. After an adapting-period of 15’ and measuring of baseline values administration (drug or control) lasts for 10’, followed by 20’ of haemodynamic measurements. S-NO-HSA is tested during solely Langendorff perfusion (0,5µmol/kg/h, n=10; 5µmol/kg/h, n=3) to evaluate the extent of vasodilation. In the second part, after 5 minutes of drug administration, hearts undergo a 2 minutes period of global ischemia to provoke reactive hyperaemia (RH). Either S-NO-HSA (0,5µmol/kg/h+RH, n=10; 5µmol/kg/h+RH, n=7) or human serum albumin (control: n=5 and n=5) are administrated. Coronary flow (CF) and heart rate (HR) are monitored under constant afterload. Tissue samples for evaluation of high-energy phosphates are taken at the end. Data are presented as mean±SEM compared to baseline (recovery in %).

Results: HR remained stable in all groups and showed no significant changes between groups. 5µmol/kg/h S-NO-HSA treatment increased CFrecovery compared to 0,5µmol/kg/h S-NO-HSA (144,71% vs. 85,86%, p=0,011). Upon reperfusion, there is a trend of reducing RH with 0,5µmol/kg/h S-NO-HSA compared to control (25,94% vs. 74,04%, p=0,076). No significant changes were observed with 5µmol/kg/h S-NO-HSA in RH compared to control. HEP showed no significant changes between groups.

Conclusion: S-NO-HSA is able to dilate coronary arteries dose-dependently and is likely to decrease the extent of reactive hyperaemia provoked with 2’ global cardiac ischemia.
Short- and Long-Term Mortality within different Age Cohorts of Patients with ST-elevation Myocardial Infarction

Paul Haller et al.

Presenter: Paul Haller

Purpose: Our senescent society leads to a growing number of elderly people suffering from ST-elevation myocardial infarction (STEMI). However, this population is often under-represented in randomized trials. Hence, our aim was to investigate the influence of age on patient characteristics, short- and long-term outcome in the Vienna STEMI registry (2003-2009).

Methods: We included patients for which we had data on following characteristics: age, gender, history of hypertension (HTN), hyperlipidaemia (HLP), diabetes mellitus (DM), smoking habit, family history (FH), and previous infarction (pMI), as well as infarct location (anterior-wall vs. non-anterior) and shock. Patients were stratified into age cohorts (≤45, 46-59, 60-79, ≥80 years respectively). Differences between cohorts were calculated with the Linear-by-Linear association test for trend. Mortality was calculated with log rank test and shown with Kaplan-Meier plots. A backward-eliminating Cox-regression model was build up to adjust mortality for risk factors, treating age as a continuous variable.

Results: A total of 2452 patients fulfilled criteria for further investigation. Mean age was 60,7 years, overall 30-day and 3-years all-cause-mortality were 6,3% and 12,6%, respectively. With rising age cohorts, the account of females, DM, HTN, pMI, shock, no reperfusion and anterior-wall infarction increased significantly, in contrast, the account of patients with FH, smoking and HLP declined significantly (p<0,0001 for all, except infarct location p=0,03). Log-rank shows significant differences between age cohorts for short and long-term mortality (p<0,0001 for both). Cox-regression analysis for short-term mortality revealed an independent association for age (p<0,0001), HTN (p=0,001) and shock (p<0,0001). Long-term mortality was independently influenced by age (p<0,0001), smoking (p=0,003), DM (p=0,006), HTN (p=0,009), HLP (p=0,002), pMI (p=0,001) and shock (p<0,0001).

Conclusion: Several risk factors are independently associated with age. Higher age cohorts face higher mortality rates in short- and long-term follow up. The Cox-regression model confirmed an independent association of age with short- and long-term mortality, however, the highest predictor of death in both follow-ups was shock.
Adherence to Current ESC Heart Failure Treatment Guidelines in a Tertiary Referral Centre and University Teaching Hospital in Central Europe

Christina Hauser et al.

Presenter: Christina Hauser

Background: The corner stones of modern heart failure (HF) treatment are an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB), a beta-blocker (BB) and a mineralocorticoid receptor antagonist (MRA). The European Society of Cardiology (ESC) HF treatment algorithm published in 2012 introduced Ivabradine as the next step of pharmacological intervention in patients with systolic HF. The aim of this study was to document guideline adherence - or lack thereof - in terms of optimal medical treatment (OMT) for HF.

Methods: This is a prospective registry of patients admitted for worsening heart failure to our department. We used descriptive statistics to analyse the extent of guideline adherence and logistic regression to calculate possible predictors for receiving OMT.

Results: To this day, 560 consecutive patients have been included in this study, 39% were female. 56% were diagnosed with ischaemic cardiomyopathy (CMP) and 43% with non-ischaemic CMP. At hospital admission 56% of patients were on ACEIs/ARBs, 61% on BBs, 30% on MRAs and 0.4% on Ivabradine. At discharge, 65% of patients were on ACEIs/ARBs, 78% on BBs, 49% on MRAs and 1% on Ivabradine. In addition to the low number of patients on appropriate HF therapy, only 33%, 47% and 13% of patients on ACEIs, ARBs and BBs had reached target doses of their respective medication at admission. There was no difference between patients with first admission for HF and those with recurrent admissions. There was a significant difference between patients with OMT upon admission (18%) and discharge (27%). In univariate analysis, there was a tendency towards male gender for the prediction of OMT compared to female gender. The intra-hospital mortality was 4.2%.

Conclusion: Despite very clear ESC recommendations and a well-documented benefit for HF patients, very few of them are in fact treated accordingly. In comparison to international registry data, the percentage of heart failure patients with OMT was low. At discharge from hospital 10% of patients would have been suitable candidates for Ivabradine therapy. However, only 1% of them was in fact prescribed Ivabradine. Therefore we plan to invite patients who were “undertreated” at discharge to our clinic in order to adapt HF therapy accordingly.
Ezetimibe-Statin Therapy compared to Statin Therapy alone

Barbara Nußbaumer-Streit et al.

Presenter: Barbara Nußbaumer-Streit

Background: Most clinical studies compared ezetimibe-statin combination therapy vs. statin monotherapy focusing entirely on surrogate variables. In this systematic review, we assess the efficacy and safety of ezetimibe-statin combination therapy in comparison to statin monotherapy by focusing on patient-relevant outcomes in hyperlipidemic patients with atherosclerosis and/or diabetes mellitus.

Method: We conducted a systematic literature search, looking for studies published between 1995 to July 2015 in PubMed, the Excerpta Medica Database (EMBASE), the Cochrane Library, and the ClinicalTrials.gov registry.

Results: Nine randomized, controlled trials with data from 19,461 patients were included in our systematic review. Ezetimibe-statin combination therapy was associated with a lower risk of cardiovascular events than statin monotherapy: absolute risk reduction of 2%-points (35% vs. 33%) within 7 years resulting in a number needed to treat of 50 over 7 years (hazard ratio [HR] 0.94, 95% confidence interval [0.89; 0.99]; p = 0.016). Patients suffering from diabetes mellitus benefited the most from combination therapy rather than monotherapy with respect to cardiovascular morbidity (HR 0.87 [0.78; 0.94]). Despite reduced risk of morbidity, the addition of ezetimibe to statin therapy did not reduce either cardiovascular or overall mortality. Serious adverse events occurred in 38% of the patients taking the combination therapy and in 39% of the patients taking a statin alone (relative risk 1.09 [0.77; 1.55]).

Conclusion: In high-risk patients with an acute coronary syndrome, combination therapy with ezetimibe and a statin lowered the risk of cardiovascular events in comparison to statin monotherapy. However, the risk of dying couldn’t be reduced by combination therapy and the risk of suffering an adverse events was similar in the two treatment groups.

The results of this systematic review were recently published in Deutsches Ärzteblatt International:

Continuous monitoring of physical activity and cardiac hemodynamics of patients with a left ventricular assist device implanted

Christoph Gross et al.

Presenter: Christoph Gross

Monitoring of daily physical activity is an emerging tool to assess health status both in normal and pathologic conditions. Such monitoring can be also important in patients with a left ventricular assist device (LVAD) implanted. This study aims at the validation of activity detection based on accelerometer data and at its first application to monitor outpatient hemodynamics combined with patient activity.

A 3-axial accelerometer was embedded in a previously developed recording device for LVAD data, which is placed in the patient’s shoulder-bag. The measured signals (sampled at 10Hz) provided a binary classification of activity/rest based on device position and acceleration magnitude. During ambulatory visits the accelerometer activity was compared to data recorded in a log-sheet including the time course of active and resting periods. Once validated the post-operative course of total daily activity (min/day) was also analyzed.

Preliminary validation of the accelerometer activity and protocolled activities was performed with 30 datasets from 15 patients and resulted in a sensitivity of 96.2±3.8% and a specificity of 93.8±6.0%. An average of 113 days of activity data were recorded in 16 patients within the first 200 days post-implant. At post-operative days 50, 100, 150, 200 average daily physical activity was 60, 77, 88, 65 min/day. Two rehospitalizations in one patient were correlated with a drop in detected activity.

Activity derived from the accelerometer can be useful to examine LVAD therapy. Combined with hemodynamic pump monitoring, it will give a more comprehensive picture of the interaction between LVAD and the remaining cardiac function during daily living.
Effects of purified coagulation factor concentrates on hypoperfusion related endotheliopathy

Nikolaus Hofmann et al.

Presenter: Nikolaus Hofmann

Background: Recent studies indicated that fresh frozen plasma (FFP) transfusion in hemorrhagic shock (HS) mitigates inflammation and preserves endothelial glycocalyx shedding. Treatment of HS related endothelial injury with purified coagulation factor concentrates has not been investigated so far.

Objective: We investigated the effects of different fluids in combination with initial Fibrinogen concentrate (FC) or prothrombin complex concentrate (PCC) on endothelial cell damage (VEGFR1), glycocalyx degradation (Syndecan-1) and sympatho-adrenal activation (Adrenalin) in a rat model of HS.

Methods: Anesthetized Sprague Dawley rats were subjected to a severe model of HS followed by resuscitation. At the end of shock (EOS) animals were randomly divided into 4 groups: PCC+Ringer’s lactate (RL), PCC+Human Albumin (HA), FC+RL and FC+HA. Animals were initially resuscitated with a bolus injection (3.5mL/kg/BW) of FC (70mg/kg/BW) or PCC (25IU/kg/BW). Resuscitation was maintained with the infusion of vehicles as RL (75mL/kg/BW) or HA (15mL/kg/BW) for 60 minutes. The experiment was terminated 60 minutes later at the end of observation (EOO).

Results: Syndecan-1 in FC+HA group (12.1±5.5 ng/mL) was significantly higher compared to FC+RL (8.2±3.4 ng/mL; p<0.05), PCC+RL (5.4±1.2 ng/mL; p<0.001) and PCC+HA (3.4±1.6 ng/ mL; p<0.001, respectively). VEGFR1 was significantly higher in the FC+RL group (19.7±3.3 ng/mL) compared to the PCC+HA group (14.6±5.14 ng/mL; p<0.05). FC+RL caused significantly higher adrenalin concentration than administration of FC+HA (2794±1098 vs 2146±1035 pg/mL; p<0.05)

Conclusion: Our findings suggest that resuscitation with FC in combination with albumin appears to deteriorate degradation of the endothelial glycocalyx and endothelial injury after HS.
Experimental models of endotheliopathy: Impact of shock severity

Nikolaus Hofmann et al.

Presenter: Nikolaus Hofmann

Background: Shock related hypoperfusion results in substantial sympatho-adrenal activation, endothelial injury, organ damage and increased mortality.

Objective: The aim of the current study was to compare moderate versus severe hemorrhagic shock (HS) in regard to the magnitude of symphto-adrenal activation and consecutive endothelial damage.

