Poster Session – Day 1
Thursday, 13th June, 14:45 – 16:00

Group 1: 1 - 14
Thematic Program: Inflammation and Immunity

(P1) NKG2D on mature NK cells is dispensable for the control of murine Abelson-induced leukemia

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NKG2D is an activating receptor expressed on a wide range of immune cells including NK, NKT, [gamma][delta]T cells, activated CD8[up+] T cells and activated macrophages. It has been implicated in autoimmune disease, infection and cancer. Transformed cells express ligands for NKG2D making tumor cells visible for NK cell mediated tumor surveillance. In line NKG2D[up-/-] mice are tumor prone and develop more aggressive tumors. Meanwhile, numerous escape mechanism for NKG2D-mediated cytotoxicity have been described. A new twist was added, by showing that persistent receptor-ligand interaction downregulates the NKG2D receptor and impairs NK cytotoxic functionality. Importantly, it was found that tumor stoma cells also express NKG2D ligands contributing to the desensitization of NK cells accompanied by decreased tumor surveillance. A recent report also showed, that NKG2D sets the activation threshold early in NK cell development by restricting NKp46 dependent signal transduction.

NK cells are of particular importance for surveillance of hematopoietic cancers. It is unclear if and how NKG2D-dependent receptor-ligand interactions interfere with tumor surveillance of hematopoietic malignancies as both, stromal cells and cancer cells express NKG2D ligands. To study this complex NK cell mediated relations we employed a mouse model specifically deleting the NKG2D locus (KLRK1) in NK cells under the control of the NKp46 promotor (NKG2D[up[Delta]NK] mice). We find that NK cells lacking the NKG2D receptor display no major differences in expression of effector molecules in-vitro and ex-vivo. In contrast to other tumor models, survival of NKG2D[up[Delta]NK] mice is comparable to that of NKG2D[upfl/fl] littermates in a model of slowly evolving B cell leukemia despite ligand expression by tumor cells. Our data underscores the complexity of NKG2D receptor mediated effects on tumor surveillance and the need to further investigate its heterogeneous relation in anti-tumor immunity.

(P2) Effect of Epidermal Growth Factor Receptor inhibition on tumor cell metabolism

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Efficiency of Epidermal growth factor receptor (EGFR) inhibition in cancer treatment has primarily been reported to impinge on tumor cell proliferation and survival. It was demonstrated that EGFR can either directly act on the metabolism of a cell by phosphorylation of rate limiting enzymes or indirectly via activation of MYC and AKT. For example, EGFR plays an important role during alteration of glycolysis in many different cancers or on lipid metabolism in glioblastoma.
Recently, EGFR inhibitors were shown to lead to reversion of the Warburg phenotype. However, in hepatocellular carcinoma (HCC) and colorectal cancer (CRC) it was demonstrated that EGFR is tumorigenic when expressed in tumor-associated macrophages/myeloid cells. It is presently unknown how EGFR expression is upregulated in tumor-associated myeloid cells/macrophages and whether factors secreted by tumor cells are important in this process.
This research project will aim to investigate the metabolic changes induced by EGFR inhibition in tumor cells and whether these changes can modulate the transition from non-tumorigenic EGFR- myeloid cells to pro-tumorigenic EGFR+ myeloid cells. Therefore, the metabolic changes induced by EGFR inhibition in tumor cells will be analyzed to compare the metabolic phenotypes of CRC treated either with EGFR inhibitors or harboring genetic deletion of EGFR in tumor cells to untreated CRC and/or normal colonic epithelium. Moreover, the effect of EGFR-mediated starvation on innate cell recruitment in vivo will be evaluated. Finally, the findings will be validated in human patient material.
By determining the metabolites and metabolic pathways altered by EGFR inhibition, a gain of information is expected that will presumably further explain how immune infiltrating cells are affected and might pose a potential second hit target for future therapeutics.

(P3) Mechanisms of tumor killing by activated Plasmacytoid Dendritic Cells

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pDCs are specialized and fast type I IFN-producers after TLR7/8 stimulation with ssRNA or synthetic agonists such as Imiquimod (IMQ). In tumors, pDCs have a controversial function as immune mediators. In this scenario, pDCs can be poor antigen-presenters and immune-suppressive; thus, pDCs are considered as a marker of poor prognosis in several tumor types. On the other hand, if properly stimulated, pDCs can boost the tumor regression. Accordingly, it has been shown previously by our lab that pDCs can acquire tumor killing effector properties by production of cytotoxic and pro-apoptotic molecules in an orthotopic melanoma mouse model after IMQ stimulation and in an IFNR1-dependent manner but without the need of the adaptive immune system.
Activation of pDCs by IMQ and their education to become tumor-suppressive effector cells is a promising strategy but more mechanistic investigations are needed. To define expression patterns that correlate with acquisition of killing capacity we are currently performing a genome-wide RNA expression profiling to generate expression signatures of pDCs at various time points after IMQ stimulation *in vitro* and *in vivo*. So far, pDCs have been purified from 7-day-old Flt3L-dependent BM cultures of C57Bl/6-WT mice and activated with IMQ at different time points (6h, 16h). These results will be important to identify the most optimal spatio-temporal expression signature conferring maximal tumor-killing capacity to pDCs. They will also reveal how well *in vitro*-derived pDCs represent the *in vivo* situation and how the presence of tumor cells modulates the expression profile of pDCs. Most importantly, the differentially expressed genes identified here will also serve as a basis to construct a focused shRNA-based library targeting the most promising genes identified in the RNA profiling analysis. The shRNA library will be tested *in vitro* in a killing-assay standardized in our lab, which already allowed us to successfully demonstrate that TLR7 and IFNAR1 signaling are important to confer the tumoricidal phenotype to pDCs.

(P4) Chromatin Dynamic Immune regulation of anti-tumor functions of plasmacytoid Dendritic Cells

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Plasmacytoid dendritic cells (pDCs) are innate immune cells with the unique capacity to produce big amounts of antiviral type I IFN, and are implicated in several inflammatory diseases, like psoriasis, Systemic Lupus Erythematosus and in immune regulation of cancer (1,2). The TLR7/8 agonist Imiquimod (IMQ) is able to activate pDCs and transform them into killer cells capable of killing tumor cells. This anti-tumor immunity of pDCs is independent of the adaptive arm of the immune system but requires TLR7 and IFNAR signalling (3). On the other hand previous studies have shown a pro-tumorigenic function of pDCs in several different types of cancer, making them a potential target for immunotherapy. So far, it is still unclear how this functional switch from pro- to anti-tumorigenic pDCs is regulated. Also, very little is known about the role of epigenetic chromatin regulation of
pDCs function. Existing studies relied on the use of Histone Deacetylases (HDAC) inhibitors (4,5), identifying HDACs as important factors for pDC development and function in vitro. We are going to employ genetically engineered mouse models (GEMMs), which harbour the HDAC1/2 floxed allele, either under control of the dendritic cell (DC) specific CD11c or the interferon responsive, inducible Mx1 promoter. For an initial look on the epigenetic regulation of pDCs after IMQ stimulation, we used Romidepsin, a class I HDAC inhibitor with more specificity for HDAC1 and 2, before IMQ stimulation, showing that HDAC inhibition causes a defect on pDC maturation. Also, co-culture of pDCs with B16 melanoma cells in vitro showed that HDAC inhibition leads to reduction of pDC's anti-tumor effector molecules. In vivo, in an orthotopic melanoma tumor model treated with 5% IMQ cream (Aldara), pDCs are significantly increased in the tumors and their draining lymph nodes. Altogether, our results point for a function of HDACs in the IMQ induced pDC immune response.

(P5) EGFR and Innate Cell Metabolism

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EGFR is a transmembrane protein that functions as a tyrosine kinase receptor for many ligands, including the Epidermal Growth Factor (EGF) family. It has been found to be deregulated in a variety of cancer types such as lung, breast cancer and glioblastoma (Yarden and Sliwkowski 2001). In metastatic colon cancer, EGFR inhibitors are the first-line therapy for patients with wild type KRAS. Although EGFR is detected in these tumors, the response of the patients does not correlate with its expression (Porru et al. 2018). Previous work from our lab showed that EGFR expression in myeloid cells has a tumor promoting role in colorectal cancer (Srivatsa et al. 2017). This was also confirmed by other groups which additionally showed decreased Arginase (Arg1) and Nitric Oxide Synthase (iNOS) expression in Bone Marrow Derived Macrophages (BMDMs) that lacked EGFR signaling, indicating a link to the metabolic and polarization status of these cells (Hardbower et al. 2017).
It is currently unknown how EGFR signaling affects the metabolic behavior of myeloid cells that are present in tumors. Importantly, we will try to unravel under which circumstances EGFR is expressed and activated in myeloid cells of the tumor microenviroment. For our studies we will use a Egfrf/f; LysM-Cre mouse model in which EGFR can be depleted specifically in myeloid cells while for a more specific deletion of EGFR in macrophages in an inducible manner we will also use the Csf1R-CreERT2 model. Using subcutaneous tumors of a C57BL6 colon adenocarcinoma cell line (MC38) we will isolate macrophages and investigate as a starting point how EGFR influences the expression of key glycolytic genes, glucose consumption and lactate production. We will also evaluate our results in sorted macrophages from colorectal tumors of the genetically induced ApcMin/+ or of the chemically induced azoxymethane (AOM) mouse model. Moreover, we will perform RNA sequencing on these macrophages to investigate how EGFR alters key pathways that are related to glycolysis, oxidative phosphorylation and fatty acid metabolism. We will try to correlate these differences to macrophage polarization and function and we aim to identify how these changes affect growth of the surrounding tumor cells.

(P6) The role of allergen-specific IgG antibodies in the induction of clinical tolerance for the birch pollen-related apple allergy

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Background Birch pollen-related apple allergy [BPRAA] results from immunological cross-reactivity between the major birch-pollen allergen Betv1 and its apple-homolog Mald1. Yet, birch pollen-immunotherapy has no convincing benefits on BPRAA. We recently showed in a double-blind placebo-controlled pilot study that 16 weeks of sublingual immunotherapy with recombinant [r] Mald1 [rMald1-SLIT] significantly improved BPRAA, whereas rBetv1-SLIT did not. To investigate the immune mechanisms underlying the induction of clinical tolerance to apple we characterized the levels, blocking capacity, and primary specificity of SLIT-induced Mald1-specific IgG antibodies [Abs].Methods Serum levels of Mald1-specific IgG subclasses were measured by ELISA and ImmunoCAP, respectively. The presence of IgE-blocking Abs in post-SLIT sera was evaluated as their ability to inhibit rMald1-induced activation of basophils. IgG1 and IgG4 Abs were depleted from post-SLIT sera samples to study their contribution to IgE blocking. The primary specificity of Mald1-specific IgG4 Abs was assessed by competition ELISA.Results Mald1-specific IgG1, IgG2 and IgG4 significantly increased during rMald1- and rBetv1-SLIT. Mald1-induced basophil activation was only inhibited by post-rMald1-SLIT sera, and preliminary data suggest that this blocking potential was mediated by both IgG4 and IgG1 Abs. Pre-incubation of post-rMald1-SLIT sera with rMald1 but not with Betv1 completely abrogated IgG4-binding to rMald1. Pre-incubation of post-Betv1-SLIT sera with rMald1 and rBetv1 completely abrogated IgG4-binding to rMald1, suggesting that these Abs bind to common cross-reactive epitopes on both allergens.
Conclusion Both Mald1- and Betv1-SLIT induced Mald1-specific IgG Abs. Yet, only post-Mald1-SLIT sera prevented Mald1-induced mediator release and contained IgG4 Abs primarily specific for Mald1. We conclude that Mald1-SLIT induces Mald1-specific Abs with blocking activity, mediating clinical tolerance to apples.

(P7) The ERBB-STAT3 Axis Drives Tasmanian Devil Facial Tumor Disease

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The marsupial Tasmanian devil (Sarcophilus harrisii) faces extinction due to transmissible devil facial tumor disease (DFTD). To unveil the molecular underpinnings of DFTD, we designed an approach that combines sensitivity to drugs with an integrated systems-biology characterization. Sensitivity to inhibitors of the ERBB family of receptor tyrosine kinases correlated with their overexpression, suggesting a causative link. Proteomic and DNA methylation analyses revealed tumor-specific signatures linked to oncogenic signaling hubs including evolutionary conserved STAT3. Indeed, ERBB inhibition blocked phosphorylation of STAT3 and arrested cancer cells. Pharmacological blockade of either ERBB signaling or STAT3 prevented tumor growth in a xenograft model and resulted in recovery of MHC class I gene expression. This link between the hyperactive ERBB-STAT3 axis and MHC class I-mediated tumor immunosurveillance provides mechanistic insights into horizontal transmissibility and led us to the proposition of a dual chemo-immunotherapeutic strategy to save Tasmanian devils from DFTD.

(P8) The role of the Protocadherin CDHR5 in intestinal tissue homeostasis and CRC

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(P9) Characterization of the affinity of Mal d 1-specific antibodies induced by sublingual immunotherapy with recombinant Bet v 1 or Mal d 1

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High structural similarity between the major birch pollen allergen Bet v 1 and the major apple allergen Mal d 1 results in immunological cross reactivity, causing allergic reactions to apple in 70% of birch pollen-allergic individuals. While birch pollen immunotherapy induces clinical tolerance to birch pollen, its effect on birch pollen-related apple allergy (BPRAA) is controversial. Bet v 1_sensitized individuals with BPRAA were treated with sublingual immunotherapy (SLIT) using either recombinant (r)Bet v 1, rMal d 1 or placebo for 16 weeks to investigate the clinical effect on BPRAA and to study underlying immune mechanisms. Significant clinical improvement to apple was recorded in rMal d 1-treated individuals, in contrast to those who received rBet v 1 or placebo. Successful SLIT has been associated with the induction of allergen-specific IgG4 antibodies with IgE-blocking activity. Notably, rMal d 1-specific IgG4 antibodies were found in post_SLIT sera of both verum groups, however, only those induced by rMal d 1-SLIT showed IgE blocking capacity. Our aim is to investigate whether functional IgE blocking is dependent on the affinity of rMal d 1-specific antibodies. To this end, rMal d 1-specific IgG4 antibodies contained in post-SLIT sera of both treatment groups were compared for their binding strength to rMal d 1 in an ELISA-based avidity protocol. This rough approach revealed a tendency for a stronger avidity of rMal d 1-specific IgG4 antibodies of the rMal d 1-SLIT group. Currently, more sensitive and advanced techniques are being established to confirm these findings, to shed light on binding characteristics and to observe the kinetics of the binding reaction. Subsequently, we will correlate the binding behavior with clinical efficacy of SLIT. Thereby, we will gain a deeper understanding of the prerequisites for functional IgE-blocking and the immune mechanisms of tolerance induction in allergy treatment.