Methods: Anaesthetized Sprague Dawley rats were subjected to either a fixed-volume hemorrhage model close to 40% of the estimated blood volume (moderate HS: mHS group) or to a severe shock (sHS group) induced by fixed-pressure to meet predefined shock criteria (base deficit (BD) >5.5mmol/L, lactate >2.2mmol/L). Circulating markers of endothelial cell damage (Vascular endothelial growth factor-1; VEGFR1), glycocalyx shedding (Syndecan-1) and symphto-adrenal activation (Adrenaline) were measured at baseline (BL) and at the end of shock (EOS).

Results: At EOS mean arterial pressure was significantly higher in the mHS group compared to the sHS group (36±5.6 vs. 30±3.0 mmHg; p<0.01, respectively). Accordingly, base deficit and lactate were higher in animals subjected to sHS group compared to the mHS group (BD: 9.5 ±2.5 vs. 3.0 ±1.0 mmol/L; p<0.001; lactate: 4.1±1.3 vs 1.6±0.6 mmol/L; p<0.001; respectively). VEGFR1 and syndecan-1 were approximately 50% higher in the sHS group compared to the mHS group (% changes vs BL: 160±10 vs 116±36; p<0.01; 170±37 vs 113±27; p<0.001, respectively). Adrenalin was twofold higher in sHS group compared to the mHS group (1815±1136 vs 855±451; p<0.02, respectively).

Conclusion: Our findings revealed that the intensity of endothelial damage is strongly related to the severity of shock and consecutive symphto-adrenal activation. Thus, experimental models investigating endothelial damage should use BD or lactate as surrogate for substantial hypoperfusion rather than blood pressure.
Angiotensin-II-induced tissue inflammation and fibrosis are distinct from its hemodynamic effects and involve TNFR1 signaling

Sandra Haudek et al.

Presenter: Magdalena Mayr

Brief systemic infusion of Angiotensin-II (Ang-II) in wild-type (WT) mice initiates the development of cardiac fibrosis by regulating a chemokine-dependent uptake of bone marrow cells and induction of proinflammatory and profibrotic factors that drive maturation of myeloid precursors into fibroblasts; this development is absent in TNF receptor-1 (TNFR1) deficient mice (Circulation Heart Failure 2015;8:352).

In this study, we investigated the long-term effects of Ang-II on heart and kidney. In WT hearts, cardiac fibrosis persisted throughout the 6 week Ang-II infusion despite the disappearance of monocytic precursors and decline in inflammation, while TNFR1-KO hearts remained protected. At this time point, WT hearts developed clear evidence of accelerated cardiac hypertrophy and left ventricular remodeling; these changes were less severe in TNFR1-KO hearts. In the kidney, 1 week of Ang-II infusion to WT mice did not evoke a fibrotic response. However, after 6 weeks, WT kidneys displayed minimal, but significant tubulointerstitial collagen deposition. This development was associated with the appearance of myeloid fibroblast precursors, proinflammatory M1 and profibrotic M2 cells, and myofibroblasts, similar to the cell populations observed in the 1-week Ang-II-infused heart. Transcriptional expression of proinflammatory and profibrotic genes was also increased. These changes were not seen in Ang-II-infused TNFR1-KO kidneys. By contrast, both WT and TNFR1-KO mice responded with identical elevations in serum markers for renal failure. Systolic blood pressure increased and cardiac function decreased more slowly in TNFR1-KO than in WT mice, but both were equally abnormal at 6 weeks.

We conclude that Ang-II-infusion induced an immediate fibrotic response in the heart while fibrosis in the kidney developed slowly; both were initiated by chemokine-driven uptake of myeloid fibroblast precursors. TNFR1-KO mice were protected from the Ang-II-induced cardiac and renal fibrosis, despite similar increases in blood pressure and cardiac and renal dysfunction. These results suggest that the fibro-inflammatory effects of Ang-II are distinct from functional consequences in early hemodynamic and renal function (Physiological Reports 2016, e12765, doi:10.14814/phy2.12765).
Acute phase cytokines do not contribute directly to liver damage through mitochondrial reactive oxygen species during systemic inflammation

Andras T. Meszaros et al.

Presenter: Andras T. Meszaros

Introduction: Systemic inflammatory response (SIR) is a defense reaction of the body against pathogens, but it can also cause damage to host tissues. Recently we have shown that this damage can be effectively reduced by inhibition of mitochondrial reactive oxygen species (mtROS) generation. The specific aim of this study was to clarify whether or not acute phase inflammatory cytokines, such as TNF-alpha, directly induce liver damage via mtROS-mediated mechanisms.

Methods: Sprague-Dawley rats were challenged with an intravenous bolus of lipopolysaccharide (LPS, E. coli, 2.5 mg/kg body weight) to elicit SIR. To examine the impact of mtROS, we treated animals with MitoTEMPO (mT), a mitochondria-targeted ROS scavenger. At 2, 4, 8 and 16 h after LPS injection we determined blood cytokine levels and organ damage parameters. In addition, ROS generating capacity of immune cells isolated from different compartments of the body were examined. In an in vitro study hepatocytes were incubated with different patterns of inflammatory mediators to further elucidate effects of acute and later mediators on mtROS production.

Results: LPS challenge resulted in an increase of inflammatory cytokine levels only at early phase (2 and 4 hours) and severe liver damage only in the late phase (16 h) of SIR. Liver damage was successfully prevented by mT treatment. In contrast, early elevation of IL-1 and TNF-α, as well as ROS production of polymorphonuclear (PMN) leukocytes was not affected by mT. To clarify whether acute or late phase cytokines elevate mtROS levels, we incubated blood cells with LPS for 6, 12 and 24 hours to create acute (6h) and late phase (12, 24h) patterns of inflammatory mediators. Incubation of hepatocytes with these cytokine patterns showed that although acute phase cytokines were completely released within the first 6h they did not elevate hepatocyte mtROS levels, while 12 and 24h cytokine patterns substantially elevated mtROS levels.

Conclusion: Acute phase cytokines do not directly induce liver damage, but they are more likely to facilitate the release of late phase damage mediator(s) of liver damage mediated by mtROS. This damage occurs due to intracellular signaling within hepatocytes rather than due to overwhelmed activation of immune cells.
**Importance of kynurenine in pulmonary hypertension**

Bence Nagy et al.

Presenter: Bence Nagy

**Rationale:** Pulmonary hypertension (PH) patients may exhibit changes in tryptophan/kynurenine metabolites that might alter the pulmonary vascular tone and serve as biomarkers of PH.

**Objectives:** To investigate circulating tryptophan and its metabolites in idiopathic pulmonary arterial hypertension (IPAH) and other PH-related diseases, and to explore the effects of the tryptophan-metabolite kynurenine on pulmonary vascular tone.

**Methods:** Tryptophan, kynurenine and kynurenic-acid levels were measured by HPLC in serum from 20 IPAH patients, 20 healthy controls, and 20 patients without PH (10 with chronic lung disease and 10 with metabolic syndrome). Kynurenine effects were investigated on human pulmonary arterial smooth muscle cells (hPASMCs), isolated rat pulmonary arteries, mouse isolated-perfused lungs and in two independent animal models of PH.

**Measurements and Main Results:** In IPAH, kynurenine levels were elevated vs. controls (3.6±0.2 µM vs. 2.6±0.1 µM, p<0.0001) and significantly correlated with mean pulmonary arterial pressure (p: 0.770, p<0.0001). Kynurenine had a strong predictive value for the disease (AUC=0.86). Tryptophan levels were decreased, and kynurenic-acid was unchanged. In hPASMCs, kynurenine significantly increased intracellular cAMP and cGMP, in preconstricted intrapulmonary arteries it caused relaxation, furthermore it decreased pulmonary arterial pressure ex vivo. In the animal models of PH, kynurenine caused a significant acute decrease in right ventricular systolic pressure in vivo.

**Conclusions:** Kynurenine is an intrinsic pulmonary vasodilator, that may ameliorate the pathologic mechanisms involved in pulmonary hypertension. The increased serum levels of this metabolite might indicate elevated pulmonary arterial pressure.
Visualisation of post-mortem vessels in MRI: Development of an imaging approach to improve detection of cardiovascular causes of death

Bridgette Webb et al.

Presenter: Bridgette Webb

Sudden cardiac death (SCD) is the leading cause of death in Austria. Generally, cause of death is conclusively determined by post-mortem examinations of the deceased. In recent years medical imaging techniques such as CT, CT angiography and MRI have been implemented to aid in forensic investigations. For the diagnosis of natural causes of death, such as SCD, the role of these techniques is still being defined. Determination of cardiovascular causes of death generally requires a thorough assessment of the coronary arteries and myocardium. We are developing an MRI-based imaging approach to visualise these elements in reperfused hearts using a liquid which is both highly visible in MRI, and can be retained within delicate post-mortem vessels without leading to tissue extravasation.

Ex situ porcine hearts were filled with paraffin oil via a Foley catheter placed in the aorta just above the origin of the coronary arteries. A second catheter was inflated in the left ventricle via one of the pulmonary veins to prevent backflow of the injected oil out of the heart. For MR imaging, hearts were placed in a glass container surrounded by flour to avoid artefacts due to the tissue-air interface. MR images were acquired at 3T (Skyra, Siemens AG) using clinical sequences (FLASH, Dixon) to visualise the reperfused vasculature. Technical image quality was assessed.

The investigated sequences were suitable for imaging the coronary arteries in ex situ porcine hearts.

Both sequences delivered excellent contrast between hyperintense perfusates and surrounding tissue. Comparison between images revealed that the Dixon sequences suffered from a slightly poorer spatial resolution than the FLASH which could not easily be counteracted within a reasonable scan time.

As the filling procedure was still being optimised, MR images of the reperfused porcine hearts were carefully interpreted. Initial results indicated that for the visualisation of the coronary arteries and the potential characterisation of occlusions in the arteries, FLASH images would be most suitable, due to the obtainable resolution. However, when considering the simultaneous evaluation of myocardium and the presence of oedema, Dixon sequences may offer additional insight.
Targeted antioxidant treatment with mitochondrial Ros-Scavengers SKQ1 and Mitotempo is detrimental in the mouse abdominal sepsis

Pia Rademann et al.

Presenter: Pia Rademann

Altered mitochondrial function by excessive production of reactive oxygen species (ROS) has been considered an important factor in pathogenesis of organ failure in sepsis. We investigated effects of two specific mitochondria-targeted antioxidants (SkQ1: lipophilic; Mitotempo: hydrophilic) on outcome, inflammatory response and organ homeostasis in a mouse model of cecal ligation and puncture (CLP) sepsis.

3-month old female CD-1 mice (n=90) were subjected to moderate-severity CLP, and treated intraperitoneally with SkQ1 (5 nmol/kg), Mitotempo (50 nmol/kg) or saline at 1h, 12h, 24h, 36h, 48h post-CLP. We assessed 28-day survival, and circulating parameters over 0 h-72 h post-CLP. Additional SkQ1/saline-treated CLP mice (n=24) were sacrificed within the first 48h for peritoneal lavage fluid (PLF) and spleen characterization.

SkQ1 exacerbated mortality by 29 % (to 67 %; p=0.04) and Mitotempo by 15 % (to 53 %; p=0.24). CLP induced a systemic protracted cytokine release (IL-1b,-5,-6,-10,-12p70, CXCL-1, MCP-1, MIP-1a, IFN-c,TNF-a) and deregulation of organ function (urea, ALT, LDH, glucose), but antioxidant treatment failed to further modify them. Similar was true for CLP-induced lymphopenia/neutrophilia and NO-release in the blood. Dying CLP mice had approximately 100-fold more CFUs in the spleen than survivors, but this effect was not SkQ1-related. In PLF, macrophage (CD11b+/F4/80+) and granulocyte (Ly6G+) counts and intra-/extracellular ROS release were similar, irrespectively of the SkQ1 treatment.

This CLP study shows that even refined, target-tailored antioxidant treatment is detrimental rather than beneficial. It is suggestive that the negative role of mitochondrial ROS as the contributory factor to MODS is overestimated, at least in the young mice in acute CLP peritonitis.
Protective effects of interleukin-33 in critically ill patients

Stefan Stojkovic et al.