(P10) An anti-inflammatory role for ALK3 in Langerhans Cells

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Langerhans Cells (LCs) represent the epidermal contingent of dendritic cells (DCs). Development and maintenance of the LC network is critically dependent on the cytokine TGF-β1. Using in vitro differentiation models, our group previously demonstrated that human LC differentiation can be induced by bone morphogenetic protein (BMP)-7, another member of the TGF-β superfamily. Conversely, silencing of BMP receptors in TGF-β 1-driven LC-cultures resulted in significantly decreased numbers of LCs. In order to investigate the role of BMP signaling in LCs in vivo we generated a number of conditional knock-out mouse models.
In ALK3Δvav-mice, the BMP receptor type 1 (BMPR1a, ALK3) is deleted already in early LC progenitors. Analysis of the epidermis of these mice showed a reduced number of $CD207^+$ cells and decreased expression of CD207 in existing LCs. Furthermore, bone marrow from these mice generates reduced DC numbers in GM-CSF/TGF-β 1-cultures.
In ALK3ΔCD11c-mice, ALK3 is deleted only in late stages of DC/LC differentiation. In the steady state, these mice display no changes in the LC network. However, in two different models of skin inflammation ALK3ΔCD11c-mice showed stronger and longer lasting inflammation. A migration assay showed that LCs from these mice emigrate faster than from wildtype mice with exfiltrated LCs showing a higher expression of MHCII and CD86.
Therefore, in line with our in vitro findings we conclude that ALK3 plays a critical role in the early steps of LC differentiation, but is dispensable for the maintenance of the already established LC network. Furthermore, we identified a previously unknown role of ALK3 in DCs/LCs in counteracting and resolving skin inflammation. Further investigation of these anti-inflammatory properties could yield new insights not only in LC biology but also provide new targets for therapies in inflammatory skin diseases.

(P11) The role of RNA modification in innate immunity and inflammation signaling

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Pathogen associated molecular patterns (PAMPs) such as nucleic acids are recognized by pattern recognition receptors (PRRs) with high specificity. Interestingly, nucleic acids from fungal pathogens can also be specifically recognized by TLRs such as TLR7/TLR9, thereby triggering proinflammatory type I interferon (IFN) responses, which can be both protective and detrimentale. Unique features such as cap structure, double strandedness, base content or nucleotide modifications help to distinguish self from non-self.
Adenosine to inosine deamination by ADARs is the most abundant type of RNA editing in metazoans. A-to-I RNA editing has been shown to play an essential role in mammalian embryonic development and tissue homeostasis, and is implicated in the pathogenesis of many diseases including skin pigmentation disorder, autoimmune and inflammatory tissue injury, neuron degeneration, and various malignancies. ADAR1 is constitutively expressed but also has a IFN-inducible isoform. Mice lacking ADAR1 show embryonic lethality at day 12.5, accompanied by a strong IFN signature. Interestingly, ADAR1 deficiency is rescued by ablation of MDA-5 or MAVS, both of which sense viral RNAs and proper balance is also required during self-versus non-self-discrimination by innate immune receptors, such as melanoma differentiation-associated gene 5 (MDA5). Of note, oligonucleotides with consecutive IU base pairs can suppress IFN signaling in ADAR1-deficient cells. This suggests that inosines in endogenous RNAs prevent immune signaling through MAVS. We hypothesize that cellular double-stranded RNAs can activate MDA-5 in the absence of inosines. Here we aim to understand how Inosine prevents the activation of MDA-5 signaling and to identify specific RNAs that can activate MDA-5. We will identify MDA5 associated RNAs by an RNase-protection by MDA5 followed by RNA-seq. In parallel we will test how inosine in dsRNA can prevent multimerization of MDA5 on dsRNA substrates.

(P12) Oncogenic germline mutations of TYK2 in acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is a frequently diagnosed cancer in children. Recently, two germline tyrosine kinase 2 (TYK2) activating mutations were reported in pediatric patients developing multiple ALL forms. The TYK2 mutations are located at the highly conserved ATP binding site within the pseudokinase domain and attenuate the inhibitory function of this domain. As a consequence, TYK2 is constitutively phosphorylated and activates downstream STAT signaling. Our goal is to study the oncogenic potential of these TYK2 mutations and to gain insight into the interactome of constitutive active TYK2.
In a first step the described germline mutations (P760L, G761V) were introduced into murine and human TYK2 cDNA. Upon expression in cell lines constitutive phosphorylation of TYK2 and downstream STAT 1, 3 and 5 was observed. Surprisingly, only human mutated TYK2 exhibited transforming capacities in IL-3 dependent murine cell lines. Injection of TYK2-transformed cells caused cancer formation in immunodeficient mice. Treatment with TYK2 inhibitors reversed the strong activation of STATs and inhibited growth of TYK2-expressing cells in vitro.
Having proven the oncogenic potential of the TYK2 germline mutations, we extend our research to human ALL cell lines or primary thymocytes and bone marrow expressing mutant TYK2. Finally, we will generate a mouse with tissue-specific expression of human mutated TYK2. These disease mimicking tools will allow to define the interaction partners of oncogenic TYK2 and will be important for understanding the molecular pathology of the genetically predisposed ALL patients. Ultimately, we will test the efficacy of TYK2 inhibitors in vivo which may be applicable for treatment of these genetically predisposed ALL patients.

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(P13) Loss of bile salt export pump (Bsep/Abcb11) aggravates lipopolysaccharide induced hepatic inflammation in mice

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Bsep is the main canalicular transporter for biliary bile acid (BA) secretion. Although lack of BSEP causes severe cholestasis in human, Bsep KO mice are protected from acquired cholestasis because of metabolic preconditioning with a hydrophilic BA pool. This study aims to investigate whether a hydrophilic BA pool may counteract development of hepatic inflammation. Wild-type (WT) and Bsep knockout (KO) mice were challenged with lipopolysaccharide (LPS) to induce hepatic inflammation. Serum biochemistry and liver histology were assessed. Hepatic markers of inflammation were analyzed by qPCR, Western blotting and immunohistochemistry. Bsep KO mice developed more severe LPS-induced liver inflammation than WT mice. LPS treatment increased serum levels of ALT and AST in Bsep KO mice compared to corresponding control group ($p \leq 0.05$), but remained unchanged in WT mice. AST, ALT and AP were elevated 6fold, 3fold, 3fold in LPS treated Bsep KO mice compared to challenged WT mice, respectively $p \leq 0.05$). NF-kB was significantly higher in LPS treated Bsep KO mice compared to WT mice at mRNA 1.7fold, $p \leq 0.05$) and protein level (1.8fold, $p = 0.052$). Inflammation was reflected by elevated mRNA expression of proinflammatory cytokines Vcam (1.3fold), Icam (1.7fold), IL1b (2fold), Mcp1 (2.5fold), Tnfa (2.7fold), IL6 (3.2fold) and iNos (5fold) ($p \leq 0.05$). Immunohistochemistry of MAC2 showed increased immune cell number in livers of Bsep KO compared to WT mice. Ppara and Nrf2 were significantly reduced upon LPS challenge in Bsep KO mice at RNA level 60%, 40%, respectively, $p \leq 0.05$). Fxra was down-regulated in Bsep KO mice at mRNA level 53%, ($p \leq 0.05$). Of note, Cyp2b10 was reduced by 53% ($p \leq 0.05$) in Bsep KO LPS treated mice compared to Bsep KO controls and by 40% compared to WT LPS challenged mice. Absence of Bsep aggravates hepatic inflammation upon LPS, potentially by reduction of anti-inflammatory signaling via Fxra, Ppara and Nrf2 as well as reduction of BA detoxification.

(P14) The Kinase Inhibitor BX-795 is a TCR signaling modulator that stimulates IL-2 while attenuating Th2 response

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BX-795, is a putative inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK1), TANK-binding kinase-1 (TBK1) and inhibitor of kappa B kinase epsilon (IKK□□, involved in T cell receptor (TCR) and innate receptor signaling. We investigated the immunomodulatory effects of BX-795 on splenocytes of TCR/DR1 double transgenic allergy mice, human polyclonal T cells and in a murine *in vivo* model of allergic asthma. We assessed viability, activation marker expression and transcription factor activity of T cells by flow cytometry and proliferation by methyl-^{3}H-thymidine uptake upon TCR ligation. Cytokine secretion was measured by multiplexing of culture supernatants, RT-qPCR and intracellular cytokine staining. Most notably, BX-795 strongly inhibited secreted IL-4 and IL-13 (92.6% and 83.9% mean reduction, $p<0.05$) levels after 72 hours in murine allergen-specific splenocytes. Although non-significant, BX-795 showed a clear trend to reduce secreted IL-5, IL-10, TNF-□ and IFN-□ (66.0%, 94.8%, 76.0% and 94.5% mean reduction) levels. Interestingly, Th17 cytokines were not reduced while IL-2 levels were strongly increased peaking at 72 hours (4.3-fold, $p<0.05$). Similar results were derived from hPBMCs stimulated with superantigens *via* their TCR. Upon TCR triggering in Jurkat T cells, BX-795 increased secreted IL-2 levels as well as NFAT (ns), NF□B ($p<0.05$) and AP-1 ($p<0.05$) reporter activity, and IL-2 mRNA levels (2.3-fold, $p<0.01$) while IFN-□ mRNA levels remained unchanged upon TCR triggering. In a murine *in vivo* model of house-dust mite-induced allergic asthma, BX-795 significantly decreased relative numbers of lung $CD3^{+}IL\text{-}13^{+}$ T cells and eosinophils along with $CD3^{+}CD4^{+}GATA\text{-}3^{+}$ T cells (35.9%, 52.5% and 60.5% mean reduction, $p<0.05$) while numbers of $CD3^{+}IFN\text{-}□^{+}$ T cells remained unchanged. In summary, BX-795 and related compounds might represent a novel treatment modality for Th2-associated diseases such as allergic asthma. Future studies will provide a better understanding of the responsible signaling pathways altered by BX-795 upon TCR stimulation.

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Group 2: 15 - 27
Thematic Programs: Immunology and Allergology

(P15) BET V 1-SPECIFIC ADAPTIVE IMMUNE RESPONSES IN MICE AND MAN

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More than 100 million patients worldwide are allergic to the major birch pollen allergen, Bet v 1. These patients suffer from respiratory allergy to pollen from birch and related trees as well as from oral allergy syndrome caused by plant food due to IgE and T cell cross-reactivity. Since mouse models are frequently used as mimics of human disease we analyzed the adaptive immune response in BALB/c mice which were sensitized classically with Aluminum-hydroxide-adsorbed Bet v 1. Such as allergic patients, sensitized mice developed cross-reactive IgE antibodies to related pollen and food allergens and showed immediate allergic reactions as demonstrated by basophil degranulation. However, IgE antibodies of sensitized mice were directed against C-terminal sequential peptide epitopes whereas allergic patients IgE recognized only intact folded allergen. In mice IgG recognized the same sequential epitopes as IgE whereas patients IgG was also directed against sequential epitopes. The mapping of T cell epitopes using CFSE-dilution and six peptides spanning the Bet v 1 sequence indicated the presence of T cell epitopes at the Bet v 1 C-terminus but also to other peptides as in allergic patients. Our results thus demonstrate profound differences regarding the antibody recognition of Bet v 1 in sensitized mice and allergic patients. Thus it is not possible to directly compare mice and patients regarding mechanisms of class-switching and antibody-mediated inflammation or treatment approaches whereas the similarities regarding T cell recognition may allow studying general mechanisms of T cell-mediated tolerance in mice.

(P16) Bi-specific antibody conjugates binding ICAM1 and allergens prevents transepithelial allergen migration and rhinovirus infection

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Passively administrated human monoclonal allergen-specific IgG antibodies significantly reduce allergic symptoms in allergic patients. However, the efficacy of systemic antibody-based treatment depends on the ability of antibodies to block patient's IgE binding to allergen. To develop a non-invasive topical form of treatment with the aim to prevent the penetration of allergens through the respiratory epithelial barrier, we hypothesized that such a treatment can be performed even with an allergen-specific IgG antibody which does not inhibit allergic patients' IgE binding to the allergen.
Antibody conjugates bispecific for the major grass pollen allergen Phl p 5 and ICAM1, a major group rhinovirus receptor on respiratory epithelial cells, were generated by coupling a monoclonal Phl p 5—specific, IgE non-blocking antibody and ICAM1-specific antibody. Immobilization of allergens on the surface of the human bronchial epithelia cell line 16HBE14o- by these conjugates was investigated by immunofluorescence microscopy and FACS analysis. The ability of the conjugates to inhibit transepithelial allergen migration through 16HBE14o- cell monolayers and basofiel activation was examined. The capacity of the conjugates to prevent rhinovirus infection of 16HBE14o- or HeLa cell monolayers was tested. The engineered bi-specific antibodies immobilized Phl p 5 on the surface of epithelial cells, significantly reduced allergen penetration and decreased allergen-induced basophil activation. In addition, antibody conjugates prevented rhinovirus infection of 16HBE14o- and HeLa cells. Our study shows that antibody conjugates consisting of allergen-specific, IgE non-blocking antibodies and ICAM1-specific antibodies prevent allergen penetration trough respiratory epithelium, allergic inflammation as well as rhinovirus infections and may be used for treatment of allergen- and rhinovirus-induced respiratory diseases.
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(P17) Soluble Fcε RI disrupts cell-bound chimeric IgE

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Soluble FcεRI is a truncated form of FcRI which is released upon receptor activation by antigen cross-linking and is able to bind circulating IgE. Its physiological role in allergic reaction is still incompletely understood. We aimed to assess the effect of sFcRI as compared to anti-IgE antibody (omalizumab) on cell-bound IgE. MelJuSo (human melanoma-derived) cells expressing FcRI-/- and loaded with chimeric IgE (cIgE) over night were stimulated with sFcRI/omalizumab (1 µM) for 1.5-6 hours, or treated for three days with cIgE (6 ng/mL) and sFcRI/omalizumab (62.5 nM) per day. Cell bound cIgE was detected by flow cytometry. Cell-bound cIgE is cleared 3 hours after application of sFcRI and reaches 41±13% of disruption at 6 hours. After 24 hours 89±8% disruption of cIgE:FcRI complexes is achieved and subsequent application increases disruption level despite the constant exposure to cIgE. Results are similar for both cell types, and comparable to the effect of omalizumab in the same conditions. As soluble FcRI shows substantial and fast cell-bound cIgE disruption function, we speculate on its role as a negative feedback modulator of allergic reactions. Whether sFcRI is an intrinsic factor that discriminates patients with same IgE levels and different clinical symptoms is still to be revealed.