Presenter: Stefan Stojkovic

Purpose: Patients admitted to a medical intensive care unit (ICU) are characterized by an activated immune system and exhibit a high mortality rate irrespective of the underlying cause of admission. Interleukin-33 (IL-33) has been shown to be protective in experimental sepsis models and it has been demonstrated that plasma levels of its “decoy” receptor soluble ST2 (sST2) are associated with outcome in critically ill patients. The aim of the present study was to investigate whether circulating IL-33 is associated with 30-day mortality in patients admitted to a medical ICU.

Methods: In this prospective, observational study, both IL-33 and sST2 levels were assessed in 223 consecutive patients at ICU admission using specific ELISAs.

Results: During the 30-day follow-up, 58 patients (26%) died. Median level of acute physiology and chronic health evaluation score II (APACHE II) was 25. Circulating IL-33 was detectable in 166 patients and in 57 patients IL-33 was below the detection limit. Both detectable IL-33 and sST2 below the median were strong predictors of survival in critically ill patients independent of APACHE II score. IL-33 and sST2 predicted risk independent from each other. Patients with both, non-detectable levels of IL-33 and sST2 levels above the median, showed a dramatically increased mortality risk (HR 6.9 95% CI 3.0-16.2; p<0.001).

Conclusion: In summary, we show for the first time that loss of circulating IL-33 is an independent predictor of 30-days all-cause mortality in an unselected cohort of patients admitted to a medical ICU. Assessment of circulating levels of IL-33 and sST2 in addition to APACHE II score could improve risk stratification of critically ill patients.
Head-to-head comparison of GDF-15 and soluble ST2 in prediction of sudden cardiac death in patients with dilated cardiomyopathy

Stefan Stojkovic et al.

Presenter: Stefan Stojkovic

Purpose: The aim of the present study was to investigate the utility of two established prognostic biomarkers of heart failure, growth differentiation factor 15 (GDF-15) and soluble ST2 (sST2), for prediction of sudden cardiac death in patients with dilated cardiomyopathy.

Methods and results: We prospectively enrolled 52 patients with non-ischemic dilated cardiomyopathy and left ventricular ejection fraction <50. The median follow-up was 7 years. Endpoints were time to arrhythmic death (AD; primary) and all-cause mortality (secondary). AD was observed in 12 patients, whereas cardiac death was observed in 20 patients, and 1 patient died because of non-cardiac death. In a univariate model, GDF-15, but not sST2, was associated with increased risk for AD (HR=1.9, CI=1.06-3.5, p=0.03). GDF-15 remained an independent predictor of AD after adjustment for left ventricular ejection fraction (LVEF) and NYHA class. Both GDF-15 and sST2 were independently associated with long-term mortality in patients with non-ischemic dilative cardiomyopathy. In a model including GDF-15, sST2 and LVEF, increased GDF-15 levels were associated with increased mortality risk (HR=2.1, CI=1.18-4.08, p=0.01).

Conclusion: In patients with non-ischemic dilated cardiomyopathy, GDF-15 is superior to sST2 in prediction of AD and all-cause mortality. Assessment of GDF-15 provides additional information which could help identify patients at risk of future arrhythmic events.
Risk stratification in acute coronary syndrome: evaluation of the GRACE and CRUSADE scores in the setting of a tertiary care centre

Katharina Tscherny et al.

Presenter: Katharina Tscherny

Background: The management in acute coronary syndrome (ACS) is influenced by risk assessment. The GRACE and the CRUSADE scores are among the most frequently used risk assessment tools. A recently published study on 1,587 patients suggested a clear superiority of the GRACE vs. the CRUSADE score to predict in-hospital mortality and major bleeding. These results were noted controversially in the scientific community.

Objectives: We aimed to assess the performance of the GRACE and CRUSADE risk scores to predict in-hospital mortality and major bleeding in a contemporary ACS population at a high-volume centre.

Methods: All patients treated for ACS from January 1, 2006 to December 31, 2015 at our tertiary care centre were prospectively included in our registry. GRACE and CRUSADE risk scores were calculated. The discrimination capacity of both scores for in-hospital mortality and major bleeding were compared using receiver operating characteristic curves and the method suggested by DeLong et al.

Results: In total 4,087 patients (874 (21.4%) female; age 62±14 years) were included, 2218 (54.3%) were diagnosed with ST-elevation myocardial infarction, 2973 (72.7%) underwent acute percutaneous coronary intervention (PCI), 92 (2.3%) received thrombolytic therapy, 113 (2.8%) died, and major bleeding occurred in 65 (1.6%). Based on GRACE risk categories 1,031 patients (25.2%) had low risk, 1,401 patients (34.3%) had intermediate risk, and 1,655 patients (40.5%) had high risk. Risk based on CRUSADE categories was very low/low in 1,505 patients (36.8%), moderate in 924 patients (22.6%), and high/very high risk in 1,658 patients (40.6%). Discrimination capacity for in-hospital mortality of the GRACE score was superior to the CRUSADE score (area under the curve (AUC) 0.91 (95% CI 0.89 - 0.93) vs. 0.83 (95% CI 0.80-0.86); p<0.05). Performance for major bleeding was poor for both scores (AUC 0.71 (0.65-0.76) for GRACE vs. 0.61 (0.55-0.68) for CRUSADE; ns).

Conclusion: Our findings support a superiority of the GRACE over the CRUSADE score to predict in-hospital mortality. Major bleeding is rare in the era of primary PCI and performance of both scores to predict it was poor, however there was a trend towards superiority of the GRACE score for this outcome, too.
Copeptin levels in patients with chest pain with type 1 and type 2 myocardial infarction

Mona Kassem et al.

Presenter: Mona Kassem

Background: Copeptin, a C-terminal end of pre-pro-vasopressin, is used in emergency medicine as a stress biomarker for the early ruling out of acute coronary syndrome (ACS) (two marker strategy, ESC NSTEMI guidelines 2015). Whether Copeptin determination allows differentiation between type-1 and type-2 myocardial infarction (MI) or not, has not yet been investigated.

Objectives: This study evaluated whether there is a difference between Copeptin concentrations of type-1 and type-2 MI.

Methods: We examined 99 consecutive patients presenting with chest pain, at the emergency department of Wilhelminen hospital in Vienna. The subjects underwent a Troponin I and a Copeptin test at presentation and underwent further diagnostic measures to differentiate between type-1 and type-2 MIs. Furthermore, patients with a negative Troponin I at two consecutive blood samples (0, 3 hour strategy) were included into the group of no-MI.

Results: Median (25th; 75th percentile) Copeptin levels were 35,4 pmol/l (10,0; 132,6) in patients with type-1 MI, 23,3 pmol/l (8,7; 48,9) in patients with type-2 MI and 5,3 pmol/l (2,9; 11,7) in no-MI patients. There was a highly significant difference in Copeptin concentrations between both MI-types and no-MI subjects (p<0,001), while the difference between type-1 and type-2 MI was not significant (p=0,68). Using a cut-off of 10 pmol/l, 76,5% of patients with type-1 MI, 69,2% of patients with type-2 MI, but also 31,9% of patients with no-MI showed elevated Copeptin level at presentation.

Conclusion: Copeptin as a stress biomarker has an equal increase in patients with type-1 and type-2 MI, which does not allow a differentiation between MI-types. Unspecific elevation in almost 1/3 of no-MI patients deserves further investigation.
Potential role of platelet-leukocyte aggregation in trauma-induced coagulopathy: ex vivo findings

Johannes Zipperle et al.

Presenter: Johannes Zipperle

Background: Platelet dysfunction has been identified as an important contributor of trauma-induced coagulopathy (TIC). The underlying mechanism still remains speculative. Pro-inflammatory stimuli triggered by hypoperfusion strongly activate leukocytes, which in turn bind activated platelets.

Formation of platelet-leukocyte aggregates (PLA) might eventually affect platelet mediated haemostatic function.

Objective: We aim to investigate which selective states of cellular activation favored PLA and whether PLA were associated with impaired platelet function.

Methods: Whole blood from 10 healthy donors was stimulated with selective and collective platelet and leukocyte agonists to simulate differential states of activation. Leukocyte CD11b expression as well as PLA formation were determined by flow cytometry. Platelet-mediated hemostatic function was measured by thromboelastometry (ROTEM®) and impedance aggregometry (Multiplate®).

Complete blood counts were carried out to determine changes in platelet numbers.

Results: Stimulation of platelets resulted in the formation of PLA and did not require the up-regulation of leukocyte activation markers. Specifically, aggregation of platelets with monocytes rather than granulocytes resulted in a reduction of haemostatic function. None of the observed phenomena was paralleled by a reduction of measurable platelet counts.

Conclusion: PLA formation can be associated with impaired haemostatic function and largely occurs with monocytes in a state of platelet activation. PLA formation cannot be estimated by blood counts.
**Activation of Protein C and the formation of Neutrophil Extracellular Traps in non-traumatic hyperfibrinolysis**

Johannes Zipperle et al.

Presenter: Johannes Zipperle

Background: Pro-fibrinolytic activation as a consequence of sustained hypoperfusion has been reported in trauma and out-of-hospital cardiac arrest (OHCA). Activation of the plasmin system and the a number of other anti-coagulant pathways are considered key factors in the manifestation of fibrinolytic activation in hyperfibrinolysis (HF). Activated protein C (APC) is an anti-coagulant mediator that is regulated by inflammatory pathways and that also has cytoprotective properties by preventing neutrophils from undergoing cell death and Neutrophil Extracellular Trap formation (NETosis). NETs are known to bare a number of neutrophil (PMN) serine proteases, including elastase, which is suspected to interfere with hemostasis and which might amplify fibrinolytic activation.

Objective: We aimed to investigate 1) whether the plasmin system was the primary pro-fibrinolytic pathway in non-traumatic HF. 2) How APC was regulated in HF in the absence of a traumatic injury and 3) whether its inhibition was associated with features of NET formation.

Methods: Blood samples of 41 patients who suffered from non-traumatic OHCA were drawn on scene and were analyzed by standard coagulation tests and thromboelastometry (ROTEM). HF was defined by EXTEM maximum lysis (ML) >15%. Plasma levels of t-PA, PAI-1 and APC-PCI-complex were determined by ELISA. PMN elastase alpha1-Pi and histonylated (h)DNA fragments were measured as an indicator for NET formation. Measurements were correlated and patients with HF (ML>15%) were compared with non-HF (ML<15%) patients.

Results: Based on ROTEM measurements, 15 patients met the criteria for HF. When compared to non-HF, there was no significant difference in the levels of t-PA and PAI-1 in HF. APC-PCI complex was significantly higher in the HF group. There was a strong correlation between elastase and hDNA levels in all patients but elastase was not elevated in HF.

Conclusions: Activation of the APC pathway is associated with HF in OHCA. In contrast, activation of the plasmin system does not appear to play a prominent role in non-traumatic HF. Although all patients displayed features of NET formation there was no correlation between APC and NETosis. There was no indicator for an effect of neutrophil elastase or NETosis on the amplification of fibrinolysis.
Gender-Related Differences in the Perception of Prodromes of Out-of Hospital Cardiac Arrest caused by Myocardial Infarction

Elisabeth Lobmeyr et al.

Presenter: Elisabeth Lobmeyr

Background: Out-of-hospital cardiac arrest (OHCA) is still one of the main reasons of death in the western world, 70% caused by cardiovascular complications. While bystander CPR and early defibrillation proved to be most beneficial for favorable positive outcome, these measures are often delayed due to neglect and ignorance of leading cardiac symptoms. This implies a crucial role for relatives and friends to recognize symptoms early.

Objectives: We aimed to assess differences in the perception of prodromes by relatives and friends of male and female victims of OHCA due to acute myocardial infarction (AMI).