(P18) Fast production of human monoclonal antibodies (IgE, IgG1 and IgG4) against beta-lactoglobulin by PIPE cloning

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IgE-mediated milk allergy is a serious threat for children during their first years of life. Understanding how milk allergy is developed and how tolerance against cow milk can be re-established is pivotal. Antibodies specific for beta-lactoglobulin (BLG), one of the major milk allergens, are therefore useful tools in this research question. The latest approach to produce human monoclonal antibodies is Polymerase Incomplete Primer Extension (PIPE) cloning (Ilieva et al., 2017). In this study, we aimed to produce IgE, IgG1 and IgG4 antibodies specific for the major milk allergen beta-lactoglobulin by Polymerase Incomplete Primer Extension (PIPE) cloning. Vectors of the antibodies, containing the variable region against BLG (Jylhä et al. 2016) were assembled through PIPE cloning, transformed into E. coli and transfected into Expi293F cells for expression. The newly produced antibodies were purified with affinity chromatography, their assembly was controlled by SDS-PAGE and their specific binding to BLG was investigated by dot blot, ELISA and ISAC 112 allergen microarray. Furthermore, the function of IgG4 was checked by inhibition immunoassays for its IgE-blocking capacity. We could harvest 2.2 mg of IgE, 0.8 mg of IgG1 and 1.9 mg of IgG4 antibodies after one transfection (30 ml). All antibodies were correctly assembled and their specificity to BLG was confirmed in dot blot and in an allergen microarray with 112 allergens. Concentration-dependent specific binding of all antibodies was observed in ELISA. Additionally, the binding of IgE to BLG was significantly inhibited by IgG4 antibodies in a concentration-dependent manner. In summary, we could produce high amounts of anti-BLG IgE, IgG1 and IgG4 antibodies with high specificity using the PIPE cloning method. The fully functional antibodies can be applied to investigate the mechanism underlying milk allergy. Furthermore, they can also be useful for quantifying BLG levels in milk products.

(P19) Establishment of surrogate ELISA-based assays for studying rhinovirus-receptor interactions

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Rhinoviruses (RVs) are the major cause of the common cold, a usually mild disease, but they can also trigger severe respiratory illnesses such as exacerbations of asthma and chronic obstructive pulmonary disease (COPD). So far, no prophylactic and/or therapeutic vaccines have been developed for RV infections. Conventional neutralization assays used to determine the ability of antibodies to inhibit the binding of RVs to their human target cells are laborious and time-consuming because they are tissue culture-based and require human target cells and infectious virus. Therefore, our goal was to develop an ELISA-based surrogate assay to study the interaction between RV strains representative for the major and minor RV groups and their corresponding cellular receptors, ICAM-1 and LDLR, respectively.
ELISA microplates were coated with recombinant human ICAM-1 or LDLR, respectively and different amounts of purified RV stains representative for major and minor groups were added to the plates. The binding of RVs to their receptors was detected by RV-strain specific antisera. Furthermore, the ability of ICAM-1 and LDLR-specific antibodies to inhibit the binding of major and minor RV strains to their corresponding receptors was tested. We found that major and minor RV strains can specifically bind to their corresponding cellular receptors in an ELISA-based assay and that the binding can be inhibited using anti-ICAM-1 and anti-LDLR specific antibodies. Thus, the developed surrogate ELISA assay can be used to test the ability of RV-specific antibodies to inhibit the attachment of RV strains to their receptors which is a crucial first step during RV infection. The ELISA assay may therefore be useful for the development of passive and active vaccination strategies for RV infections and the identification of anti-RV compounds.

(P20) Influence of obesity on the development of allergy and mucosal tolerance

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Along with allergic diseases obesity is a serious health problem that is remarkedly increasing in prevalence worldwide. Obesity is associated with several disorders and dysfunctions including impairment the immune system. It is known that hypertrophic adipocytes and expanded populations of proinflammatory immune cells cause inflammation in the adipose tissue. Obesity has been recognized as an important risk factor for enhanced allergic diseases, such as allergic rhinitis or asthma. However, the causal link between obesity and allergy has not yet been fully elucidated.
The aim of this study is to investigate whether obesity is associated with a changed susceptibility to allergic sensitization and induction of mucosal tolerance.
4-5-week-old C57BL/6 mice were fed on high-fat diet or standard diet. After 9 weeks on respective diet, mice were immunized with ovalbumin (OVA). Body weight was measured once a week and fasting blood glucose level was measured every second week. To evaluate the lung function, airway hyperresponsiveness (AHR) was assessed. Determined metabolic parameters included serum insulin, leptin and amyloid P. Immunological analyses consisted of allergen-specific antibody detection in sera, investigation of immune cell populations in bronchoalveolar lavage (BAL), lung, spleen and intestine (by FACS), determination of cytokine production in bronchial lymph nodes, spleen, lung, and BAL by ELISA.
Based on the data obtained so far, we have shown that high-fat diet induced obesity in C57BL/6 mice and thus we implemented this model for subsequent analyses. We now aim to determine how obesity may cause changes in humoral and cellular immune responses compared to responses in lean mice. This might enable us to gain better understanding of how the obesity influences the development of allergy as well as whether the susceptibility to mucosal tolerance induction is altered under this condition.

(P21) Suppression of experimental allergic airway inflammation by nasal application of probiotic E. coli O83

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In clinical trials oral supplementation with probiotic bacteria prevented the development of certain types of allergic diseases such as atopic dermatitis but exerted only moderate effects on allergic lung inflammation. We hypothesized that nasal delivery of probiotic bacteria might be more effective in protection against allergic airway disease.To test this, wild-type or TLR4-deficient mice were treated intranasally with E. coli O83 at the time of allergic sensitization and challenge with ovalbumin (prophylactic approach) or only after sensitization and before allergen challenge (therapeutic approach). Prophylactic intranasal application of E. coli O83 suppressed airway hyperreactivity (AHR), eosinophilic infiltration and Th2 responses in the lung and this effect required signaling via TLR4. Mechanistically, intranasal exposure to E. coli O83 triggered IL-1[beta] and IL-23 production in the lung and induced pulmonary IFN[gamma]/IL-17A+ [gamma][delta] T cells in a TLR4-dependent manner. Importantly, therapeutic intranasal treatment of allergen-sensitized mice with E. coli O83 reduced AHR and Th2 immunity in the airways. This study provides experimental evidence that intranasal application of E. coli O83 can suppress airway allergy, in both, a prophylactic and therapeutic setting. The protective effect of E. coli O83 was associated with the TLR4-dependent recruitment of [gamma][delta] T cells to the lung. IL-1[beta] and IL-23 induced by E. coli O83 might play an important role in suppressing allergic inflammation by promoting cytokine production by [gamma][delta] T cells. Collectively, our findings show both proof of concept and mechanistic insight into probiotic bacteria-mediated suppression of allergic airway inflammation. Supported by the Austrian Science Fund (FWF) grants SFB F4609, Doctoral Program MCCA W1248-B13, an FWF Erwin Schroedinger Fellowship J3332-B21 and the OeAD grants CZ 15/2015 and CZ 13/2016.

(P22) Neutrophils promote T-cell-mediated inflammation in allergy.

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Lately we identified HLA-DR-positive neutrophils in allergen-induced cutaneous late phase reactions (LPR) in vivo. Moreover, we demonstrated in vitro that allergen-pulsed neutrophils from allergic individuals that had been activated with cytokines present in the allergic LPR were capable of inducing proliferative and cytokine responses of HLA-DR-restricted allergen-specific CD4+ T cells. Notably, HLA-DR-positive neutrophils did not express CD80 and CD86 which represent primary ligands for CD28. CD28 is relevant for optimal activation and clonal expansion of CD4+ T lymphocytes. T cell receptor (TCR) signaling in the absence of CD28-costimulation results in clonal anergy. Anergic T-cells poorly proliferate to subsequent TCR stimulation even in the presence of costimulation. Here we studied whether neutrophils induced clonal anergy in allergen-specific T cells. Allergen-specific T cells were at first stimulated with either allergen-pulsed autologous HLA-DR-positive neutrophils or PBMC or synthetic peptides containing their epitopes. When re-stimulated with PBMC plus allergen, T cell cultures that had been primarily stimulated with peptide poorly proliferated. In contrast, T cells stimulated with neutrophils plus allergen demonstrated similar responses to allergen-specific re-challenge as those initially exposed to PBMC plus allergen. Neutrophils constitutively expressed CD58. The addition of anti-CD58 antibodies resulted in a markedly reduced proliferative response of T cells to specific stimulation by neutrophils. Together, these results indicate that neutrophils engage CD58 for productive T-cell activation which might amplify T-cell-mediated allergic inflammation. The latter was confirmed in a chimeric human/mouse model of birch pollen allergy. We conclude that neutrophils are relevant amplifiers of T cell-mediated inflammation in allergic diseases.

(P23) The AIT- adjuvants alum and MPLA as trigger for NET release in human neutrophils

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Insoluble aluminium salts (alum) are still the most widely used adjuvants. In allergen-specific immunotherapy (AIT) besides alum, also the TLR-4-ligand monophosphoryl-lipid A (MPLA), a detoxified derivate of LPS, is used as adjuvant. Neutrophils are the first cells to arrive at the site of vaccine injection. In response to strong or particulate stimuli they can simultaneously release cellular DNA and granular material. These so-called neutrophil extracellular traps (NETs) are not only able to trap and kill microbes, but, as modified endogenous DNA, may also represent danger-associated molecular patterns (DAMPs) that activate APCs. Therefore, adjuvant-induced NETs may play an important role in the initiation of immune responses to AIT vaccines.

Here, we used a human in vitro test system to investigate whether different adjuvants esp. alum and/or MPLA and also authentic vaccine preparations are capable to induce NET formation. NET release of stimulated neutrophils was verified by confocal fluorescence microscopy and quantitatively assessed by DNA release assays. Alum induced NETs which showed the typical co-localization of DNA and granular proteins, while very few neutrophils released typical NETs upon stimulation with MPLA or LPS. Stimulation with aluminium hydroxide induced marked DNA release, whereas aluminium phosphate was less potent, most likely due to their different surface charge. MPLA and LPS only very weakly induced DNA release and a synergistic effect of alum and MPLA was observed. Vaccines containing novel adjuvants like saponin or squalene had no such effect. The vaccines containing alum as adjuvant induced NET release but less than alum alone, whereas the one with MPLA lead to a higher response than MPLA alone.

So far, our data indicate that especially alum is a potent trigger for NET release, while MPLA alone only weakly induces NET formation. The synergistic effect of alum + MPLA may result in a superior adjuvant effect.

(P24) PIPE-cloned recombinant antibodies against the major birch pollen allergen Bet v 1 engineered with Fc domains of different isotypes

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Tailor-made antibodies of several classes and subclasses targeting the same epitope on an allergen are both interesting for functional studies and diagnostic assays in allergology. In this study we aimed at the creation of IgE, IgG_1 and IgG_4 targeting the major birch pollen allergen Bet v 1 using the cutting-edge method PIPE (Polymerase Incomplete Primer Extension) cloning (Ilieva et al., 2017). Variable region sequences (Levin et al., 2014) were engineered with constant region sequences for IgE, IgG_1 and IgG_4 using PIPE cloning. Recombinant antibodies were expressed in Expi293 cells and purified using affinity chromatography. Concentration was measured using a BCA protein assay and correct assembly was confirmed by SDS PAGE. Specificity was tested in an ELISA, dot blot and ISAC112 microarray (IgE only). Antibodies were then used in a quantitative blocking ELISA: Briefly, Bet v 1 was immobilised onto an ELISA plate and pre-incubated with IgG_1 or IgG_4, respectively. Plasma of allergic individuals was added and IgE binding to Bet v 1 was assessed with Anti-IgE antibodies. IgE concentrations were quantified using our PIPE-cloned IgE as a standard. Expression of PIPE-cloned constructs in Expi293 cells and affinity chromatography yielded high amounts of pure antibody in the range of several hundred micrograms to milligrams. SDS PAGE confirmed correct assembly and all of the used test systems (ELISA, dot blot, ISAC112) showed specific binding to Bet v 1. Furthermore, IgG_1 and IgG_4 were able to block epitopes on Bet v 1 for IgE from birch pollen allergic individuals in a concentration-dependent manner. PIPE-cloning and expression in Expi293 allows fast production of recombinant antibodies with different Fc regions targeting the same epitope on Bet v 1. As these antibodies share the same variable region, they are valuable tools for class-specific functional studies in type 1 allergy, as well as assay development.

(P25) CD47 targeted immunogene therapy for tumor eradication via immune cell activation

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The ubiquitously expressed CD47 surface protein is up-regulated in leukaemia and many solid cancers. It serves as a 'marker of self' or 'don't eat me' signal enabling cancer cells to evade the immune system, since the binding of CD47 to the cell surface receptor SIRPα on macrophages and dendritic cells (DCs) prevent cancer cell eradication. Antibodies evaluated in advanced clinical trials to block CD47 and trigger antibody directed cytotoxicity (ADCC) cause side effects like anemia, as CD47 is also expressed on non-malignant cells including erythrocytes. Our approach of immunogene therapy is the tumor restricted expression of CD47 blocking protein fused to a human IgG Fc part. Our plasmid based gene therapy vector encoding for this SIRPα-Fc fusion protein (SIRPα-Fc) consists of an IL2 sequence for secretion into the intracellular space, a SIRPα sequence as CD47 ligand and an IgG1 Fc sequence which is highly active in inducing ADCC and triggering immune response. In vitro transfection studies of triple negative human breast cancer cells with SIRPα-Fc gene therapy vector confirmed the expression, secretion and CD47 blockage of SIRPα-Fc after direct transfection or treatment with supernatant from cells transfected with SIRPα-Fc encoding plasmid. After orthotopic implantation into CB.17 SCID mice, reduced tumor growth and also tumor eradication was observed with SIRPα-Fc transfected cells when compared to controls. Luciferase-marking allowed in vivo tumor growth detection by bioluminescence imaging (BLI) and histological ex vivo analysis. In summary, we could demonstrate that tumor-restricted secretion of the CD47 binder SIRPα-Fc is a potential gene therapy approach to prevent side effects in non-target organs, while unleashing the full antitumoral potential and immune activation. This concept could be applicable for the development of novel gene vectors adapted to any kind of immune checkpoint.

(P26) Chronic CTLA4-Ig therapy leads to permanent heart allograft survival in the absence of CD40-CD40L interaction

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Costimulation Blockers as new immunosuppressive agents are challenging the predominance of calcineurin inhibitors (CNI). Regimens based on CTLA4-Ig (Belatacept) mediated blocking of the CD28-B7 pathway have demonstrated superior combined patient and graft survival, and preserved graft function, compared to CNI in kidney transplant recipients. However, Belatacept treatment was associated with an increased incidence of acute rejection episodes. We therefore sought to determine whether the concomitant blockade of the CD40-CD40L costimulatory pathway would improve the efficacy of CLTA4-Ig in a clinically relevant murine heart transplant model.

Balb/c fully mismatched donor hearts were transplanted cervically into wildtype BL/6 or $CD40L^{-/-}$ BL/6 mice. 0,25mg CTLA4-Ig (10mg/kg bodyweight) were administered i.p. on days 0, 4, 14, 28, 56 and 84. 0,2mg (low dose; LD) and 0,5mg (high dose, HD) anti-CD40-L monoclonal antibodies (MR1) were injected on days 0 and 4. The follow up was conducted for 100 days, or until cessation of the heartbeat, followed by subsequent histological analysis.

Anti-CD40-L induction monotherapy led to a prolongation of graft survival with a median survival time (MST) of 15 days in the LD (n=6) and 63 days in the HD group (n=5). The combination of anti-CD40-L induction and chronic CTLA4-Ig administration displayed a MST of 53 days in the LD MR1 (n=6) and 64 days in the HD MR1 (n=5) group. This increase was not significant compared to CTLA4-Ig monotherapy (n=7; MST 22 days). In complete absence of the CD40-CD40L interaction ($CD40L^{-/-}$ recipients) chronic CTLA4-Ig treatment led to long-term allograft survival (n=6, p=0.021 vs. chronic CTLA4-Ig), whereas hearts were rejected acutely (MST 13 days) in untreated CD40L-/- recipients.

Anti-CD40-L induction did not synergise with CTLA4-Ig treatment. However, the continuous blockade of the CD28-B7 and CD40-CD40L pathway led to excellent long-term allograft survival.

(P27) Significance of genotype-specific immunity against Helicobacter pylori antigens

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Background: Even with the best therapeutic agents available for eradication of Helicobacter pylori (HP), the re-infection risk cannot be eliminated. The best alternative would be the prophylactic vaccines- therefore identification of the HP proteins which elicit a strong immune response in human plays a crucial role in analyzing these proteins' potential to confer protective immunity. In this project, we aim to understand the clinical relevance of HP genotypes in different gastric diseases mediated by this pathogen, and evaluation of the potential cross-reactivity of subtype-specific antibodies in polyclonal sera. Methods: A protein microarray for the comprehensive screening of HP virulence factors was designed. The genes encoding the chosen virulence factors of HP were amplified from clinical HP isolates and sequenced. Phylogenetic analyses were made and most diverse strains were chosen. The genes of interest were then cloned into a mammalian expression vector. Using a cell-free protein translation system protein were translated from different genotypes and immobilized on the self-prepared Ni-NTA (nitrilotriacetic acid) glass slides. In parallel, serum samples were collected from gastritis, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma patients and the immune reactivity of these sera against HP antigens were evaluated with use of a commercially available kit. Results: We have established a protein array with several genotypes of three virulence factors of HP: OipA, CagA, and SabA. Besides, we have identified 48 serum samples (14, 5, and 29 from gastritis, gastric cancer and MALT lymphoma patients, respectively) with HP-specific antibodies so far. Conclusions: In the next step, we will screen the immune response of the clinical serum samples with use of our protein array in order to seek for disease-specific patterns, and/or cross-reactivity of subtype-specific antibodies, which would provide a strong basis for vaccine development.

Group 3: 28 - 40
Thematic Program: Molecular Signal Transduction

(P28) In vitro feeding of Ixodes ricinus and Amblyomma variegatum ticks using silicon membranes

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Studying ticks under laboratory conditions has always proven to be difficult due to their need of a blood host several times during their lifespan. Over the years, alternatives for the direct use of laboratory animals have been developed. During this study we implemented one of such alternative feeding methods, using silicon-based membranes, to feed ticks. Both *Ixodes ricinus* larvae and *Amblyomma variegatum* nymphs were successfully fed.
On average, *I. ricinus* larvae feed five to seven days and *A. variegatum* nymphs feed five to ten days on a host. During *in vitro* feeding assays, we observed an increase in feeding time for both tick species. For *I. ricinus* larvae the average feeding time was seven to ten days, while *A. variegatum* nymphs fed for fourteen to seventeen days. This prolonged feeding time is also seen in other studies and signifies the difficulties to optimise artificial feeding of ticks.
Both *I. ricinus* larvae and *A. variegatum* nymphs successfully moulted into nymphs and adults respectively. It took *I. ricinus* larvae approximately 40 days and *A. variegatum* around 45 days to moult.
Using artificial feeding systems to rear and study ticks, introduces the possibility to standardise conditions and monitor the feeding proceedings in closer detail. Likewise, transmission dynamics of pathogens transmitted by ticks can be studied without the use of laboratory animals. Namely, while ticks feed, DNA from tick-borne microorganisms can easily be detected in the blood reservoir. Blood samples, taken from the blood used to feed ticks, can then be used for culture attempts of the specific microbes to proof that viable organisms are transmitted by the studied ticks.

(P29) Novel inborn error of immunity linking aberrant cytoskeletal dynamics and severe immune dysregulation

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Disorders of immune regulation comprise a poorly characterized group of inborn errors of immunity with predominant autoimmunity and/or autoinflammation. Recently, study of patients with autoinflammatory syndromes carrying mutations in actin regulators have suggested that actin cytoskeleton integrity is key to prevent the onset of autoinflammation. However, discovery of additional molecules linking actin cytoskeleton with specific inflammatory pathways will be necessary to decipher the complex network controlling immune homeostasis in humans.

We discovered a novel gene defect affecting actin cytoskeleton dynamics in a patient with impaired immune regulation. Clinically, the patient suffered from recurrent fever, hepatosplenomegaly, infections with unknown etiology and severe amyloid A amyloidosis very early in life. Using whole exome sequencing, we identified a missense mutation in a gene encoding a guanine nucleotide exchange factor. We were able to show that this mutation leads to impaired downstream signaling indicating a loss-of-function mutation. Moreover, patient primary cells showed morphological abnormalities, including reduced polarization and decreased formation of actin-rich protrusions. Our study identifies a hitherto poorly studied protein as a key regulator of actin dynamics. Naturally occurring variants in actin-related genes will allow us to further dissect the molecular mechanisms by which the highly controlled cytoskeleton meshwork interacts with the inflammatory machinery. To complement our findings from these monogenic diseases, we are using CRISPR/Cas9-based screens to discover additional actin-related genes involved in immune homeostasis.

(P30) The role of DNA methyltransferase 1 in macrophages

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DNA methylation is the addition of a methyl group primarily on cytosine residues (5C) in CG dinucleotides and is catalyzed by DNA methyltransferases (DNMTs). In mammals, it is associated with transcriptional repression, genomic imprinting, genomic instability, and X-chromosome inactivation.

DNMT1 is a maintenance methyltransferase and has an approximately 30-fold preference for hemimethylated DNA. Maintenance transferases enable the establishment of the methylation pattern on newly synthesized DNA. DNMT1 at the replication fork interacts with cell cycle regulators, for instance PCNA, and acts as a scaffold for proteins involved in DNA replication, facilitating its function on the newly synthesized DNA.

Several knockout studies of DNMT1 have shown that ablation of DNMT1 in mice is embryonic lethal at embryonic day E9.5 and leads to a vast reduction in overall genomic methylation levels compared to those of controls. Knockout embryos also showed reduced proliferation, developmental defect, ,and increased level of apoptosis. Inhibition of DNMTs by small inhibitors such as 5-Aza-2-deoxycytidine is used as a treatment in acute myeloid leukemia as well as in myelodysplastic syndrome. Aberrant DNA methylation is implicated in autoimmune diseases, as well as inflammatory diseases such as psoriasis, sepsis and atherosclerosis. In this project, our aim is to investigate the role of Dnmt1 in macrophages using a conditional knockout mouse model. Our preliminary data show that treatment with the DNMT1 inhibitor 5-Azacytidine-2-deoxycytidine results in downregulation of DNMT1 in bone marrow derived macrophages, and that upon activation with LPS, proinflammatory cytokines exhibition differential expression in inhibitor-treated macrophages.

(P31) Allergen exposure modulates the microbial composition in the gut

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In industrialized countries, the prevalence of allergic airway diseases is constantly rising. The modified microflora hypothesis suggests a link between altered gut microbiota and the susceptibility to develop allergies in distant organs, such as the lung. In this project we aim to determine whether allergic inflammation in the lung influences the gut microbiome. We tested the impact of ovalbumin-induced allergic airway inflammation and orally-induced allergen-specific tolerance on the composition and function of the intestinal microbiome in mice. The microbial communities in cecal and fecal samples were assessed with Fourier transform infrared spectroscopy (FT-IR) and Illumina MiSeq sequencing of 16S rRNA gene amplicons. Additionally, the metabolic pattern in serum was analyzed with hydrophilic interaction chromatography/mass spectrometry (HILIC-MS). FT-IR measurements indicated shifts in the gut microbiome of allergic and tolerized mice compared to naïve mice. Analysis of 16S rRNA gene sequence data indicated an increase in relative abundances of the families Prevotellaceae and Rikenellaceae in feces of both allergic and tolerized mice compared to sham controls, while Bacteroidaceae were reduced in tolerized mice compared to allergic mice and sham controls. HILIC-MS revealed a distinct metabolite pattern in serum of sensitized mice, which exhibited reduced levels of L-carnitine and its alkylated forms compared to sham-controls. Our results indicate shifts in the microbial composition of the gut are stronger associated with tolerance induction than with airway inflammation. Characterizing the underlying mechanisms might pave the way for novel intervention strategies to prevent or treat allergic diseases targeting the gut microbiota.

(P32) Back to Basics - Characterizing the Cells of the Respiratory Tract Brings Us One Step Closer to Understanding Pneumonia

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Pneumonia is the most prevalent nosocomial infection on many intensive care units (ICU). Due to a lack of specific diagnostic tools, the approach to pneumonia on the ICU remains problematic. At the same time, it is well known that any delay in administering appropriate antimicrobial therapy leads - in the case of various nosocomial infections - to an increase in morbidity and mortality. Thus, a prompt and correct diagnosis of pneumonia is essential. Our two main aims consist of: (1) characterizing the changes occurring in the cell population landscape in pneumonia and (2) identifying correlations between cellular and clinical profiles. Together, these aims lead to a rapid identification of patients at risk of getting pneumonia, ideally before its clinical manifestation. By multicolor flow cytometry, we provide a comprehensive analysis of over 35 cell surface markers in sputum, tracheal aspirate and bronchoalveolar lavage (BAL). The study includes (a) lung-healthy volunteers, as well as (b) mechanically ventilated ICU patients without pneumonia and (c) mechanically ventilated ICU patients with pneumonia. Thus far, we were able to detect marked and constant differences in the cellular profiles of the three above-mentioned patient cohorts (a, b, c). By characterizing these patterns in pneumonia patients and by discriminating them from those induced by mechanical ventilation, this study will provide a basis for identifying individual risk factors and biomarkers of pneumonia on the intensive care unit.

(P33) Comprehensive phenotypic immune monitoring in a prospective randomized controlled trial of prophylactic use of extracorporeal photopheresis (ecp) in lung transplantation

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A prospective open randomized single center trial is currently being carried out to investigate the addition of prophylactic use of Extracorporeal Photopheresis (ECP) to a tacrolimus-based immunosuppressive regimen after lung transplantation (LuTx). To determine the immunomodulatory mechanisms of ECP a comprehensive phenotypic immune monitoring is performed including analysis of regulatory T cells and B cells. To date, 29 bilateral LuTx recipients with end-stage chronic obstructive pulmonary disease (COPD) were randomized into 2 treatment arms: standard triple immunosuppressive therapy with or without additional ECP treatments (calculated sample size: 62 patients). Each patient of the ECP group received 16 ECP treatments (8 cycles on 2 consecutive days) over a period of 13 weeks, starting within 72 hours after surgery. For monitoring leukocyte subsets such as regulatory T cells ($CD4^+CD25^+FoxP3^+$ Tregs) and B cells ($CD19^+CD5^-CD1d^+$ Bregs), polychromatic flow cytometry analysis is performed on fresh whole blood samples using validated, lyophilized monoclonal antibody panels (DuraClone). Samples were acquired before and 3 months after transplantation, when the last ECP treatment was conducted. Statistical analysis was performed using paired and unpaired t-tests. Twenty patients have reached their 3-month-visit and are analyzed here. No significant difference in the frequency of Tregs (p=0.106) or Bregs (p=0.407) between both groups was found at baseline. At 3 months, Tregs have significantly decreased in the non-treated population (p=0.001) while in the ECP-treated group no significant decline was seen (p=0.176). Regarding Bregs a trend towards a post-transplant increase was found in both groups (control p=0.075; ECP p=0.058). Preliminary data from an interim analysis of double lung transplanted patients receiving ECP as prophylactic treatment suggest that ECP prevents the posttransplant decline in Treg frequency seen with standard immunosuppression.

(P34) Isolation of exosomes from body fluids by differential ultracentrifugation

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Exosomes are 30-150nm diameter cell-derived vesicles present in all body fluids and essential for inter-cell communication. They carry specific cargoes (proteins, RNA, DNA and lipids) and so act as fingerprints of the cells they originate from. Potentially exosomes from patients could aid diagnosis and monitoring of disease. This would require robust methods for isolating pure exosomes from patient samples, and current commercial isolation kits are unable to do this. Here we have compared two principal methods of exosome isolation: ultracentrifugation (UC) and the Exoquick kit (Systems Biosciences) in order to identify a method suitable for purifying exosomes for functional and biomarker studies and for biobanking. Initial studies defined the optimal UC protocol for exosome isolation from human serum, plasma and urine: namely pre-clearing the sample by centrifuging (3000g; 30 mins); filtration through a 0,22 [my]m filter; and then ultracentrifugation (30000g; 2 h). Exosomes were pelleted (UC at 100000g; 2 h) and washed before re-pelleting and resuspending in PBS. Exoquick purification was performed as described by the manufacturer. Samples purified by Exoquick contained abundant exosome-like vesicles but these were contaminated with larger vesicles and protein/lipid clusters; Western blotting identified many additional proteins as well as exosome markers. By contrast, UC isolation resulted in a uniform population of vesicles with the expected diameter of exosomes on NTA analysis. TEM images confirmed a single population of intact vesicles with the characteristics of exosomes that expressed the exosomal proteins CD9 and CD63 by immuno-gold labelling and Western blotting without contamination. Vesicle numbers varied with the biological fluid, but the purity remained the same. In conclusion, differential UC is suitable for isolating pure exosomes from human biological fluids with high efficiency and is ideal for purifying them for a broad spectrum of applications.