Methods: From January 1, 2006 to December 31, 2015, we prospectively collected data of all patients treated for OHCA due to AMI at our high-volume tertiary care centre. Demographic and clinical characteristics, as well as details on history, diagnostic findings and therapy were analyzed. The frequency of any suspected cardiac prodromes observed by relatives was compared between women and men using the chi-square test.

Results: In total, 531 patients (101 (19%) women; age 59+/-12 years) met the inclusion criteria. Cardiovascular risk factors, risk profile according to GRACE score (182 (IQR 161-210) in women vs. 178 (151-204) in men), cardiac arrest-related factors (e.g. witnessed arrest 38% vs. 37%; lactate level on admission 7 (IQR 4-9) vs. 6 (4-9) mmol/l) as well as AMI-related characteristics (e.g. STEMI 63% vs. 62%; most frequent location of infarction anterior wall 45% vs. 44%) were very similar between women and men.

Suspicious prodromes were, however, remembered significantly more often in male than in female patients (10% vs. 3%, p=0.03).

Conclusions: Despite very similar risk profiles to male victims, family and friends of female victims with OHCA far less often remember prodromes which were interpreted as potentially of cardiac origin. The general public thus needs to be encouraged further to listen attentively, especially to women with cardiac risk factors.
**In vitro effects of anti-anginal drugs on inflammation and coagulation in endothelial cells. A comparative study of nicorandil, trimetazidine and ranolazine**

Max Lenz et al.

**Presenter: Max Lenz**

Objective: Atherosclerosis is an inflammatory disease and increased expression of adhesion molecules and pro-inflammatory cytokines is associated with an increased risk for acute cardiac events that are associated with coronary thrombus formation. Three different anti-anginal drugs are approved for symptom relieve in patients with stable coronary artery disease: nicorandil, trimetazidine and ranolazine. Considering the emphasized role of inflammation in atherogenesis and atherosclerosis the aim of this study was to test whether these drugs have direct effects on inflammation, coagulation and fibrinolysis in vascular cells in vitro.

Methods: Human umbilical vein endothelial cells (HUVEC) were stimulated with interleukin-1β (IL-1β: 200U/ml) and treated with nicorandil, trimetazidine or ranolazine (500µM respectively) for 2-24 hours. Expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin) and tissue factor (TF) was measured using flow as well as real time-PCR. Further interleukin 6 (IL-6), interleukin 8 (IL-8) and phospho-I-kappa-B-alpha was quantified by specific enzyme-linked immunosorbent assays.

Results: Treatment with ranolazine strongly attenuated IL-1β-induced expression of adhesion molecules, TF, IL-6 and IL-8. Further phospho-I-kappa-B-alpha was significantly reduced. Treatment with trimetazidine resulted in a decreased expression of mRNA levels of VCAM-1 and IL 6 as well as an up-regulation of ICAM-1 and E-selectin mRNA but had no significant effect on phospho-I-kappa-B-alpha levels. In contrast, treatment with nicorandil had no significant effect on expression of adhesion molecules, TF and the cytokines IL-6 and IL-8.

Conclusion: Our findings indicate that ranolazine exhibits anti-inflammatory effects on endothelial cells in vitro by inhibition of NF-kappa-B. Whereas nicorandil has no effects on inflammatory and procoagulant proteins in vitro, the effects of trimetazidine need further studies in detail.
Therapeutic potential of APOSEC against trauma/haemorrhage-induced inflammation, organ failure, and mortality in rats

Arian Bahrami et al.

Presenter: Arian Bahrami

Background & Aim: Trauma & haemorrhage (TH) is a leading cause of death worldwide and is often associated with general inflammatory response, cell and organ injury. Recent preclinical studies have shown that APOSEC, a newly developed compound (made in Austria), exerts cytoprotective and/or immune modulating effects. We evaluated the therapeutic potential of APOSEC in a TH model in rats.

Material and Methods: Anesthetized rats were subjected to TH and a resuscitation protocol mimicking pre-hospital setting with a restrictive reperfusion phase followed by an adequate reperfusion phase. Animals received either APOSEC or vehicle intravenously 20 minutes after onset of reperfusion. Blood samples were taken at baseline, end of resuscitation (EOR), 24 and 48h after shock and survival was followed for 28 days.

Results: 28-day mortality (25%) was prevented by APOSEC treatment (100% survival). APOSEC treatment modulated the immune response reflected in IL-10 (decrease by 26% at EOR) compared to vehicle group. Changes in IL-6 and MCP-1 were not different between groups. APOSEC attenuated apoptosis reflected by plasma histone release up to 24h. Cell injury assessed by lactate dehydrogenase was 40% decreased by APOSEC treatment 24h post shock. HTS-induced liver injury determined by plasma alanine aminotransferase was 1.6 and 1.5 fold higher in vehicle compared to APOSEC group at EOR and 24h respectively.

Discussion: Our data show for the first time that APOSEC supplemented resuscitation ameliorates the TH-related inflammation, apoptosis, cell and organ injury, and prevents TH-induced long term mortality in rats.
Shock wave treatment of 3D cardiac model systems activates ERK1/2 signaling pathway and influences cardiomyogenesis

Christiane Fuchs et al.

Presenter: Christiane Fuchs

In the last years, accumulating evidence for the positive effects of shock wave treatment (SWT) on myocardial regeneration has gained growing attention of clinicians as a possible alternative treatment method after myocardial infarction (MI). Previous experimental and clinical studies have shown that SWT significantly improved systolic function of the heart, increased the number of blood vessels and myocardial blood flow. Although there is evidence that SWT can improve regeneration of the myocardium after MI to some extent, to date the underlying mechanisms are not entirely understood.

Therefore, our aim was to investigate the effects of SWT on in vitro cardiomyogenesis using embryonic stem cell-derived embryoid bodies (EBs) and cardiovascular progenitor cell-derived cardiac bodies (CBs) as 3-D cardiac model systems to elucidate the underlying molecular mechanisms. We analyzed expression levels of cardiac markers and signaling pathways involved in mechanotransduction, proliferation and differentiation. We could show an energy-dependent effect of SWT on signaling pathways which play a role in mechanotransduction and differentiation. SWT of EBs resulted in an evident, dose-dependent activation of the ERK1/2 pathway immediately after treatment, with the effect gradually fading with time. Gene expression analysis of EBs subjected to SWT revealed that the initial upregulation of an early cardiac marker Nkx2.5 decreased between days 7 and 14 of differentiation.

Noticeably, this effect was more pronounced in the control group, suggesting that SWT may be involved in the prolongation of the expression of Nkx2.5. In CBs we observed that, within a time frame of 24 hours, the ERK and rS6 signaling pathways were induced upon SWT, with an initial rise of ERK activation which was followed by rS6 activation. Moreover, on the gene expression level, SWT significantly upregulated lineage-specific and cardiac markers compared to untreated controls. Our intention is to provide clinicians with a solid base to pave the way for SWT into the clinics as an alternative or additive therapeutic approach in the treatment of MI.
Characterization of early left ventricle dysfunction in a relevant model for human rheumatoid arthritis

Kiss Attila et al.

Presenter: Kiss Attila

Background: Rheumatoid arthritis (RA) is associated with left ventricle (LV) hemodynamic dysfunction which may be due to the upregulation of the circulatory levels of cytokines and chemokines. However, their expression and contribution to LV dysfunction in RA heart are unknown. The activation of neuregulin-1 (NRG1)/receptor Tyrosine Kinase 2 and 4 (Erbb2 and Erbb4) pathway is considered to protect the myocardium and the impairment of this pathway, ultimately contributes to the development of heart failure. Aim: Characterize LV function and determine the expression of inflammatory cytokines, chemokines, NRG1/ErbB in hearts of a TNF-driven inflammatory, erosive arthritis model.

Methods: Anaesthetized and intubated male and female 14-15 weeks old human TNF-alpha transgenic (hTNFg; n=7) and their wild type (Wt) littermates mice (n=7) were used. LV function was evaluated by inserting the catheter tip retrograde into LV. mRNA levels of cytokines and chemokines (IL-6, IL-1β, MCP-1, MIP2 and KC), NRG1, Erbb2 and Erbb4 in LV samples were determined by RT-qPCR. Results: hTNFg mice showed severe arthritis such as paw swelling in association with smaller body and heart weight in comparison to Wt littermates (P<0.01). LV systolic pressure and the rate of LV pressure rise was decreased in hTNFg mice (P<0.01).

In comparison with Wt littermates mRNA expression of MCP-1 and KC were increased in hTNFg mice (P<0.01). NRG1 expression gradually increased in hTNFg mice compared to Wt littermates. Female mice showed attenuation of ErbB2 expression, irrespectively of RA. Conclusion: hTNFg mice have showed impaired LV systolic function in association with the upregulation of MCP-1 and KC. NRG1 expression increased in both gender which might be a compensatory mechanism and downregulation of ErbB2 was observed in female mice, irrespectively of RA. These results may represent a potential novel therapeutic point to improve LV function and reduce risk of cardiovascular disease in RA.
Impact Of The HeartWare Ventricular Assist Device Lavare Cycle On Intraventricular Flow Patterns

Philipp Aigner et al.

Presenter: Philipp Aigner

Background: Thromboembolic events in mechanical cardiac support patients are often related to blood flow stagnation within the supported ventricle due to altered flow patterns. The HeartWare® Ventricular Assist Device (HVAD®) includes a periodic pump speed modulation (Lavare cycle) to ameliorate flow patterns and reduce areas of potential blood stasis. Effects of these speed changes were investigated in an in-vitro model.

Methods: The impact of the Lavare cycle was investigated in a transparent left ventricular model and flow patterns were analyzed with Particle Image Velocimetry. A baseline speed of 2800 rpm was used and situations compared where the Lavare cycle was on (LON) and where the Lavare cycle was off (LOF). Fluid dynamics parameters (standard deviation $\sigma$ over the measurement period for characterization of flow variation; angular dispersion $r$ of the flow direction for identification of constant flow directions, stagnation index $SI$ for washout) were calculated and compared for the two situations.

Results: Overall the flow patterns for both investigated cases were similar but the variation of flow was higher, when the Lavare cycle was turned on. With the Lavare cycle on the variation in the flow patterns was higher (LON $\sigma=0.027\pm0.019$ m/s vs. LOF $\sigma=0.015\pm0.007$ m/s ). Additionally a less aligned and consistent flow orientation was identified by the angular dispersion (LON $r=0.84\pm0.18$ vs. LOFF $r=0.70\pm0.19$). Finally also the stagnation index showed a reduction (LON $SI=3.05\pm2.14$s vs. LOFF $SI=3.92\pm4.98$s).

Conclusions: Fluid dynamical measurement showed that the activation of the Lavare cycle resulted in improved ventricular washout and higher flow variations that can reduce areas of stasis and might improve the patients outcome. This is congruent also with an elsewhere published analysis of the ReVOLVE clinical registry, where a statistically significant lower stroke rate was found in patients in case the Lavare cycle was turned on.
Impact of Time of Admission on Short- and Long-term Mortality in the Vienna-STEMI-Registry

Maximilian Tscharre et al.

Presenter: Maximilian Tscharre

Introduction: Several studies have shown contradictory findings regarding mortality and hospital admission-time in patients presenting with ST-elevation myocardial infarction (STEMI). The aim of this study was to assess the impact of “on- or off-hour-admission” on short- and long-term mortality of patients in the advanced Vienna-STEMI network between 2003-2009.

Patients and Methods: The study population consisted of 4587 patients. Exact admission and delay times as well as treatment modalities were recorded in 2829 patients, who finally were included into this analysis. Patients were stratified according to their admission time into “on-hours”-admission (07:30 until 15:00) and “off-hours”-admission (15:00-7:30 and weekends). As endpoint all-cause mortality was investigated after 30 days and 3 years of follow-up (all patients and Landmark analysis for survivors of the index event).