(P35) Studies on the mechanism of beneficial effects of probiotic *E. coli* O83 in a mouse model of airway inflammation

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E. coli strain A0 34/86 (serotype O83:K24:H31; *E. coli* O83) is a non-pathogenic bacterial strain that is commercially available as an oral live vaccine (Colinfant New Born) and used to treat nosocomial infections and diarrhea in infants. Recently, we have shown that intranasal application of *E. coli* O83 suppressed ovalbumin (OVA)-induced allergic airway inflammation in mice in a prophylactic as well as a therapeutic setting.
In this Master thesis, the cellular and molecular mechanisms of the beneficial effect of *E. coli* O83 on allergy prevention and treatment will be further investigated. Firstly, we will study the impact of *E. coli* O83-exposure on i) the uptake of the experimental allergen OVA by hematopoietic and structural cells in the lung and on ii) the maturation and function of lung cells. Cell surface activation, maturation, and intracellular expression of cytokines will be analyzed as well. Furthermore, we will isolate and characterize *E. coli* O83-derived outer membrane vesicles (OMVs), and test them for the interaction with the host immune system and allergy prevention. Our preliminary data show that *E. coli* O83 elevated the uptake of OVA by antigen-presenting cells in the lung, nevertheless, these cells exhibited reduced levels of surface MHCII, suggesting their reduced capability to present antigen. Furthermore, we could show that OMVs isolated from *E. coli* O83 stimulated IL-8 production in the intestinal cell line HT-29. The characterization of the crosstalk between *E. coli* O83 and the host immune system is fundamental for the development of novel, effective and safe microbiota-targeted treatment strategies for the prevention and treatment of allergy and other immune-mediated disorders.

(P36) A genome-wide FACS based screen in macrophages uncovers genes involved in phagocytosis and cellular pH homeostasis

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Macrophages embody the first defense mechanisms of the immune system during the invasion of pathogens. In our recent publication, Sedlyarov et al. performed a FACS-based solute-carrier (SLC)-focused CRISPR/Cas9 mediated mutagenesis screen that employs pH sensitive reporter latex beads taken up by macrophages. Interestingly, we found that the bicarbonate transporter SLC4A7 is not only critical for phagosome acidification, a key step in the deactivation of pathogens, but also that it acts through homeostatic regulation of the cytoplasmic pH. This discovery highlights the fact that pH is one of the key drivers of cellular homeostasis. Thus, it is involved in the regulation of many cellular processes, which makes it an interesting target for future studies and potential new therapies. We followed up on these results by expanding our approach to a genome-wide scale, targeting every human gene by four guide RNAs and thereby apply FACS-based CRISPR/Cas9 mediated mutagenesis screening in human monocytic cell lines. Our dual labeled pH sensitive latex beads enter macrophages during phagocytosis and serve as a reporter to discriminate cells at different stages of the phagocytic process. FACS sorting of defined populations and subsequent NGS analysis allows us to map the genes responsible for different stages of phagocytosis and for phagosome acidification. Furthermore, employing new pH-sensitive reporters and making specific changes of the cultural milieu will allow us to look even deeper into cellular processes and get a better mechanistic understanding of how acidification in the lysosomes but also phagocytosis itself is regulated.

(P37) Identification and characterization of almond allergens

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Almond, a drupe of Prunus dulcis tree is one of the most commonly produced and consumed nuts all over the world. An accurate diagnosis in patients with suspected almond allergy is important because almond is a source of nutrition and milk products for children with other food allergies. Current diagnostic often made on the basis of routine skin prick testing and/or almond specific -IgE levels testing with whole almond protein extract is complicated by a low specificity and thus high rate of false positives. Our aim was to isolate individual known and unknown IgE-binding proteins from almond nuts and evaluate their clinical importance and involvement in cross-reactivity with other tree nuts. For the isolation of seed storage proteins, a combination of precipitation and chromatographic techniques was used. MALDI-TOF and high accurate tandem mass spectrometry were applied in order to identify the mass and the amino acid sequences of the purified proteins. Further, the immunological activity was evaluated by IgE-ELISA using sera from 31 almond allergic patients. Preliminary results showed that we purified three IgE binding almond proteins: putative almond vicilin, legumin Pru du 6) and a 13 kDa antimicrobial peptide. When tested in ELISA, 68% of almond allergic patients' sera had sIgE to almond extract. Of those, 76% contained IgE specific for Pru du 6. Initial testing showed that almond vicilin and 13 kDa antimicrobial peptide were recognized by majority of sera containing almond-specific IgE. Further biochemical and immunological characterization of the purified proteins will be performed to evaluate their relevance for diagnosis of almond allergy based on the concept of component-resolved diagnosis. We will include in the study an equal number of patients who are only sensitized but without symptoms to almond to try to distinguish between allergens specific for clinical reactivity and those involved in cross-reactivity with allergens from peanut or other tree nuts.

(P38) Rinl - a novel modulator of CD4$^+$ effector T-cells

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(P39) Reduced antibody titers and higher seronegativity rates against most common vaccine preventable diseases in adult cancer patients

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Advances in cancer therapy caused longer survival of cancer patients, leading to the necessity to adapt treatment concepts in regard to prevention of concomitant infectious diseases. This is important, because tumor disease and the intense chemotherapy make cancer patients more susceptible to infectious complications. Studies in oncologic children showed impaired seroprevalence to vaccines preventable diseases (VPDs), yet studies in adult cancer patients are scarce. In this study we examined the antibody titers and seroprevalence of antibodies against common VPDs in adults with cancer and compared them to healthy controls. Sera from 478 cancer patients and 117 controls were evaluated for antibodies against measles, mumps, rubella, varicella, hepatitis A and B, diphtheria, tetanus, pertussis and tick-borne encephalitis (TBE) by established ELISAs. In regard to age- and gender-matched controls, significantly lower antibodies in patients with solid cancers (SC) were found against measles (-15%, p=0.0002), hepatitis B (-80%, p<0.0001), diphtheria (-17%, p=0.0001), tetanus (-41%, p<0.0001) and TBE (-81%, p<0.0001), while in patients with haematological cancers (HC), antibody titers against all examined VPDs were significantly decreased, ranging from -33% (p=0.0452) for rubella to -90% (p<0.0001) for TBE. In concordance, cancer patients showed higher seronegativity rates than controls, ranging from +10% for measles to +46.61% for hepatitis B in patients with SC, and +1.12% for measles to +33.72% for hepatitis B in patients with HC. Our data show, that the susceptibility to vaccine preventable diseases in adult patients with SC and HC is higher than in healthy controls. These data indicate the necessity to include control of the vaccination status as well as vaccination programs into the treatment concept of cancer patients.

(P40) Structure based epitope grafting indicates the IgE specific to the major birch pollen allergen, Bet v 1, binds a subset of potential epitopes in a patient specific manner

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Knowledge of the epitopes bound by allergen specific IgE may aid in predicting symptom severity, cross-reactivity and efficacy of allergen immunotherapy. Most conformational IgE epitopes of the major birch pollen allergen, Bet v 1, have not been characterized, yet. We aimed to identify relevant IgE epitopes by grafting epitope-sized surface patches of Bet v 1 onto TTHA0849, a non-IgE-binding structural homologue from Thermus thermophilus. Based on a structural alignment, surface-exposed residues of TTHA0849 were replaced by corresponding ones of Bet v 1 while preserving the hydrophobic core. Thereby, we created 14 chimeric proteins (TB1-TB14), each carrying a different Bet v 1-derived surface patch. Synthetic genes were expressed in Escherichia coli as 6xHis-tagged proteins and purified by metal chelate affinity chromatography. The chimeras were characterized via SDS-PAGE, MALDI-MS, circular dichroism (CD) spectroscopy and dynamic light scattering. IgE binding was assayed by ELISA using sera from 20 Bet v 1-sensitized, birch pollen-allergic patients. Until now, six chimeras were expressed as soluble proteins. Purification yielded between 2 and 58 mg per liter bacterial culture. MALDI-MS analysis revealed that the chimeras matched their theoretical masses. CD spectra showed mixed alpha-beta structures indicating correct folds. Dynamic light scattering showed <2% aggregation for 5 of 6 chimeras. IgE ELISA revealed low frequencies of IgE recognition of individual chimeras, ranging from 5% for TB14 to 15% for TB1 and TB6. TB2 did not bind IgE from any tested serum. In conclusion, structure based design of single-epitope carrying chimeric proteins yielded soluble, folded proteins. The low frequencies of IgE binding to each chimeric protein indicate that Bet v 1-specific IgE from individual patients recognizes only a minority of the potential epitopes on Bet v 1.

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(P41) Devising a dual chimeric antigen receptor system to prevent on-target off-tumor effect of engineered T cells

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CAR-T cell therapies have shown to be potent tools in the fight against cancer as validated by the recent FDA approval of two such therapies against B cell malignancies. However, a complication associated with all current CAR designs is limited tumor-specificity due to the non-existence of tumor-specific antigens for the CAR to act upon. As a consequence, CAR-T cells attack invariably healthy tissue. To overcome this impediment, we are devising a logic NOT gate CAR-T cell system based on the combinatorial recognition of at least two antigens, which we expect to confer sufficient discriminatory power for cancer therapy with much reduced off-target toxicity. To this end we co-express in addition to the tumor-associated antigen-specific CAR a second CAR with inhibitory properties iCAR) binding to a surface antigen exclusively present on healthy tissue to be protected from CAR-T-cell attack. For proof of concept and system optimization we derive the iCAR from the well characterized high affinity 1G4 TCR, which targets HLA-A201/NY-ESO-1 as model antigen and which can be precisely fine-tuned through the use of NY-ESO-1-derived altered peptide ligands within an affinity spectrum covering 6 orders of magnitude. Tumor-specificity, killing capacity and intracellular signaling characteristics will be assayed through i) conventional immunological assays and ii) a preclinical molecular imaging platform, which involves the use of protein-functionalized planar supported lipid bilayers serving as target cell surrogate in combination with advanced microscopy affording single molecule resolution.

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(P42) Vaccination with preS-based grass pollen allergy vaccine BM32 induces protective antibody responses against hepatitis B

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Hepatitis B infection (HBV) is a major public health problem. HBV virion envelope contains three surface proteins: preS1, preS2 and HBsAg. Available HBV vaccines are based mainly on the HBsAg although preS1 has been shown to be crucial for the entrance of the virus into hepatocytes. More than 10% of the population does not respond to HBsAg-based vaccines and no therapeutic vaccines are available for the treatment of chronic HBV infection. We recently found that immunotherapy with the preS1- and preS2-containing recombinant grass pollen allergy vaccine BM32 induced antibodies in immunized subjects which inhibit HBV infection in vitro. We expressed preS in E. coli and purified it to homogeneity. Furthermore, we synthesized three preS-derived peptides: peptide A comprising the epitope, involved in liver cell attachment; peptide B containing the epitope, thought to be required for inhibition of infection; and peptide C, including both regions. Sera from 130 grass pollen allergic patients, who had received 3, 4 or 5 injections of BM32 or placebo in monthly intervals, were tested. Serum levels of IgG[down]1 and IgG[down]4 antibodies against preS, preS-derived peptides were measured in a quantitative ELISA assay. We detected the induction of a robust preS-specific IgG antibody response consisting of an early induction of IgG[down]1 and a sustained IgG[down]4 response. PreS-specific IgG[down]1 and IgG[down]4 antibodies reacted with all three peptides and thus were directed against the sequences important for liver cell attachment and inhibition of infection which indicated that BM32 indeed may protect against HBV infection.

(P43) Protein phosphatase 1 as a modulator of innate immunity during CMV infection

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(P44) Impairment of TBE vaccine-induced antibody response by previous yellow fever vaccination

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Flaviviruses have an increasing global impact as arthropod-transmitted human pathogens, exemplified by outbreaks or endemic diseases caused by Zika, dengue, yellow fever YF), West Nile, Japanese encephalitis and tick-borne encephalitis TBE) viruses. Since all flaviviruses are antigenically related, they are prone to phenomena of immunological memory 'original antigenic sin') that can modulate immune responses in the course of sequential infections/vaccinations. In our study, we addressed this question by analyzing the influence of pre-existing YF vaccine-derived immunity on the antibody response to vaccination with a TBE vaccine. We compared the antibody responses of two cohorts of 28 YF pre-vaccinated and 44 flavivirus–naïve individuals to TBE vaccination. TBE virus-specific as well as broadly cross-reactive antibodies were determined by ELISA, and virus neutralizing antibodies by neutralization tests. To determine the proportions of type-specific and cross-reactive antibody subsets, depletion experiments with recombinant envelope proteins of different flaviviruses were performed. We show that pre-existing YF immunity caused a significant impairment of the neutralizing antibody response to TBE vaccination, while broadly flavivirus cross-reactive antibodies were boosted in the initial course of vaccination. These cross-reactive antibodies, however, did not contribute to neutralization of TBE and YF viruses. Considerable individual deviations from the mean values were observed in both groups. Our data point to a negative effect of pre-existing cross-reactive immunity on the outcome of flavivirus vaccination that may also pertain to other combinations of sequential flavivirus infections and/or vaccinations. These results are likely to encourage corresponding studies with other flavivirus vaccines on the market or in clinical development.

(P45) Potency of IL-2/anti-IL-2 complexes to induce tolerance in a skingraft model

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Regulatory T-cells (Treg) are commonly known as suppressor T-cells with a key role in autoimmune disease and allotransplantation models. Therefore, enhancing Treg cell numbers in vivo became an exciting strategy for tolerance induction in transplantation. Since ex vivo preparation and adoptive transfer of Tregs is accompanied by limitations, in situ expansion is an attractive research approach. Preliminary data showed that induction of tolerance towards islet allografts could be achieved by specific in vivo activation and expansion of Tregs using Interleukin-2 (IL-2) coupled to a specific antibody against IL-2 (IL-2 complexes). According to preliminary data, however, only little success was shown in a fully mismatched skin transplantation setting. To investigate the potency of IL-2 complexes to induce tolerance in a skin allograft model, fully mismatched Balb/c or minor mismatched Balb/b skin was transplanted onto C57BL/6 mice. Mice were treated with IL-2 complexes in combination with rapamycin and anti-IL-6 mab. To study the mechanisms of tolerance, anti-donor T-cell reactivity was assessed by in vitro proliferation assays (= mixed lymphocyte reaction) and donor-specific antibodies were detected by flow crossmatch. In addition, flow cytometric analyses for evaluation of specific leucocyte subpopulations within lymph nodes, spleen and skingraft were performed. We could show that the combined treatment with IL-2 complexes, mTOR inhibitor rapamycin and anti-inflammatory treatment with an IL-6 neutralizing antibody significally prolonged survival of fully mismatched skin allografts. In order to enhance the period of skingraft survival and to gain deeper knowledge of the mechanisms responsible for acute and chronic allograft rejection further experiments are warranted. Nevertheless, we consider these results encouraging and are confident to develop a protocol leading to tolerance towards an allograft without the need of lifelong immunosuppression.