Results: Mean age was 60.5 ± 13.3 years, 2048 (72.4%) were male and 1260 (44.5%) patients presented with anterior wall infarction. In total, 683 (24.1%) patients were admitted during “on-hours”, whereas 2146 (75.9%) patients were admitted during “off-hours”. All-cause death occurred in 176 (6.2%) patients after a follow-up of 30 days and in 337 (11.9%) patients after 3-years. Survivors of the index event exhibited a 3-years mortality of 5.7%.

For short- and long-term mortalities no significant differences could be detected between on- and off-hour-admission in univariate, multivariate Cox proportional hazard analyses and propensity score adjusted outcome analysis.

Conclusion: In the Vienna STEMI network, “on- or off-hour” admission had no impact on short- and long-term mortality for patients with acute STEMI.
Use of P2Y12-inhibitors in patients with acute coronary syndrome undergoing PCI in Austria: Insights from the ATTAIN registry

Maximilian Tscharre et al.

Presenter: Maximilian Tscharre

Background: Prasugrel and Ticagrelor have been shown to be superior to Clopidogrel in the setting of acute coronary syndrome (ACS). However, recent data from different national registries showed a reluctant prescription policy with rates of Clopidogrel usage at discharge ranging from 35 to 55%.

Methods: In this prospective, multi-centre registry we sought to assess the prescription rates for P2Y12-inhibitors in patients presenting with ACS in four Austrian tertiary PCI centres. Furthermore the predictive parameters for the use of Clopidogrel were evaluated in multivariate binary logistic regression analysis.

Results: Between January and June 2015, 990 patients presenting with ACS were included, and 808 considered for further analysis. Of those patients, 416 (51.5%) presented with STEMI and 392 (48.5%) with NSTE-ACS. Mean age was 65.7 ± 12.4 and 240 (30.9%) were female. At discharge, 212 (26.2% of all) received Clopidogrel, 260 (32.2%) Prasugrel and 297 (36.8%) Ticagrelor, while 11 (1.4%) did not receive any P2Y12-inhibitor. Twenty-eight (3.5%) died during the hospital stay.

Of those patients, who were discharged with Clopidogrel, 117 (55.2% - 14.4% of all patients) had no absolute contraindication against a new P2Y12-inhibitor.

Conclusion: The ATTAIN registry clearly showed the high quality of care of patients presenting with acute coronary syndrome in urban Austria with regard to dual antiplatelet therapy compared to other western societies. Nevertheless, a considerable rate of patients were still not treated with the highest standard available.
Epicardial Adipose Tissue and its Predictive Effect on Cardiovascular Outcome in Patients with Acute Coronary Syndromes Undergoing Percutaneous Coronary Intervention

Maximilian Tscharre et al.

Presenter: Maximilian Tscharre

Aims: We sought to investigate the association between epicardial adipose tissue (EAT) thickness and cardiovascular outcomes in a cohort of high-risk acute coronary syndrome (ACS) patients undergoing percutaneous coronary intervention (PCI).

Methods and Results: Of 1198 patients undergoing PCI, 438 had a transthoracic echocardiography performed during index hospitalisation. EAT thickness was measured in the parasternal long-axis view, perpendicularly on the free wall of the right ventricle at end-systole in 3 consecutive cardiac cycles and was then averaged. As primary outcome measure, a composite of major adverse cardiovascular events (MACE), including cardiovascular death, non-fatal myocardial infarction (MI) and non-fatal stroke, was investigated after 3 years of follow-up.

Patients were included between 2004-2012, 293 (66.9%) were male. Median EAT thickness was 2.65 mm [IQR 2.00-3.00]. EAT was correlated with body-mass-index (R=0.404; p<0.001) weight (R=0.314; p<0.001), baseline creatinine (R=0.118; p=0.014) and baseline glucose (R=0.129; p=0.007). After a follow-up of 3 years, MACE occurred in 64 patients (14.6%) corresponding to 36 (8.2%) with cardiovascular death, 21 (4.8%) with MI and 7 (1.6%) with stroke. Regarding the primary endpoint, EAT thickness revealed a significant predictive effect upon univariate Cox-regression (HR=1.479 [95% CI 1.192-1.953]; p=0.006) and multivariate Cox-regression analysis (HR=1.524 [95% CI 1.011-2.267]; p=0.038) after adjusting for established cardiovascular confounders.

Conclusions: In a high-risk cohort of ACS patients undergoing PCI, EAT was associated with established markers of cardiovascular death. Moreover, EAT was an independent predictor for 3-year cardiovascular outcome.
A (poly)trauma hit requires functional verification of its immuno-inflammatory characteristics and outcome effect upon secondary sepsis

Susanne Drechsler et al.

Presenter: Susanne Drechsler

Polytrauma activates and then impairs the immune response, predisposing patients to secondary sepsis. Only severe mouse polytrauma models seem to reproduce those immunedysfunctions. New polytrauma models combine various elements (as 1st hit). Yet, we often fail to verify the true impact of the 1st hit (polytrauma) on the 2nd hit sepsis outcome. Thus, we compared the effect of two mouse polytrauma models of increasing severity on 1) the immuno-inflammatory phenotype, 2) phagocytic capacity and 3) sepsis outcome. We also studied the impact of splenectomy as a polytrauma element.

Female Balb/c mice (n=84) underwent 1) severe polytrauma (SPT) by femur fracture (FF), hemorrhagic shock (HS; 30% blood loss) and splenectomy, or 2) moderate polytrauma (MPT) by FF and HS. Blood was sampled at 0, 24 and 48h post-trauma. Absolute/relative counts of granulocytes, lymphocytes, CD4+, CD8+ and regulatory T-cells, monocyte MHC-2 expression and leukocyte phagocytic capacity were assessed. Additional mice underwent 2nd-hit cecal ligation and puncture (CLP) 48h after the 1st-hit.

CLP sepsis alone caused 32% mortality by day 28. The MPT exacerbated the CLP mortality to 56% (p<0.05). The SPT reduced the 28-day mortality to 8% (p<0.05). Within 48h, both SPT and MPT induced a distinct neutrophilia (5 and 2.5-fold increase) and lymphocytosis (1.3 and 2-fold increase; p<0.05). Only the more severe SPT induced a 1.5-fold increase in the absolute CD4+ and CD8+ T-lymphocyte counts (p<0.05) at 48h; contrasted by a 30% decline in the relative CD4+ and CD8+ counts (p<0.05). In SPT mice at 48h, absolute and relative counts of regulatory T-cells (CD4+CD25+CD127-) increased by 1.5-fold (p<0.05). Monocyte MHC-2 expression increased by 46% within 48h post-SPT. Phagocytic capacity of macrophages/granulocytes isolated from whole blood increased by 54% in SPT by 48h.

The greater SPT severity lowered the post-traumatic sepsis mortality, while milder MPT worsened it. Splenectomy caused a strong immunosuppressive T-reg phenotype coinciding with immune system activation. We advise a mandatory end-effect verification of the existing (poly)trauma hits (as the whole and of individual elements) before they are deemed as clinically relevant and used in any 2-hit model system. Supported by FWF Hertha-Firnberg.
Homocysteine modulates mineralization of murine cell cultures

Norbert Hassler et al.

Presenter: Norbert Hassler

Hyperhomocysteinemia is associated with several pathologies such as cardiovascular disease, diabetes, and atherosclerosis. We have recently demonstrated that homocysteine (Hcys) alters collagen cross-linking, perturbs triple-helix formation and regulates expression of genes found in osteoblastic cells, possibly via the inflammation related gene SAA3. Concerning cardiovascular disease it was demonstrated that Hcys is related to aortic mineralization in patients with ischemic heart disease. For a better understanding of the mineralization process itself, we studied the influence of Hcys on the deposition of mineral in murine bone cell cultures.

For our experiments, we used the pre-osteoblastic cell line MC3T3-E1, which in long-term culture differentiated into mature, mineral depositing osteoblasts. As a second system we cultured MLO-A5 cells. These cells are late osteoblasts, which also deposit mineral, however, already after 10 days.

MC3T3-E1 and MLO-A5 cells were cultured up to 5 and up to 3 weeks, respectively. The cultures were treated either with Hcys or β-glycerophosphate (βGP) or in combination and mineralization was determined by Alizarin-red staining. Gene expression was addressed by genome-wide expression analysis (GeneChip, Affymetrix) and expressions of interesting genes were confirmed by RT-qPCR.

Long-term cultures of MC3T3-E1 cells revealed that Hcys in combination with βGP strongly increased the deposition of mineral after 4 and 5 weeks of culture. In MLO-A5 cultures, however, the sole treatment with βGP stimulated the deposition of mineral, and Hcys had no additional effect.

Genome-wide expression analysis and RT-qPCR of Hcys treated cells demonstrated an increase of Phospho1 (phosphatase, orphan 1) and Alpl (alkaline phosphatase), both genes, which are involved in the mineralization process.

Our data suggests that Hcys by up-regulating the expression of phosphatases increases the concentration of inorganic phosphate, which accelerates mineralization of osteoblastic MC3T3-E1 cell cultures. These results also suggest that Hcys can modulate physiological as well as pathological mineralization processes.
Development of a Flow Estimator for Left Ventricular Assist Devices.

Martin Maw et al.

Presenter: Martin Maw

Introduction: Left ventricular assist devices (LVAD) are increasingly implanted in end-stage heart failure patients. Due to the lack of long term stable pressure sensors for monitoring hemodynamics and the interaction between pump and circulation, the pump flowrate needs to be estimated. This can be achieved using signals of the LVAD, such as current uptake and rotational speed, after identification of the relationship of these parameters with a measured flowrate.

Methods: Based on hydraulic testing in a mock circulation, which included a powerful linear motor for load variation, two methods of developing a flow estimator were compared. Both methods used the observable parameters pump current and pump rotational speed, that are supplied by the LVAD control unit. Low frequency behaviour could be estimated with a third order polynomial. This was used in both flow estimators. In one flow estimator ($Q_{in}$) the higher frequency dynamics were approximated by a differential term modelling to the inertia of pump speed. In the other flow estimator ($Q_{mo}$) the higher frequency dynamics were estimated based on a linear model of a dc motor.

Results: Both estimators were validated in an in vitro setup and proved to be able to estimate flow correctly with a root mean square error of 0.42±0.08 L/min ($Q_{mo}$) and 0.35±0.06 L/min for ($Q_{in}$).

Discussion: This shows that flowrate can accurately be estimated from internal pump signals, without need for sensors and can be used for other signal processing means to estimate hemodynamic parameters.
**Age dependent changes in lung vessel morphology in healthy women and men**

Michael Pienn et al.

Presenter: Michael Pienn

Background: Since thoracic computed tomography (CT) is associated with exposure to X-rays, typical morphologic readouts of healthy subjects are rarely reported. However, these are necessary as a reference for computer aided diagnosis (CAD) algorithms. In this study we examine lung vessel morphology in a large cohort of healthy subjects and examine changes with sex and age.

Methods & Materials: This study includes subjects, who underwent CT pulmonary angiography because of suspected pulmonary embolism, but in whom no major thoracic pathologies were detected. Lung vessel morphology was assessed with our fully-automatic vessel segmentation algorithm, which determined the tortuosity for each individual vessel segment in a diameter range between 2 and 10 mm. To validate the algorithm, wrongly identified structures were marked by a radiologist in 15 randomly selected subjects. The distribution of tortuosity values for the individual vessel segments was examined using histogram analysis. Differences between men and women were analysed by Mann Whitney test. Correlation with age was assessed using linear regression analysis.

Results: The algorithm was applied to 136 subjects (72 women). Mean age was 59±17 years (range 17-94 years), with no significant difference between women and men. Wrongly identified structures were present in 3 of 15 subjects with a percentage of below 5% of vessel segments. Histograms of the sum-of-angles metric (SOAM), a measure for tortuosity, were skewed to the right (mean skewness 1.0±0.3) likewise for women and men. The median of SOAM was significantly higher in women than in men (0.0416±0.0016 vs. 0.0407±0.0012 rad/mm, respectively; p=0.0011). In women the median and the 95th percentile of SOAM were positively correlated with age (R²=0.06; p=0.04 and R²=0.20; p<0.001, respectively). The 5th percentile was negatively correlated with age in women (R²=0.08; p=0.014). In contrast, these readouts were not correlated with age in men.