(P46) Myeloid PTEN Promotes Obesity-Induced Insulin Resistance

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(P47) A platform to functionally test T cell sensitivity

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(P48) Anti-tumor activities of Chinese herbal medicine and their possible mechanism in prostate cancer cells

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Prostate carcinoma is one of the most commonly diagnosed types of malignancy and the second most common cause of cancer mortality among men in the US and in Europe. There are many different therapeutic approaches in treating the prostate cancer. As for the unfortunate group of patients which enter the progressive metastatic disease the resources remain very limited. Therefore, novel therapeutic approaches that demonstrate efficacy in prostate cancer treatment with less associated toxicities and side effects are still in an unmet need. Chinese medicinal herbs have been proven to have an anti-cancer effect, both in-vitro and in-vivo. Panax quinquefolius (PQS), Gynostemma pentaphyllum (GpM), Coptis chinensis (CC) have all been shown to exhibit anti-cancer activities in various cancer cell-lines. In this study human prostate cancer cells DU145, PC3 and immortalized benign prostate cells PNT2 cells were treated with PQS, GpM and CC herb extract. Upon cell viability assays and flow cytometric analysis of the cell cycle were performed. We were able to demonstrate the dose-dependent inhibition of the cell viability in DU145 and PC3 cells. On the contrary, the PNT2 cells were unaffected by our treatment with GpM and PQS herb extracts. Our flow cytometric analysis indicated G1/S cell cycle arrest when treated with the GpM and PQS, whereas the CC herb extract induced the G2/M cell-cycle arrest. Furthermore, we performed real-time quantitative PCR with multiple prostate cancer related proteins and were visualized by the western blot analysis. Significant changes could be observed in the TMPRSS2, ETV5, TMEM79 and ACOXL proteins, which could be the novel biomarker candidates for prostate cancer diagnosis in the future. We demonstrated that PQS, GpM and CC had good stability and quality, which might be the effective herbal remedies playing a complementary or alternative role for treating prostate cancer. This work is supported by the BMWFW (GZ 402.000/0009-WF/V/b/2016).

(P49) AID and KI67: interrelated partners in germinal center biology

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It is now well recognized that ectopic (or tertiary) lymphoid structures (ELSs) can be formed in diseased organs in association with cancer, autoimmune diseases, or infections and play important role in disease progression, either beneficial or damaging. ELSs are characterized as highly structured aggregates with separated areas of various B-cell subsets populated by T cells and follicular dendritic cells. To understand the biological mechanisms that lead to ELS formation, maintenance, and function in target disease tissues we used an integrative approach for comparing them with active lymphoid structures, known as germinal centers, GCs, which are formed in secondary lymphoid organs. Meta-analysis (GENEVESTIGATOR platform) for genes showing co-regulation with AICDA, encoding activation-induced cytidine deaminase – B-cell molecule responsible for sustained antibody diversification, pointed to MKI67 (co-expression score 0.82). MKI67 encodes a nuclear protein with complex biology, which may play a role in chromatin organization and that is often associated with cellular proliferation. Ki67 overexpression is considered as a marker of tumor cells of different origins; the role in B-cell/GC biology is much less known/understood. Immunohistochemical staining of tonsil tissue sections for AID and Ki67 revealed similarity in staining patterns with pronounced dark zone staining of GCs. Immunofluorescent double staining furthermore showed that AID+ extra-follicular B cells, with not-yet-known function, are Ki67+. In contract to B cells, the tonsillar CD3+ T-cell populations are predominantly Ki67-. In support to staining-derived information, correlation and regression analyses for AID and Ki67 within GCs (n=30) revealed significant association. Thus, Ki67 can be proposed as marker of active ELSs in diseased tissues. Supported by OeNB project N 17278

(P50) Treatment with IL-2 Complexes Effects Frequency of Plasma Cells

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Treatment with specific IL-2-anti-IL-2 antibody complex (IL-2-cplx) is known to allow the selective expansion of regulatory T cells (Tregs) and has been demonstrated to impair allograft rejection. Here, under IL-2-cplx treatment, we focused on the effect on B cell subpopulations and possible changes in the expression of markers relevant for immune response activation. C57BL/6 mice received intraperitoneal injections of either PBS (control) or IL-2-cplx (1µg IL-2/5µg anti-IL-2) on five consecutive days and were sacrificed on day 7. We used flow-cytometric analysis to investigate the frequency of Tregs and B Cells subpopulations [Naïve mature, Germinal Center (GC) B cells and Plasma Cells (PC)] with focus on the expression of MHC-II and important costimulatory molecules on B cells that are known to play a key role in immune response activation, namely CD80, CD86, and PDL-1 in samples taken from spleen and lymph nodes. Beside an expected significant increase in Tregs (CD4+CD25+FoxP3+) within spleen and lymph nodes (19.4% vs 4.2%, $p<0.001$ in spleen; 13.5% vs 5.9%, $p=0.03$ in lymph nodes; vs naïve) treatment with IL-2-cplxs resulted in elevated numbers of B220-CD138+Blimp-1+ PCs in spleen (1.57% vs 0.44%, $p=0.03$; vs naïve). Beyond that, administration of IL-2-cplxs led to an increase of B220+GL-7+Fas+ GC B cells in spleen (0.72% vs 0.53%, $p=0.01$; vs naïve) and a decrease of Bcl-6 expression within the GC B cell compartment in treated mice (30.3% vs 16.8%, $p=0.01$; vs naïve). Moreover, IL-2-cplx treatment significantly enhanced the surface expression of MHC-II (MFI=109326 vs 79233, $p<0.001$) and PDL-1 (MFI=7026 vs 5010, $p=0.001$) gated on B220+ B cells when compared to the naïve mice.

These data suggest an important role for Treg cells in PC biology that may have further implications in immune cell modulation protocols for human organ transplantation. Further research to explore the mechanism between PCs and Tregs are warranted.

(P51) NFκB signaling is dispensable for suppressive function of human regulatory T-cells

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Regulatory T-cells (Treg) and effector T-cells (Teff) share a broad range of common features while simultaneously exerting opposing functions. Parts of this discrepancy are well understood and mediated by the difference in transcription factor expression (e.g. the expression of the Treg-master regulator forkhead-box protein 3 FOXP3). However, other mechanisms still remain elusive and the overall complexity of Treg is not fully understood. In this context, NFκB signaling has been defined as an integral pathway for Teff activation and proliferation. Target gene transcription is induced through integration of different extracellular signals, including TCR signaling, and down-stream signaling through the IκB kinase and the inhibitory IκB complex. However, in contrast to Teff, little is known about NFκB signaling in human Treg. Here we show that the nuclear translocation of the active signaling compound p65 is nearly absent in human thymic-derived Treg (tTreg) after activation. By using a mutagenic IκB construct, which abrogates NF[kappa]B signaling, we could show that tTreg do not require NFκB signaling for their suppressive function. Furthermore, blocking of the NFκB pathway during in vitro induction of Treg from total $CD4^+$ T-cells with the mTOR inhibitor rapamycin, does not only create induced Treg (iTreg) with a distinct surface marker profile, but also more potent iTreg with a higher FOXP3 expression. Thus, our data add to the understanding of signal integration in Treg and might provide new strategies to selectively manipulate peripheral blood Treg and create more potent iTreg for use in therapy.

(P52) WFDC12, a potential epidermal protease inhibitor, contributes to skin homeostasis

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(P53) Osteoclasts Metabolically Adapt to Arginine Restriction

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(P54) Epithelioid cell differentiation in granulomatous disease

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Granuloma formation is central to several infectious or sterile diseases of significant morbidity and economic impact, including tuberculosis, sarcoidosis, and schistosomiasis. Granulomas are defined as aggregates of enlarged, tightly interdigitated epithelioid macrophages that form as a result of continued antigen exposure. Neither the initial stimulus for granuloma formation nor the metabolic changes occurring within macrophages as they shift towards an epithelioid phenotype have been extensively characterised to date, although these are crucial for understanding and treating granulomatous disease. We are using an in vivo ex vivo mouse granuloma model as well as bone marrow-derived macrophage cultures to study and define epithelioid granulomas in the context of pharmacological inhibition, metabolomic changes, and histological markers, with the aim of characterising the cellular and molecular changes that occur within macrophages when granulomas are formed. This presentation aims to introduce and give a summary of the current knowledge about epithelioid cell differentiation in the infectious setting of schistosomiasis, with a particular focus on nutrient sensing and metabolic alterations and their phenotypic consequences in macrophages. The results presented will pertain to our metabolic analyses, immunofluorescence of ex-vivo granulomas, and macrophage aggregation assays to identify potential drug targets.

(P55) Exploring the Functions of Metabolic Enzymes in the Nucleus

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A growing number of metabolic enzymes, which are usually localized to the cytoplasm or to mitochondria, have also been found to localize to the nucleus. In the nucleus, they can either have canonical functions which is the production of the metabolites they normally produce or have novel functions outside of their established metabolic roles. Some of these nuclear functions of metabolic enzymes are exploited by cancer cells and therefore present interesting therapeutic opportunities. However, the investigation into the nuclear role of metabolic enzymes has been complicated by the fact that some of them are essential enzymes or that knockout of the enzymes could have unintended cytoplasmic effects. Therefore, we have developed a novel screening approach to specifically degrade the nuclear fraction of more than 2000 metabolic enzymes by combining a genetic CRISPR screening with nuclear protein degradation using PROTACs. Using this strategy, we aim to assess the roles that these enzymes play in proliferation and transcription and we aim to uncover novel nuclear metabolic dependencies or vulnerabilities that could be therapeutically exploited.

(P56) Screening for cardiac amyloidosis in aortic stenosis scheduled for TAVR

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A significant number of patients with aortic stenosis (AS) also suffer from cardiac amyloidosis (CA). Since both conditions lead to significant left ventricular (LV) hypertrophy, CA in AS is difficult to detect. The present study characterized parameters for a reliable differentiation between pure AS and AS with concomitant CA.

238 consecutive patients (82.0±8.2 years; 50.2% female) were prospectively enrolled between October 2017 and February 2019. Assessment included echocardiography with strain analysis, ECG, cardiac magnetic resonance imaging (CMR), 99mTc-DPD bone scintigraphy, and serum and urine free light chain measurement. Myocardial biopsy was performed in AL-CA. ROC curve and binary logistic regression analysis were performed to evaluate the discriminative power of respective parameters.

CA was found in 11.0% (n=21), including TTR-CA (n=19), AL-CA (n=1), and combined TTR-AL-CA (n=1). Native T1 time and LV mass index on CMR, as well as relative apical longitudinal strain on echo did not differ between AS and CA-AS (P for all>0.05). Typical pattern of late gadolinium enhancement was only present in 28.6% of CA. Voltage/mass-ratio (VMR; Sokolow-Lyon index on ECG/LV mass index on echo) as well as out-of-proportion-hypertrophy (OPH; aortic peak gradient/LV mass index x stroke volume index on echo) showed excellent discriminative power for the detection of CA (AUC 0.770 and 0.812, respectively). By multivariate binary logistic regression, VMR (OR 0.304; 95% CI 0.130-0.710; p=0.006) and OPH (OR 0.121; 95% CI 0.038-0.389; p<0.0001) were significantly associated with CA.

VMR and OPH based on ECG and echocardiography allow the identification of the majority of CA among AS patients scheduled for TAVR. This is particularly important in centers lacking nuclear medicine, CMR, or hematologists/oncologists. We furthermore show here that not only TTR-CA, but also AL-CA can be present in AS.

(P57) In vitro characterization of 1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one (PVP) enantiomers.

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1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one (PVP) is a synthetic analog of the naturally-occurring stimulant cathinone, a main psychoactive compound in the Khat plant. PVP is manufactured in Asian laboratories and sold worldwide by internet vendors and street drug dealers. After high-dose or chronic misuse, PVP can cause serious adverse effects including aggression, paranoia, and seizures (EMCDDA 2016). The PVP formulation in the recreational marketplace is composed of two enantiomers: (R)- and (S)-PVP . Previous findings with racemic PVP show it acts as a potent inhibitor of DAT and NET (Meltzer et al. 2006). While these findings imply a link between transporter inhibition and adverse effects of PVP, a thorough characterization of its enantiomers is missing. Here, we examined the activity of PVP enantiomers on monoamines transporters in vitro using uptake inhibition assays, site directed mutagenesis, whole cell patch clamp and ex vivo using fast scan cyclic voltammetry (FSCV). Our findings demonstrate (S)-PVP is the bioactive enantiomer in the racemic mixture and that its binding site at DAT differs from the one of cocaine.

(P58) Implementation of real-time image-based respiratory motion compensation for cardiac MR spectroscopy

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In western countries, cardiovascular diseases are among the leading causes of death. Magnetic resonance spectroscopy (MRS) is the only tool to noninvasively detect and monitor pathological alterations of cardiac energy metabolism that do not require ionising radiation. MRS has a very high specificity in predicting disease progression and patient survival, in e.g. heart failure. (Neubauer, NEJM, 2007) The heart's position and the poor filling factor due to its shape lead to low sensitivity and high susceptibility to motion.

To increase the quality of cardiac MRS and decrease overall measurement time, we modified a fast-low-angle-single-shot (FLASH) sequence to acquire proton (^{1}H) navigator images in an interleaved manner prior to each MRS acquisition. (Hess et al, MRM, 2011) An in-house developed algorithm to reproducibly localize the heart in these low-resolution images was implemented into the manufacturer's online reconstruction software to update the position of the MRS voxel prospectively to the acquisition, which was triggered by each heartbeat. Data were acquired by placing the MRS voxel in the interventricular septum (T_R = 1500ms, T_M = 10ms, T_E = 20ms, 15 x 20 x 30 voxel size, 30 averages) using a 7 tesla (7T) Siemens MR scanner and a ^{31}P-^{1}H Rapid loop coil.