Conclusion: With increasing age, women show increasing vessel tortuosity and a broadening of the tortuosity distribution while in men tortuosity and distribution stay constant. These results point to fundamentally different processes of aging of the lung vasculature in women and men.
The new St. Thomas Hospital polarized cardioplegia: improved efficacy of myocardial protection in pigs

Anne-Margarethe Kramer et al.

Presenter: Anne-Margarethe Kramer

Objective: Increasingly, patients undergoing cardiac surgery are more elderly, sicker and hence require improved protection. We compared cardioprotective efficacy of a new St Thomas' Hospital Polarizing cardioplegia (STH-Pol: esmolol, adenosine, magnesium) to conventional St Thomas' Hospital cardioplegia (STH2: potassium, magnesium) in a pig model of cardiopulmonary bypass (CPB). Our hypothesis was the non-inferiority of depolarized versus polarized arrest. Material and Methods: Pigs (47±4kg) were anesthetized and monitored for baseline hemodynamic function. After sternotomy, CPB and aortic cross-clamping, hearts were arrested via antegrade warm (37°C) STH-Pol (n=7) or STH2 (n=6) for 60min ischemia followed by 60min on-pump reperfusion. After weaning from CPB, hearts were monitored for further 120min off-pump reperfusion before sacrifice and tissue sampling (for high-energy phosphates and electron microscopy). Recovery was measured as % of baseline (mean±SEM).

Results: Baseline hemodynamics were comparable. After 180min reperfusion, recovery of mean arterial pressure and heart rate were similar; however, in STH-Pol hearts had improved recovery of left ventricular systolic pressure (133±8 vs. 97±5 %, p<.01) and external heart work (145±16 vs. 88±10%, p<.05) than STH2 hearts. Coronary flow/heart weight was also higher during early (430±59 vs. 211±59%, p<.05) and late reperfusion (269±43 vs. 90±16 %, p<.01) in STH-Pol. Total creatine kinase release was lower in STH-Pol hearts during reperfusion (2016±262 vs. 1232±199 U/L, p<.05). Creatine phosphate levels in ST-POL hearts were higher (133±31 vs. 63±2 nmol/mg, p<.05). There was no difference in ultrastructure between groups. Discussion: Polarized cardiac arrest improves myocardial protection and reduces ischemic damage in a model of CPB in pig hearts. We therefore think this new concept of polarized cardioplegia should have clinical relevance.
**Contribution of mitochondrial nitrite reductase to the regulation of hemodynamics**

Peter Dungel et al.

Presenter: Peter Dungel

Inorganic nitrite is known to regulate vascular tonus via reduction to nitric oxide (NO), by several nitrite reductases (NR) under hypoxic conditions. NR can be found in both blood and tissue and there is a controversial discussion about the individual contribution of these proteins. We aimed to investigate the role of selected NR, especially mitochondrial NR, in the regulation of hemodynamics in in vivo and in vitro models.

Rats were randomly assigned to normoxia or hypoxia. Hypoxia was induced by inhalation of nitrogen/air containing 15% O2 and a bolus dose of nitrite (15µmol/kg) injected. At given time points blood samples were taken for blood gas analysis and determination of NO-Hb complexes by electron paramagnetic resonance spectroscopy (EPR). To determine the hemodynamic changes in response to nitrite pulse wave analysis was performed. In vitro experiments were performed with heart tissue homogenate, isolated RBC and HL-1 cardiomyocytes. Nitrite and total NO release were analysed by chemoluminescence assay and cGMP levels by ELISA.

Analyzing pulse waves in in vivo models of hypoxia and hemorrhage combined with nitrite infusion we showed that single NRs regulate specific hemodynamic parameters, such as heart rate, arterial stiffness, peripheral resistance etc. NR located in red blood cells regulates predominantly heart rate, while Mb and mitochondrial NR predominantly regulate peripheral resistance. Using ex vivo on-line NO-detection we determined optimal oxygen levels for single NRs. Mb was active at the lowest oxygen tension, while mitochondrial NR operates both under hypoxia and normoxia, suggesting that nitrite may contribute to physiological regulation of hemodynamics. The experiments with cell culture suggested that reduction of nitrite by mitochondria regulates cGMP synthesis and consequently contractility of cardiomyocytes.

All together our data suggest that mitochondria contribute in a hypoxia-independent manner complementary to hemoglobin, myoglobin and xanthine oxidoreductase, which are activated under hypoxic conditions. Oxygen tension and hematocrit determine which NR is active. This network of NRs regulates diverse hemodynamic parameters, complementary to the cholinergic system and opens new approaches for targeted nitrite therapy.
Angiopoietin-Like Protein 4 (ANGPTL4): a potential regulator of pulmonary fibrosis

Anita Sahu-Osen et al.

Presenter: Anita Sahu-Osen

Angiopoietin-Like Protein 4 (ANGPTL4): a potential regulator of pulmonary fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible, and mostly fatal lung disease of unknown cause. IPF has characteristics of abnormal wound healing process, fibroblast proliferation and extra cellular matrix deposition. ANGPTL4 has been shown to negatively regulate lung tissue integrity. However, involvement of ANGPTL4 in IPF disease progression is not much explored. Therefore, the aim of the study was to investigate the targets and mechanism involving ANGPTL4 in IPF.

Methods: Human lung parenchymal fibroblasts (hPFs) from donor subjects were used to perform in-vitro investigation. Bleomycin induced lung fibrosis (intra-tracheal administration) in ANGPTL4 knock out (KO) and C57B/6J (WT) animal model were used to confirm the findings in-vivo.

Results: Pro-fibrotic factor such as TGFβ and PDGF-BB induced expression of ANGPTL4 in hPFs. Stimulation with recombinant ANGPTL4 induced human hPFs proliferation, in line with the above findings silencing of ANGPTL4 strongly inhibited PDGF-BB induced cell proliferation. In-vivo bleomycin induced lung fibrosis in WT mice led to time dependent increase in ANGPTL4 protein expression followed by increase collagen content and worsening of lung function. Bleomycin treated ANGPTL4 KO mice were protected from fibrosis development as indicated by reduced collagen deposition and improved lung function.

Conclusion: In this study, we have demonstrated that ANGPTL4 exhibits fibrotic properties by inducing collagen production. Thereby is important in the progression of fibrosis in bleomycin mouse model. Hence, blocking ANGPTL4 could be of therapeutic importance.
Circulating copeptin and high-sensitivity troponin I in patients with chest pain after a recent syncope

Kris Vargas et al.

Presenter: Kris Vargas

Copeptin is useful in combination with troponin in the early rule-out of chest pain patients with suspected acute myocardial infarction. No evidence is available on the baseline characteristics and copeptin values of chest pain patients presenting with syncope in the emergency setting. We measured copeptin and high-sensitivity cardiac troponin I in 700 consecutive emergency department patients who presented with chest pain between February 2011 and January 2012. Retrospectively, we selected only patients with a concomitant syncopal episode. Copeptin cut-off values above 10 pmol/L were considered positive. Variables were measured as frequencies, means, medians and standard deviations. Sixteen patients (males = 8) presenting with chest pain and concomitant syncope were found. Their mean age was 72.3 ± 18.4 years; with time since symptom onset ≤ 6 h in 15 of 16 patients.

All patients exhibited elevated copeptin levels (median 54.3 pmol/L, IQR 31.7 to 147.0 pmol/L) and no statistical significant difference (P = 0.40) was found in copeptin values between males and females. 5/16 patients with a history of recent syncope had elevated hs-cTnI values on admission or several hours thereafter (0 / 3 hours detection strategy) and presented with the following additional diagnoses: deep venous thrombosis/pulmonary embolism in two cases and acute coronary syndromes in three. In 7/11 patients with normal troponin values, syncope was most likely due to worsening of their comorbidities including valvular insufficiency, chronic ischemic heart disease, congestive heart failure, venous insufficiency and dehydration thus most likely leading to transient cerebral hypoperfusion. The remaining four patients in this group may have experienced a vasovagal syncope as no major comorbidities were found. From the classical risk factors, hypertension was present in 63% of patients, hyperlipidemia in 25%, while past smoking habit and diabetes mellitus type 2 were documented in four and two patients, respectively. Elevated circulating copeptin levels in combination with normal troponin levels are mainly seen in patients experiencing non-coronary chest pain and syncope. Further studies need to determine associations between underlying causes for syncope and reveal potential confounding factors.
Standardized Telephone Intervention Algorithm for Improved Ventricular Assist Device Outpatient Survival and Reduced Adverse Event Rates

Thomas Schlöglhofer et al.

Presenter: Thomas Schlöglhofer

Purpose: Ventricular assist devices (VADs) are an established therapeutic option for patients with chronic heart failure. Continuous monitoring of VAD parameters and adherence to management guidelines are crucial to detect problems in an early stage to optimize outcomes. A telephone intervention algorithm for constant communication with VAD patients was developed, clinically implemented and evaluated.

Methods: An analysis of VAD outpatient outcomes without (n=71, control cohort) and with bi-weekly telephone interviews in their routine care (n=25) was conducted. During the calls a structured inquiry about pump parameters, alarms, blood pressure, INR, body weight and temperature, driveline exit-site status and symptoms of dyspnea was performed. Answers were categorized using a self-developed algorithm in 5 levels of severity: no problems, recall next day, refer to physician, follow-up visit next week and readmission. To minimize confounding, outcomes were analyzed using proportional hazard Cox regression, with risk adjustment based on telephone intervention-use propensity score model computed from demographics, risk factors and operative covariates.

Results: From February 2015 through May 2016, 25 patients (n=3 HeartMate II and n=4 HeartMate III, n=18 HeartWare HVAD) underwent 320 telephone interventions. Within the first year of support, 50% of the calls determined no problems and patients adhered to institutional guidelines of blood pressure (85.4±9.4mmHg) and INR (2.4±0.4). In 4% patients were recalled on the next day because of alarms. In 33% the VAD coordinator had to refer to the physician due to elevated blood pressure (>85mmHg), INR <2.0 or >4.0 (n=20) or edema (n=2). 13% of the calls resulted in a follow-up visit because of driveline exit-site problems and could be solved by early intervention. The average days of rehospitalization in the hospital (31.8±46.1 days vs. 18.6±21.8 days, p=0.42) within one-year post discharge were n.s. higher in the control group. Propensity-adjusted one year survival (95% vs. 67%, p=0.03) was significantly superior for the telephone intervention group.

Conclusion: Continuous, standardized communication with VAD outpatients is important for early detection of upcoming problems and leads to significant improved survival.
Increased Platelet Reactivity in Dyslipidemia in Patients on Dual Anti-Platelet Therapy

Bernhard Jäger et al.

Presenter: Bernhard Jäger

The optimal duration of dual anti-platelet therapy (DAPT) following percutaneous coronary intervention (PCI) is still a matter of debate. Biomarkers may help to identify patients who will benefit from extended or intensified DAPT. Aim of the study was to test the interaction between routine lipid parameters and platelet function in patients with coronary artery disease (CAD) on DAPT. 58 patients on DAPT (clopidogrel: n=18, prasugrel: n=17, ticagrelor: n=23) were prospectively included following PCI after an initial acute coronary syndrome (ACS) or after elective PCI in stable CAD and all patients were free from ischemic or bleeding events for at least 6 months prior to inclusion. Mean platelet volume (MPV), platelet distribution width (PDW), fraction of reticulated thrombocytes (RT) and ADP-induced multiple electrode aggregometry (MEA), as well as serum lipids i.e. HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), total-Cholesterol (TC), triglycerides (TG) and Remnant-Cholesterol (RC) were assessed 2-4 hours after intake of ASA (100mg) and a P2Y12-Inhibitor. A significant inverse correlation was found for HDL-C levels and markers of platelet activation in univariable analysis: MPV (r=-0.351, p=0.009), PDW (r=-0.391, p=0.003), fraction of RT (r=-0.402, p=0.003) and ADP-induced MEA (r=-0.345, p=0.011), respectively. Only weak or no association was found between other lipid parameters (TG, TC, LDL-C, RC) and markers of platelet activation.

After adjusting for confounders of platelet activation and lipid parameters in a linear regression model using backward elimination, HDL-C levels served as strong and significant predictors of MPV (95% CI -0.039 to -0.009; p=0.002), PDW (95% CI -0.094 to -0.034; p<0.0001), RT (95% CI -0.107 to -0.031; p=0.001) and MEA (95% CI -0.540 to -0.170; p<0.0001), while TG, LDL-C, RC and TC were not significantly associated with platelet function. Within known routine lipid parameters, only HDL-C levels show a strong inverse correlation with different markers of platelet activation in patients with CAD on DAPT. As increased platelet activation is a determinant of future cardiovascular events, detection of dyslipidemia, mainly low HDL-C levels, might indicate the need for further prolongation of DAPT.
Regenerative Medicine Research

Noval human placenta substrates for Neovascularization

Johannes Hackethal et al.

Presenter: Johannes Hackethal

To vascularize engineered organs, to revascularize infarcted tissue and to inhibit new vessel formation in cancer is one of major challenges for Tissue Engineering (TE) and clinics. In this study, we established two very mild isolation procedures from human placentas villous tissue using 0.5 M Tris-NaCl or 2 M Tris-urea buffers to provide as bioactive substrates for the feasibility of spontaneous differentiation of endothelial cell (EC) progenitors (green fluorescent-labeled human umbilical vein endothelial cells, gfpHUVECs) into interconnected networks. The substrates were characterized by using BCA, CyQuant DNA staining, 1,9-dimethyl-methylene blue (DMB) staining, polyacrylamide gel electrophoresis, western blotting and angiogenesis cytokine array. In vitro NW characteristics were assessed using Angiosys 2.0.

The mean protein content of the liquid substrates/mL was calculated as 2.25±1.33 mg/mL (Tris-NaCl) and 2.46 ± 0.63 mg/mL (Tris-Urea; n=8, mean ± SD). DNA content was significantly reduced after isolation whereas the amount of glycosaminoglycans was not. Western Blots identified Laminin-111 and collagen-4. A mix of different potent pro-angiogenic cytokines was detected.

Compared to the gold standard Matrigel, our substrates generated networks with significantly higher number of junctions or tubes in vitro. The networks remained viable up to 5 days whereas the networks on Matrigel underwent apoptosis after around 24 hours.

We have developed two simple, rapid, easy-to-reproduce and cost-efficient isolation methods for bioactive substances from human placenta tissue. The described isolation methods result in human low-cost substrate alternatives to Matrigel for in vitro neovascularization models and may have significant benefit to many neovascularization-related clinical scenarios.
In-depth characterization of vital human amniotic membrane

Asmita Banerjee et al.

Presenter: Asmita Banerjee

For over a century, the human amniotic membrane (hAM), the innermost fetal membrane, has been used in clinics for tissue repair mainly in devitalized form. Now, it is becoming more and more evident that resident cells of vital hAM substantially contribute to tissue regenerative and healing processes. Optimized application of vital hAM for clinical settings requires investigation of its basic properties on cellular, sub-cellular and extracellular level.

On the sub-cellular level, mitochondria have recently been shown to play an important role for tissue regeneration. This study aimed to characterize specific properties of two sub-regions of the hAM connected to mitochondrial activity.

We found higher mitochondrial respiration and higher ATP levels in the placental sub-region of fresh hAM. Inhibition of the ATP synthase led to elevated lactate levels in placental amnion, indicating increased glycolysis. Interestingly, this switch was not observed in the reflected amnion.

Despite higher respiratory activity, we found lower levels of intracellular reactive oxygen species (ROS) in placental amnion, however, higher levels of extracellular ROS, suggesting different regulatory mechanisms in the two sub-regions.

Regarding long-term cultivation of the hAM, measurement of mitochondrial activity showed that distension seems to be necessary to sustain cellular viability, which should impact banking strategies of the hAM.

Taken together, we found distinct metabolic differences of placental and reflected amnion. These differences should be taken into account for an optimized clinical application of the vital hAM, as an alternative to current devitalized applications.
Adipose Tissue-Derived Therapeutic Cells - Towards a Non-Enzymatic Procedure

Eleni Priglinger et al.

Presenter: Eleni Priglinger

Human adipose tissue is an attractive and abundantly available source of adult stem cells applicable in regenerative medicine and tissue engineering. Hence, prerequisite for the translation into clinics is the production of the stromal vascular fraction (SVF) under current good manufacturing practice (cGMP). A number of companies developed systems aiming for a closed, sterile, safe and reproducible cell isolation process limiting risk for contaminations and unpredictability of the cell material.

However, many of these systems are based on enzymatic digestion with collagenase which is the most expensive part of the isolation process, complicates regulatory authorization and may have negative impacts on cell potency and efficacy. Therefore, we compared classical enzymatic cell isolation methods with reduced enzyme concentration methods and a new non-enzymatic isolation method regarding cell yield, identity and potency. Our results demonstrate that enzyme treatment is necessary for applications which require large cell numbers, since reduction of enzyme concentration to 30%, 10% and 0% result in smaller cell yields. In contrast, the viability of the isolated cells as determined via cellular ATP decreased with increasing collagenase concentration. Further we found that a closed system incorporating non-enzymatic treatment steps is suitable to isolate therapeutically relevant subpopulations such as endothelial progenitor cells (CD45-/CD31+/CD34+), pericyte-like cells (CD45-/CD31-/CD146+), and supra-adventitial cells (CD45-/CD31-/CD146-/CD34+) with high cellular ATP content and elevated differentiation potency. Cells derived from our new non-enzymatic method showed stronger potential to form tube-like structures.

Our findings support the concept of using non-enzymatic closed systems which allow the isolation of therapeutically active cells in a one-step procedure.
One of the mainstays of facial rejuvenation strategies is volume restoration which can be achieved by autologous fat grafting. In our novel approach, we treated the adipose tissue harvest site with extracorporeal shock wave therapy (ESWT) in order to improve the quality of the regenerative cells in situ. The latter was demonstrated by characterizing the cells of the stromal vascular fraction (SVF) in the harvested liposuction material regarding cell yield, ATP content, proliferative capacity, surface marker profile and differentiation potential. While SVF cell yield was only slightly enhanced, viability and ATP concentration of freshly isolated cells as well as proliferation doublings after 3 weeks in culture were significantly increased in the ESWT compared to the untreated group. Likewise, cells expressing mesenchymal and endothelial/pericytic markers were significantly elevated concomitant with an improved differentiation capacity towards the adipogenic lineage.

Hence, in situ ESWT might be applied in the future to promote cell fitness and adipogenesis within the fat graft for successful facial rejuvenation strategies with potential long-term graft survival.
Extracorporeal shockwave therapy accelerates motor axon regeneration despite a phenotypically mismatched environment

David Hercher et al.

Presenter: David Hercher

Introduction: Peripheral nerve injuries are common and a frequent cause of hospitalization displaying a major burden to patients and social health-care systems. Although regeneration after autologous nerve transplantation is the target of scientific curiosity since the beginning of modern medicine, not much progress in accelerating this tedious process has been made. A possible explanation could be the experimental model chosen. Most research groups use the sciatic nerve defect as a model for autologous nerve transplantation, dismissing the influence of phenotypically different nerve grafts on regeneration. Here we investigate the effect of a this mismatch on the regeneration of motor axons using the femoral nerve model. Additionally, we evaluate the influence of extracorporeal shockwave therapy (ESWT), which has been shown to be one of very few treatment options accelerating peripheral nerve regeneration.

Methods: A femoral nerve defect model was established in the rat reflecting the phenotypical difference of transplanted autologous nerve grafts in the clinic. The effects of ESWT on motor fibers regenerating through a sensory environment have been evaluated using automated gait analysis, electrophysiology, histology and qPCR. Data were analyzed using one-way ANOVA followed by Tukey’s multiple comparison test, unless indicated otherwise.

Results: Motor nerves differ from sensory nerves regarding expression of pro-proliferative markers as well as myelination associated proteins in early and later stages of neuronal regeneration. Furthermore, electrophysiological as well as histological evaluation show inferior regeneration of motor axons through a sensory nerve graft compared to a phenotypically matched graft. ESWT increases expression of markers for re-myelination and homeostasis up to 100% 6 weeks after injury, indicating amelioration of negative effects of phenotypical mismatch.

Discussion: This study shows that ESWT is able to accelerate peripheral nerve regeneration in a model which reflects the clinical reality after autologous nerve transplantation. Hereby, providing support for the use of ESWT after surgical repair of peripheral nerve injuries.
Inpatient Rehabilitation of painful Shoulder Diseases with and without additional therapeutic Nuclear Magnetic Resonance (NMRT)

Barbara Stritzinger et al.

Presenter: Barbara Stritzinger

Chronic pain caused by osteoarthritis can affect activity and participation. Shoulder pain as a common musculoskeletal affection often arises due to disorders in the complexity of the shoulder. Most patients who suffer from shoulder pain recover with non-operative interventions, whereas the goal of non-operative interventions as those used in rehabilitation programmes, in many respects, refer to: (i) pain reduction, (ii) help in recovery and maintain a passive range of motion, (iii) to strengthen the rotator cuff in a non-impingement range of motion, and (iv) to prevent the occurrence of progressive pathological changes.

In addition to the main outcome parameter “sustainability and effectiveness of inpatient rehabilitation” our investigations should help to answer the following second question: Does NMRT, applied in addition to a standardized rehabilitation programme, influence the rehabilitation outcome of patients with painful shoulder diseases?

Nuclear magnetic resonance (NMR) as a therapeutic form of treatment applied in rehabilitation medicine represents a therapeutic technology with the potential to activate cellular processes.

In a double blinded placebo-controlled multicenter study on 150 patients with various painful shoulder diseases the influence on pain (VAS), sleep quality (Pittsburgh sleep quality index) and shoulder function (Constant Score and Quick-Dash-Score) were investigated. We included biomarkers for stress and pain (ß-Endorphin, Dynorphin, ACTH and Serotonin). While the therapeutic NMRT during inpatient rehabilitation was applied as an additive treatment, the outcome of rehabilitation with and without NMRT was analyzed. NMRT during inpatient rehabilitation was applied at series of 9 x 1 h. In fact the results point out a good outcome of the rehabilitation programme, unfortunately the notably effective NMR therapy did not result in a statistically verifiable, additive effect. Virtually all investigated parameters, mainly pain and function improved significantly during and after the inpatient rehabilitation programme in both groups. Therefore, one might consider that a possible positive effect by the NMRT on osteoarthritis is masked by the obviously effective rehabilitation programme to treat the very complex shoulder joint.
**Fast T2-mapping at 7T in patients after posterior medial meniscectomy under loading**

Sebastian Röhrich et al.

Presenter: Sebastian Röhrich

Introduction: Articular cartilage undergoes complex alterations in disease and after cartilage repair procedures. In order to evaluate these changes, fast and non-invasive diagnostic methods are pivotal. MRI-T2-mapping was shown to resemble collagen architecture and water content in knee cartilage. Therefore, the goal of this study was to demonstrate the implementation of a fast 3D-TESS sequence at 7T to measure in-vivo changes of T2-values after partial meniscectomy (APM) and under the direct application of axial pressure.