In a proof of concept we show time-efficient updates of positions using a moving phantom. Navigator feedback took less than 80ms, updating the position of the stimulated echo acquisition mode (STEAM) MRS voxel for each acquisition. In-vivo applications show significant improvements of the spectral signal amplitude compared to free-breathing acquisitions without motion compensation.

In a next step, navigation using ^{1}H images will be extended to ^{31}P MRS sequences using an in-house built device to switch dynamically between ^{1}H and ^{31}P acquisitions.

With these ongoing labour intensive and challenging developments we plan to significantly improve cardiac MRS.

(P60) Pharmacological characterization of plant-derived peptides as ligands of the kappa-opioid receptor

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(P61) Continuous Perfusion Culture Effects on the Endocrine Pancreas

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Blood glucose homeostasis is maintained through a tightly regulated signaling network that centers on the endocrine pancreas. Pancreatic islets make up the functional unit of endocrine pancreas and are composed mainly of insulin-secreting β cells and glucagon-secreting α cells. Proper secretion of these antagonistic peptide hormones is dependent on a complex interplay of numerous other secreted factors, extracellular matrix composition, and even electrical stimulation via other cells types. Numerous factors have been identified that are capable of modulating insulin and glucagon secretion – and therefore blood glucose homeostasis. GABA and serotonin are co-secreted with insulin and modulate β cell function in an autocrine fashion, for example. Others act between cell types –glucagon-like peptide 1 from α cells modulates insulin secretion, for example. Insulin and glucagon can also act on their opposing cell types in unexpected ways - glucagon can stimulate insulin secretion at low concentrations while suppressing it at higher levels. It is therefore evident that these cell types would be highly sensitive to their microenvironmental composition.
Traditional static culture techniques do not tightly control the cellular microenvironment. Cellular debris, waste, and secreted factors accumulate while various resources are depleted. This happens in a sharp, cyclic fashion as media is exchanged intermittently and cells passaged. We have begun to characterize the effects of constant media composition on murine α and β cell lines using an in-house built perfusion system at a scale suitable for simultaneous unbiased multi-omics analysis. In preliminary β cell line proteomic experiments, we have observed changes in several metabolic pathways as well as various transporters that hint at altered intracellular vesicle pH. Further characterization of these effects on cell lines and primary islets shows promise in revealing new aspects of islet biology.

(P62) Epigenome-Wide RNAi Screen Identifies a Repressor of Insulin Expression in Alpha Cells

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Within the pancreatic islets of Langerhans, alpha and beta cells tightly maintain glucose homeos previous studies have reported that loss of a single transcription factor, ARX, or gain of another, PAX4, is sufficient to impair alpha cell identity and induce beta cell characteristics. Probing this evident epigenetic plasticity could yield valuable information regarding transcriptional regulation in the endocrine pancreas. Hence, our group is interested in identifying and characterizing genes and pathways involved in the transcriptional control of insulin with the aim of inducing insulin expression in alpha cells.
We conducted a chromatin-focused RNA interference screen on the murine alpha cell line, aTC1, in search of proteins repressing insulin and Pax4 transcription. The most promising hit was an RNA-binding protein involved in mRNA splicing and degradation. Follow-up RNA sequencing confirmed the general upregulation in beta cell markers upon knockdown in aTC1 cells. Knockout experiments revealed the essentiality of the gene for alpha cell survival and proliferation. Furthermore, its function appears to be conserved in human islets, in which its knockdown induced a significant upregulation of PAX4 transcription. Further characterization of the protein via affinity proteomics revealed a strong enrichment in RNA-binding interactors, notably two known direct binders and stabilisers of Ins2 mRNA. Moreover, global splicing analysis identified multiple events (i.e. intron retention and exon skipping) in genes important for insulin secretion. Current RNA interaction and degradation experiments should provide additional information to fully functionally characterize this protein's role in the maintenance of alpha and beta cell identity.

(P63) Identification of new regulators of alpha to beta-like cell transdifferentiation

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Type 1 diabetes is characterized by the loss of pancreatic b cell mass. Regeneration of insulin-producing cells is a major target for therapy research. One promising approach is the transdifferentiation of apancreatic cells, as they are developmentally related to the beta cells. A recent study from our group reported that the anti-malarial drug Artemether is able to repress the alpha master transcription factor Arx, by inducing its translocation from the nucleus to the cytoplasm, resulting in the induction of insulin expression.
In this project we aimed to study how this drug acts “ex vivo” in pancreatic islets at a single-cell transcriptome level. We treated human and mouse islets *ex vivo* for 72 hours with artemether and studied the effects on the transcriptome at the single-cell level sing Dropseq and 10X. We were able to identify that artemether increases the proportion of alpha cells that express insulin and furthermore, express other beta-cell specific genes.
Several aspects of the mechanism by which Artemether-induced GABA signaling results in Arx translocation are still unknown. In order to elucidate this complex signaling, single-cell and genome-wide CRISPR screens will be employed using mouse pancreatic cell lines aTC1 for alpha cells, and bTC3 and Min6 for beta cells. The mouse CRISPR Brie pooled library will be in a genome-wide loss-of-function screen[6].After treatment with Artemether, GABA and DMSO, cells will be sorted according to their expression level of insulin and glucagon, which reflects their level of transdifferentiation. Genes involved in the Artemether-induced alpha to beta transdifferentiation pathway will then be identified by next-generation sequencing.
This screen will also allow us to identify key biological aspects of alpha and beta cell biology, such as factors involved in the maintenance of their identity and of insulin and glucagon expression.

(P64) T-cell - Fibroblast interactions in patients with Rheumatoid Arthritis: partners in crime?

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Rheumatoid arthritis (RA) is a chronic autoimmune disease, which affects 1% of the population and is characterized by synovial inflammation. The RA synovial tissue is composed of a variety of cell types, including fibroblast-like synoviocytes (FLS) and T-cells. Although previous publications point to a role of T-cell - FLS interactions, the main mechanism and the contribution to disease development and progression remains unknown. We therefore aimed to establish an experimental system, which allows studying T-cell – FLS interactions. RA patient derived FLS were isolated from synovial tissue that was acquired during synovectomy. Using fluorescence activated cell sorting (FACS) of patient-derived PBMCs, naïve CD4+ T-cells were gathered to establish a co-culture that allows for examining consequences of T-cell – FLS interactions. By adding different pro-inflammatory cytokines, this system allows for deciphering the effects of the inflammatory synovial environment on T-cell – FLS interaction using automated fluorescence microscopy and downstream bioinformatic image analysis. Furthermore, by re-isolation of T-cells from co-culture, effects on T-cell activation and differentiation can be investigated using flow cytometry. We successfully established a co-culture system to visualize and quantify T-cell – FLS interactions. Our data confirmed enhanced T-cell – FLS interactions, in particular when T-cells were activated using CD3/CD28 stimulation. Re-isolated T-cells from co-culture showed increased upregulation of early and late T-cell activation markers especially after pre-treatment of FLS with IFN-[gamma]. In conclusion, our data show that FLS interact with T cells, which as a consequence leads to enhanced T cell activation. These data highlight a potential role of T-cell - FLS interactions as a major driver of local inflammation. A further understanding of T-cell – FLS interactions will help to develop new therapeutic strategies for the treatment of RA patients.

(P65) Automated immuno-histo-enzymatic investigation of metabolic enzyme activity in cryosections of skin and epidermal equivalents

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(P66) Development and anticancer activity of hypoxia-activatable prodrugs of the tyrosine kinase inhibitor crizotinib

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Group 6: 67 - 79
Thematic Programs: Molecular Mechanisms of Cell Biology/ Cardiovascular and Pulmonary Disease

(P67) Functional characterization of beta-cell dedifferentiation using an endogenous reporter cell-line

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The metabolic disease type II diabetes is characterized by insulin resistance and pancreatic β -cell failure. This involves increased rates of β -cell death. However, there is a growing body of evidence that β-cell failure also includes a loss of cell identity and dedifferentiation to a progenitor or stem cell-like state. This process involves the loss of Foxo1 transcription factor activity and can be modeled pharmacologically. We aim to establish a screen to comprehensively identify all genes involved in [beta]-cell dedifferentiation and compounds that can reverse it. As a read-out, insulin and glucagon expression will be analyzed using automated microscopy and FACS-based readouts. Therefore, the insulin and glucagon gene loci in β -cell lines were edited using the PITCh (Precise Integration into Target Chromosome) system to couple expression of the respective gene to the expression of fluorescent proteins via IRES elements. We successfully generated endogenous reporter cell-lines for the expression of insulin and glucagon and showed that the fluorescent signal correlates with the expression of insulin in these edited cells. After establishing the reporter cell-lines, we started testing the PLACEBO compound library for compounds affecting the expression of insulin or glucagon in a pharmacological model of dedifferentiation. First hit compounds could be validated and were tested in human primary pancreatic islets from cadaveric donors. A further edited β -cell line expressing Cas9 will be used for a genetic screen using the CROP-seq protocol. Promising genes can then be further analyzed mechanistically.

(P68) Search for human proteins showing potential interactions with Toxocara canis MUc-3 antigen using yeast two hybrid system

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Toxocariasis is the most commonly occurring zoonotic parasitic disease in the developed countries which is caused by Toxocara sp. roundworm larvae. During infection, Toxocara larve modulates the body's immune response by producing expression-secretory products (ES). Additionally, the surface coat of this parasites is saturated with immunogenic glycoproteins. One of the main glycoproteins which is secreted by the parasite together with other excretory – secretory proteins and also expressed on the surface coat is MUC-3. The aim of the study was to identify human proteins interacting with T. canis MUC-3 antigen using the yeast two hybrid system (Y2H), which enables the detection of protein–protein (bait and prey) interactions in vivo. The bait is encoded by MUC-3 cDNA containing vector as a fusion protein with Gal4 DNA-binding domain. The prey proteins are encoded by human expression cDNA library and expressed as fusion proteins with Gal4 activation domain. Interaction between bait and pray provides the transcription activation of four reporter genes. Prey plasmids were selected on LB agar plates with ampicillin and sequenced. Obtained nucleotide sequences were subjected to bioinformatic analysis. We selected twenty nine proteins showing potential interaction with MUC-3. Among these we found proteins involved in such processes as: regulation of immune responses, cell signalling (expression and regulation of transcription factors, membrane receptors), regulation of cell cycle and apoptosis, protein modification or protein transport. The obtained data is preliminary. These interactions need to be proved by other experiments. However, the demonstrated data allows to determine possible pathways of host-parasite interactions on many different levels of the infection process.

(P69) The role of alternatively spliced STAT3 isoforms in acute myeloid leukemia

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Signal transducer and activator of transcription 3 (STAT3) is an essential transcription factor mediating cytokine signaling in various cellular mechanisms such as proliferation, cell survival, migration and differentiation. In cancer, STAT3 has been shown to act as either an oncogene or a tumor suppressor depending on the disease context. In acute myeloid leukemia (AML), STAT3 was frequently shown to be constitutively active, correlating with an adverse patient outcome. Two STAT3 isoforms are generated by alternative splicing: the full-length isoform STAT3α and the C-terminally truncated STAT3β. However, the impact of the two isoforms on cellular mechanisms involved in AML is still elusive. This study aims to investigate the distinct roles of the two alternatively spliced STAT3 isoforms on proliferation, migration and survival in AML cells. Therefore, we generate and characterize murine AML cell lines carrying the fusion oncogene MLL-AF9, one common cytogenetic abnormality involved in AML. The cell lines are derived from hematopoietic stem cells isolated from fetal livers of mice with CB57BL/6J background. Using various functional in vitro assays, we intend to elucidate the effect of transgenic overexpression or knock-out of STAT3β or STAT3α, respectively, on proliferation, tumorigenicity, survival and migration. Thus, we compare four cell lines: Stat3β transgenic, Stat3β homozygous knock-out, Stat3α heterozygous knock-out and wildtype. Preliminary results demonstrate differences in growth behavior of heterozygous knock-out of Stat3α in comparison to overexpression and knock-out of Stat3β as well as wildtype. Further functional in vitro assays are planned to investigate transwell migration behavior, methylcellulose-based colony formation and survival in presence of chemotherapeutic drugs. In addition, we aim to examine changes in STAT3 target gene expression by using RT-qPCR.

(P70) Identification of evolutionarily ancient epidermal differentiation genes by comparative analysis of organotypic models of chicken and human skin

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The skin barrier to the environment is formed by keratinocytes under the control of a specific set of epidermal differentiation genes. Comparative genomics has revealed that several mammalian epidermal differentiation genes have homologs in reptiles and birds, but comprehensive information about keratinocyte differentiation in non-mammalian species has remained elusive. Here, we established a culture protocol for chicken keratinocytes that allows, to the best of our knowledge, for the first time to model 3-dimensional differentiation of non-mammalian skin cells in vitro. By transcriptomic analysis of non-differentiated and fully differentiated keratinocytes of the chicken, we identified genes that are upregulated in their expression during skin barrier formation. Comparison with human skin models and skin in vivo showed conservation of multiple established differentiation genes, such as ABCA12 and loricrin. In addition, incompletely characterized human keratinocyte differentiation proteins, such as endonuclease U, a LY6 domain protein LY6EL), and TMEM45, were conserved in the chicken skin model. We conclude that the comparative analysis of keratinocytes from phylogenetically diverse species under defined in vitro differentiation conditions helps to identify conserved differentiation genes that are likely to control evolutionarily ancient processes during epidermal cornification.

(P71) Fish-derived low molecular weight components modify bronchial epithelial barrier properties and release of pro-inflammatory cytokines

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Background: Prevalence of fish allergy among fish-processing workers is higher than in the general population, mainly due to sensitization via inhalation. However, the response of bronchial epithelium to fish allergens has never been explored. Major fish allergens are parvalbumins (PVs) from bony fish, while cartilaginous fish and their PVs are considered less allergenic. Recent studies show that components other than proteins from the allergen source, such as low molecular weight components smaller than 3 kDa (LMC), may act as adjuvants during the allergic sensitization. Methods: We investigated the response of bronchial epithelial cells 16HBE14o- to PVs and to LMC from Atlantic cod, a bony fish, and gummy shark, a cartilaginous fish. Polarized monolayers were stimulated apically with fish PVs and/-or the corresponding fish LMC. Barrier integrity, transport of PVs across the monolayers and release of mediators were monitored. Results: Intact PVs from both fish species were rapidly internalized by the cells and transported to the basolateral side. The PVs did not disrupt the epithelial barrier nor modify the release of proinflammatory cytokines. In contrast, LMC from both species modified the physical and immunological properties of the epithelial barrier and the responses differed between bony and cartilaginous fish. While the barrier integrity was lowered by cod LMC 24 hours after cell stimulation, it was increased by up to 2.3-fold by shark LMC. Moreover, LMC from both fish species increased basolateral and apical release of IL-6 and IL-8, while CCL2 release was increased by cod but not by shark LMC. Conclusion: We demonstrated rapid transport of PVs across the epithelium which may result in their availability to antigen presenting cells required for allergic sensitization. Furthermore, different cell responses to LMC derived from bony versus cartilaginous fish were observed, which may play a role in different allergenic potentials of these two fish classes.