Methods: Knee joints of 10 subjects were scanned one year after posterior APM. An MRI-compatible compression device was used to apply a pressure of 250N (25kg). Regions-of-interest (ROIs) were drawn on T2-maps on two slices adjacent to the surgery site. All subjects completed the KOOS-questionnaire. Comparisons between mean T2-values of superficial and deep cartilage zones, as well as anterior and posterior regions were conducted (Student’s t-test). Differences between loading conditions were analysed with a repeated measurements ANOVA (Bonferroni-posthoc test).

Results: A zonal differentiation between superficial and deep ROIs was seen with T2-values in superficial ROIs being 50% higher than in deep ROIs (p=.0001 for all ROIs). T2-values of the superficial zone adjacent to the surgery site were consistently higher (femoral p=.012, tibial p=.023) than those in anterior ROIs. Increased T2-values of posterior femoral cartilage were associated with decreased KOOS-subcales (r=.762, p=.014). The most pronounced change of T2-values after applying load was seen in the central femoral ROIs (superficial: +15%, p=.036; deep: +20%, p=.002).

Discussion: A correlation of increased T2-values near the site of previous APM with worse KOOS-subcales may indicate clinically relevant cartilage alterations in this area.

Increased T2-values in the central femoral weight-bearing region under loading may resemble reduced collagen-anisotropy of degenerated cartilage when subjected to compression, as has been discussed in in-vitro studies.

3D-TESS at 7T allows for nearly 4 times faster T2-mapping than sequences from other knee cartilage compression studies (1:58min vs. 7:35min), enabling non-invasive evaluation biochemical and biomechanical properties of cartilage.
Effects of low level light therapy on endothelial cells and vasculogenesis

Peter Dungel et al.

Presenter: Peter Dungel

Low level light therapy (LLLT) with light-emitting diodes (LEDs) receives increasing interest in the fields of wound healing and angiogenesis. Recently it has been shown that both red and blue LED light improved wound healing in a single side ligation induced ischemia skin flap model in rats by increased vessel formation and enhanced perfusion. Endothelial cells play an important role in angiogenic processes. The aim of this study was to compare the effects of pulsing LED light of three different wavelengths on endothelial cells and vasculogenic processes in vitro.

The effects of pulsed LED light on proliferation and migration of human umbilical vein endothelial cells (HUVEC) were investigated in several 2D and 3D cell culture models. Cells were treated with either blue (475 nm), green (516 nm) or red (635 nm) LED light. Control cells were not illuminated. 2D proliferation was determined at given time points by manual counting. 2D migration was assessed by scratch assays, 3D migration was evaluated by Cytodex bead assays. The vasculogenic potential of HUVEC in co-culture with adipose-derived stem cells (ASC) in response to LLLT was determined by analysing the network formation in a 3D fibrin matrix co-culture model after 4 and 7 days.

Stimulation with both red and green pulsed LED light significantly increased HUVEC proliferation. Moreover, HUVEC showed increased 2D migration potential with green light stimulation. The 3D migration was significantly enhanced by green and red light. In the 3D fibrin co-culture model, HUVEC elongation as precursor of vasculogenesis was enhanced by green and red light during the first 4 days while green light stimulation led to enhanced vasculogenesis after one week.

Both red and green light enhanced proliferation, migration and vasculogenesis processes while blue light was ineffective. Several parameters showed that green light was even more potent to stimulate regenerative processes than well-established red light therapy. Further studies have to focus on intracellular signalling induced by different wavelengths in order to optimize this promising, alternative application in tissue regeneration and wound therapy.

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Fast and label-free single cell analysis in regenerative medicine

Heidi Kremling et al.

Presenter: Heidi Kremling

Introduction: In the last years, the field of regenerative medicine have opened unforeseen therapeutic opportunities. In order to ensure functionality and safety of cell based therapies used for patient care, methods allowing reliable quality control during all steps of product development are essential. Raman Spectroscopy (RS) is a highly sensitive technology increasingly used for biomedical applications like cell identification and characterization, and quality control of cell based products as it reveals detailed information on the metabolic state of living single cells in a label-free and non-invasive way. Here, we demonstrate that RS is a valuable tool in regenerative medicine – to understand basic cellular processes, to ensure quality of cell based products and to improve current gold standard methods.

Materials and Methods: In a first experiment, RS was used to investigate stem cells isolated from adipose tissue (ASCs) and exposed to standard (21% O2) or hypoxic (5% O2) oxygen conditions. In a second application, Raman spectra were taken from fibroblasts, keratinocytes and melanocytes in a 3D human skin graft model. Last but not least, RS was used to monitor the quality of blood products, i. e. erythrocyte- and thrombocyte concentrates over 30 and 8 days, respectively.

Results: In hypoxia experiments, RS was able to discriminate ASCs grown under hypoxia and normoxia. Thereby, RNA expression level was found to be the main difference. Using a 3D skin graft model, assessment and discrimination of keratinocytes, fibroblasts and melanocytes was possible, also in depths of up to 200µm. In the last test, RS enabled quality control of blood products by monitoring cell death induced changes in erythrocyte and thrombocyte samples over time.

Discussion: We could provide evidence that RS is a suitable tool to analyze cells and cell based products used in regenerative medicine. As label-free method, RS provides highly specific molecular information about the entire metabolome of single cells. Thereby, this non-invasive approach only requires a few cells to provide new insights into basic cell functions or to speed-up and improve quality control of patient specific therapeutics.

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Spatially resolved determination of Glycosaminoglycan content in bone and cartilage

Sonja Gamsjaeger et al.

Presenter: Sonja Gamsjaeger

Bone and cartilage are connective tissues with distinct organic matrix composition. Proteoglycans (PGs), non-collagenous proteins, fulfill functions that are determined by their core protein and their glycosaminoglycan (GAG) chains, are negative modulators of mineral homeostasis, participate in the osteoblast-osteoclast cross-talk, are responsible for the mineral-free maintenance of the canalicular network, and are responsible for tissue water content (water binds to GAGs). Cartilage is present throughout the body at numerous anatomical locations and classified histologically as being hyaline, elastic or fibrocartilagenous, depending on the molecular composition. Cartilage PGs are essential for normal tissue function, and alterations in their abundance or structure can have significant consequences on cartilage formation in the embryo, growth plate function in the juvenile, and articular cartilage function in the adult. Moreover, the GAG content of PG molecules undergoes significant alterations as a function of healthy aging, tissue aging within the same subject, and disease (e.g. osteogenesis imperfecta, osteoarthritis).

The purpose of the present study was to identify Raman bands representative of GAG concentration and may be used in both bone and cartilage tissues. To achieve this we analyzed a series of reference PGs and collagens, as well as turkey leg tendon to verify the laser polarization independency of the identified bands. Additionally, the applicability of these bands in both cartilage and bone tissue simultaneously was tested in a healthy femoral head by Raman imaging and hierarchical cluster analysis to describe the distribution of GAGs at the micron-level from articular cartilage to subchondral bone. The results show that a band ~1375 cm\(^{-1}\) can be used as a GAG marker in both cartilage and bone. Moreover, articular cartilage has a lower content of organic matrix (mostly type II collagen), while the middle and deep transitional zone have a higher GAG content. The calcified cartilage is characterized by lower GAG content and total organic matrix.

In summary, Raman spectroscopy is able to spatially monitor GAG content in both hard and soft tissues in health and disease, thus valuable for the evaluation of tissue-engineered bone and cartilage.
Genetic morphometrics: discovering the influence of genes on morphology
Uwe Yacine Schwarze et al.
Presenter: Uwe Yacine Schwarze

Genes control bone development and functional adaptation to loading. There are, however, few methods to objectively assess these adaptations. With geometric morphometrics we now have the means to quantify morphological traits and evaluate the information how genes influence bone form. Differences in morphology, also relating to diseases, shape-independent size, asymmetry, and distances can be analysed. To do so, high resolution computed tomography data are used to obtain three-dimensional coordinates of wild type and genetically altered mouse skulls. Principal component or regression analysis is used for statistical evaluation and, at the same time, provides morphological information. Differences in shape or form are then visualized by exaggeration. We have applied geometric morphometrics on mice with knockouts of Sost, TREM2, Dkk1 and transgene of hTNF.

We introduce geometric morphometrics as a method to understand the anatomical implication of altered gene expression, thereby supporting the applicability of mice models to study human diseases.
Fibrin is a natural material, which is biocompatible, biodegradable, nontoxic and therefore, constitutes a suitable biomaterial for skeletal muscle tissue engineering. Another attractive feature is its tunability, which makes it an ideal material to mimic the stiffness of native skeletal muscle tissue. If mechanical stimulation is applied, fibrin fibrils align along the axis of strain, which favors a guided cellular structuring. In our 3D in vitro studies we were using a bioreactor system (MagneTissue) to apply mechanical stimuli to myoblasts, embedded in ring shaped fibrin scaffolds. We could observe myotubes with a more mature phenotype in terms of sarcomeric patterning, width and length after a strain of 10 % for 6 hours and 3 % for 18 hours on 6 consecutive days.

Furthermore, we investigated a positive effect of static strain on the myogenic differentiation concerning gene expression levels. Static strain enhanced the expression of myogenic markers MyoD and Myogenin as well as Troponin T. The latest experiments highlighted that both cyclic as well as static strain are positively influencing muscle maturation concerning gene expression levels of MHC and Troponin T. After a training within 9 days both stimuli showed higher aligned and mature muscle-like constructs. This could be demonstrated on the morphological level via immunofluorescence stainings. This novel bioreactor system provides a versatile tool for testing different training parameters and regimes, which can be individually adjusted, using a custom made software to engineer mature skeletal muscle-like tissue.

A future outlook is, to examine different mechanical stimulation protocols over 21 days to achieve even more mature skeletal muscle tissue constructs for putative transplantation.
Potential Confusion in Diagnosis of Muscle Weakness In Children with Distinctive Syndromic Entities

Ali Al Kaissi et al.

Presenter: Ali Al Kaissi

Potential Confusion in Diagnosis of Muscle Weakness In Children with Distinctive Syndromic Entities

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Seven children (six boys and one girl with average age of 8 years) were referred to our department because of diverse forms of skeletal abnormalities. Three children (two boys and one girl) were compatible with the diagnosis of progressive pseudorheumatoid chondrodysplasia (PPRC). The genetic mutation was correlated with the WISP 3 gene actively expressed by articular chondrocytes and located on chromosome 6. Klinefelter syndrome was the diagnosis in two boys. Karyotyping confirmed the 47, XXY (aneuploidy of Klinefelter syndrome). And two boys were finally diagnosed with Morquio syndrome (MPS type IV A) both showed missense mutations in the N-acetyl-6 sulfatase gene.
Relation of MRI utilization with hip replacements and knee replacements in the OECD

Robert Emprechtinger et al.

Presenter: Robert Emprechtinger

MRI is known as a diagnostic instrument suitable to show very small abnormalities. This, as well as the absence of the radiation burden, makes MRI an attractive, but costly imaging procedure. Apart from that, the diagnostic performance in osteoarthritis is mediocre. Hence, the American college of radiology (ACR) does not recommend MRI for the diagnosis of osteoarthritis of the knee and it only recommends MRI under certain conditions for the diagnosis of osteoarthritis of the hip. Osteoarthritis is the main reason for hip or knee replacements. We evaluated whether there is evidence that the recommendations are being followed using data provided by the OECD. According to the recommendations of the ACR, we expected a small relation between MRI and the number of hip replacements and no relation between MRI and the number of knee replacements.

We computed two multiple regressions with the number of MRI exams, the number of CT exams, and the health care spending as predictors for either hip or knee replacements.

Contrary to what we expected, we found a statistically significant effect of the MRI utilization on the number of knee replacements ($p = 0.025$), but not on the number of hip replacements ($p = 0.68$) when the variables of CT utilization and health care spending were held constant. Our results indicate that an increase of 10 MRI exams per 1 000 population results in 8.39 additional total knee replacements per 100 000 population. If these replacements are in fact caused by an overutilization of MRI and not by a third variable that was not assessed in our analysis, these additional and potentially unnecessary knee replacements could be avoided if the ACR guidelines were being followed.
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