(P72) Assessment of tumor hypoxia with Blood and tissue oxygenation level-dependent MRI measurements as prognostic biomarkers for breast cancer aggressiveness

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Introduction: In tumors, hypoxia reflects an imbalance of oxygen delivery and consumption and is a prognostic biomarker of aggressiveness and local recurrence. To quantify the oxygenation level within a tumor, blood- BOLD) and tissue oxygenation level_dependent TOLD) MRI can be evaluated combining different intrinsic MRI contrast mechanisms T1= T[down1], T2* =T[down2up*]). Our main goal is to assess the use of both techniques to characterize the aggressiveness of different breast cancer models. Methods: Human breast cancer cells of different levels of aggressiveness were injected into the mammary gland of female nude mice. At a tumor diameter of approx. 10mm, MRI measurements were performed using a 9.4T magnet system combined with a 1H=[up1]H volume coil Bruker). The MR protocol includes a T1_RARE sequence TOLD) and T2* MGE/T2* EPI sequences BOLD). The oxygenation challenge was performed in two different ways: A stepwise increase of the oxygen level and a direct increase to 100%. Data analysis of the calculated maps was performed on a voxel-by-voxel basis using Matlab. Results First results and a comparison of the two different oxygen challenges and different aggressive breast cancer models will be presented. BOLD and TOLD provide different temporal and spatial information on breast cancer oxygenation, giving evidence of elevated hypoxia levels in aggressive tumors and providing impetus to use non-invasive BOLD/TOLD MRI for drawing conclusions regarding aggressiveness. A stepwise oxygenation or starting the measurement after oxygen saturation could lead to different information. To make the technique even more efficient, T2* EPI might also be a potential sequence for fast BOLD measurements. Conclusion Results give evidence of elevated hypoxia levels in breast cancer of high aggressiveness. First measurements provide further impetus to use non-invasive BOLD/TOLD MRI for monitoring tumor oxygenation and drawing conclusions regarding aggressiveness.

(P73) Cardiac phenotype of the Dmdmdx rat – an animal model of Duchenne muscular dystrophy (DMD)

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DMD is the most severe type among the muscular dystrophies and is characterized by progressive muscle weakness and degeneration. Besides the relatively well described skeletal muscle degeneration, DMD is also associated with cardiovascular complications. Typically DMD patients develop arrhythmias and a dilated cardiomyopathy, which account for about 40% of deaths in these patients. To date, most DMD cardiomyopathy studies have been conducted with the mdx X-linked muscular dystrophy mouse model. Even though the mdx mouse is a useful genetic and biochemical model of DMD, the human disease can only partially be mimicked. Thus, limited signs of muscular dystrophy and a milder heart phenotype occur, which results in an almost normal lifespan. A rat model of DMD Dmdmdx rats was recently generated (Larcher et al., PlosONE 2014) and might be a useful alternative animal model to study DMD. Dmdmdx rats show a more severe skeletal muscle phenotype and a reduced lifespan partly due to the development of a dilated cardiomyopathy, comparable to the human disease. The cardiac disease phenotype of Dmdmdx rats, however, has not yet been characterized in great detail. Here, we aimed to study the pathophysiology of the heart at the organ and cellular level. We found an impaired cardiac function in this Dmdmdx rats. Moreover, in dystrophic ventricular cardiomyocytes, Ca^{2+} currents were normal but intracellular Ca^{2+} transient decay and amplitude were significantly altered. Thus, the impaired cardiac function might be due to disturbed Ca^{2+} handling, independent of L-type Ca^{2+} channel abnormalities in Dmdmdx rats.

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(P74) pH dependent activation of TALK1 channel

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TALK1 channel belongs to the two pore K+ [K2P] channel family and it functions as a dimer. The individual subunit is composed of four transmembrane segments and two pore forming domains. It has been shown that the TALK1 channel is pH sensitive, but until now it was not elaborated how it senses the changes of extracellular pH. Based on the predicted structure of the TALK1 channel we hypothesize that positively charged arginine 233 [R233] or lysine 84 [K84], located near the selective pore, are required for the pH sensing of the channel. In order to investigate the importance of R233 in pH sensing, we made mutants of a human TALK1 channel in pcDNA3.1 changing the R233 to the neutral valine [V] or to the negatively charged glutamic acid [E]. In the case of the K84, we have mutated it to the neutral alanine [A]. The wild type TALK1 or one of the mutated plasmids were transfected into COS-7 cells. Activation of the TALK1 channels was measured by the patch-clamp technique in whole-cell mode. In order to test the influence of different pH values on the activation of the TALK1 or the TALK1 mutated channels we exchanged the extracellular solution with different pH values in the range between 5 and 11. Our results demonstrate that the wild type TALK1 channel senses changes in the extracellular pH, exhibiting strongly high-pH activated outwardly rectifying currents. In the R233V mutated channel, the pH dependency was greatly diminished. Even more interestingly, the R233E mutant shows reverse pH dependent activation with strong low-pH activated outwardly rectifying currents. Mutating K84 to neutral alanine did not influence the pH sensing in comparison to the wild type. We propose that the sensing of extracellular pH changes is accomplished by the positively charged arginine 233, located near the selective pore. The positively charged lysine 84 does not seem to be involved in the pH sensing.

(P75) Specific Fibroblast Populations expand in the Tumor Stroma of Skin Papillomas

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Background: Because of its increasing incidences and a lack of therapeutic options for metastasizing disease, cutaneous squamous-cell carcinoma (cSCC) has become an increasing public health concern. Cancer-Associated Fibroblasts (CAFs) have emerged as potent promotors of carcinogenesis– they therefore might represent a promising new therapeutic target. However, their origin and their mechanisms of interaction with cancer cells are yet to be elucidated. In mouse dermis, fibroblasts originate from two different lineages with distinct marker expression patterns and unique functions under homeostatic conditions. Additionally, they differently respond to signals originating from neoplastic cells. We therefore hypothesize that both lineages differently affect tumor progression and metastasis as well.
Methods: By lineage tracing of fibroblast subsets in a murine model for cSCC, in vitro characterization, and in vivo Tumorxenograft-experiments, we aim at identifying how distinct CAF-subsets are implicated in tumor progression and metastasis in the skin.
Results: Lrig1+ papillary and Sca1+ reticular fibroblasts accumulate in tumorigenic stroma of cutaneous papillomas. Analyses via flow cytometry showed, that these accumulating cells are in fact Lrig1+Sca1+- double positive fibroblasts – a very scarcely represented subpopulation under physiological conditions. Additionally, a CD26-CD24+ fibroblast subpopulation expands under tumorigenic conditions. Lrig1+Sca1+ as well as CD26-CD24+ fibroblasts might therefore represent tumorspecific fibroblast subpopulations.
Conclusion: Two potentially tumorspecific fibroblast-subpopulations expand in tumorigenic stroma of cutaneous papillomas. Further characterization of these subsets via in vitro 3D-culture, via RNA-Sequencing and via in vivo Xenograft-experiments will help us uncovering the mechanisms by which CAFs support tumor development, progression and metastasis.

(P76) Screening of Modafinil analogues: Atypical dopamine re-uptake inhibitors for neurocognition enhancement

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Modafinil is a FDA-approved schedule IV drug that directly increases cortical monoamine levels, indirectly increases glutamate, serotonin, histamine and orexin levels and indirectly down regulates GABA levels in the brain. It has been studied widely for neurocognition enhancement in healthy human subjects, and found to be safe for its widespread use. The advantage of novel analogues of modafinil is their high potency, which means lower effective doses and better bioavailability over parent compound (Battleday and Brem 2015).
HEK293 cells stably expressing human isoforms of the dopamine transporter (DAT), the norepinephrine transporter (NET) and the serotonin transporter (SERT) were used for reuptake inhibition by modafinil analogues. Reuptake was measured by increasing the concentration of these compounds on all three isoforms of monoamine transporters.
The parent compound modafinil has the IC50 6.4 µM for DAT. After screening dozens of compounds, we found one which has IC50 significantly lower than modafinil for DAT and they inhibit SERT and NET non-significantly. The most potent compound for DAT has the IC50 ~ 1 µM. IC50 for SERT and NET was 66.32 µM and 57.97 µM respectively.
Previously, we have reported similar modafinil analogues with in silico, in vitro and in vivo studies (Ilic et al. 2018) and those compounds have proven to be nontoxic in animal studies. The latest compound will be further subjected to molecular docking studies with DAT to see the interaction of functional groups with binding site, followed by in vivo studies.

(P77) Myocardial and valvular characterization of a novel closed chest model of ischemic mitral regurgitation in pigs

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(P78) Life under hypoxia improves glucose tolerance in mice exposed to a high-fat diet without any impact on the lipid metabolism and mitochondrial biogenesis

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Mountain dwellers show a lower prevalence of obesity and diabetes, which might be due to the low partial oxygen pressure at high altitude. To investigate the metabolic adaptation to hypoxia, male C57BL/6J mice were pre-fattened for three months with high fat diet (HFD) followed by another three more months under hypoxic conditions (10%O [down2]). Since hypoxia reduces voluntary food intake, we examined a control group on normal air, which was restrictively fed to obtain a weight curve similar to that of hypoxia-exposed mice. Similar caloric intake associated with parallel weight changes did not indicate hypoxia induced changes in energy expenditure or food efficiency. Long term exposure to hypoxia had an anti-hyperglycaemic effect progressively reducing basal blood glucose levels over time, with prominent effects after two and three months. Furthermore, hypoxia markedly improved the glucose tolerance independently of weight changes. Circulating lipids (triglycerides, free fatty acids) and adipokines (leptin, adiponectin) were not affected by life under hypoxia. Since obesity induced insulin resistance has been attributed to impaired mitochondrial density causing reduced lipid consumption and accumulation of ectopic fat, we examined potential reversal of such disturbances under hypoxia. Hypoxia induced lowering of blood glucose, however, did neither reduce triglyceride content in muscle and liver, nor did we find any evidence for increased expression of genes involved in mitochondrial biogenesis in these tissues. Furthermore, mitochondrial density (mitochondrial/nuclear DNA) was not affected and there was no changed in fuel preference (fatty acids/glucose) in skeletal muscle examined ex vivo. The results obtained so far thus indicate that life under hypoxia strongly counteracts obesity induced hyperglycaemia in a weight independent manner, but that this is not due to reversal of impaired mitochondrial capacity.

(P79) Functional genomic investigation of NUP98-fusion proteins in leukemia

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The Nucleoporin 98 (NUP98) gene is involved in the formation of oncogenic fusion proteins. They arise from structural chromosomal rearrangements and represent one of the largest multi partner translocation families in acute myeloid leukemia (AML). Lack of knowledge of the molecular mechanisms underlying NUP98-fusion protein driven leukemogenesis has hampered the development of efficient targeting strategies for patients who are facing poor prognosis. We aim to develop new models that allow fast and reversible manipulation of fusion protein levels in leukemia cells, as a tool to better understand NUP98-fusion-specific disease mechanisms. The auxin-induced-degron (AID) system and the degradation tag (dTAG) system represent two experimental systems that enable fast depletion of tagged proteins of interest by specific proteasome-mediated degradation after ligand-induced recruitment of E3 ubiquitin ligases. Generating NUP98-fusion models that allow degradation as well as efficient detection and isolation of the fusion protein will lay the foundation of this project. Introduction of degron-HA-tagged NUP-fusion expressing constructs into fetal liver cells by retroviral transduction will be followed by transplantation into recipient mice. Efficiency of ligand-induced fusion protein degradation will be evaluated in vivo and in vitro. These models will be important tools to investigate NUP98-fusion protein dependent processes including chromatin organization (ChIP-seq) and gene expression (SLAM seq). CRISPR/Cas9-mediated genome-wide loss-of-function screens will be used to test the functional contribution of identified molecular mechanisms. Promising hits will be validated in a wide array of cell lines and xenograft (PDX) models. Novel information of critical effectors combined with their functional link to disease progression might inform diagnostic and therapeutic strategies to improve treatment of patients suffering from NUP98-fusion protein associated leukemia.

Group 7: 80 - 93
Thematic Programs: Molecular Drug Targets/ Regeneration of Bones and Joints

(P80) The role of cytosolic and mitochondrial reactive oxygen species in the transition from quiescence to an active state in hASMCs

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The replacement of parenchymal cells by stem cells is a fundamental process at the base of tissue regeneration, in which the transition from quiescence to an active state plays a crucial role. It has been suggested that reactive oxygen species (ROS) play a key role in this transition; however, this has not been yet completely elucidated. This study aimed to determine the role of cellular ROS in the proliferation of human amniotic mesenchymal stromal cells (hAMSCs). In the first part of the study we activated hAMSCs under previously established quiescent and proliferative conditions either via conditioned medium containing inflammatory mediators (IM), 1% O2, or shockwaves, and then treated with mitochondrial complex III inhibitor, antimycin A, mitochondria targeted antioxidant mitotempo, NADPH oxidase stimulator PMA, and its inhibitor apocynin. In the second part of the study hAMSCs were treated under proliferative conditions with superoxide dismutase (SOD), catalase, hydroxyl radical scavengers mannitol and thiourea, and ferrous ions. In all experiments proliferation was determined by crystal violet assay. The activation methods applied here showed only a slight increase in cell proliferation, which in case of IM was significant under both quiescent and proliferative conditions and in case of 1% O2 only under proliferative conditions. Furthermore, the treatment with antimycin A and apocynin considerably decreased cell proliferation under quiescent and proliferative conditions but only in the cells activated by IM. A similar effect was achieved also by the treatment with extracellular SOD, catalase, mannitol and thiourea. Our results suggest that the inhibition of mitochondrial electron transport chain and thus stimulation of mitochondrial ROS does not have an impact on the transition of quiescent hAMSCs to their activated state. In contrast, this transition is stimulated by the ROS generated extracellularly by NADPH oxidase, and most likely by hydroxyl radical.

(P81) Validation of commercial dose calculation algorithms for pencil beam scanning in proton therapy

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The treatment planning system (TPS, RaySearch Laboratories, Stockholm, Sweden) offers two clinical dose calculation algorithms for pencil beam scanning (PBS) in proton therapy. One is based on the Pencil Beam algorithm (RS-PB), while the other is a Monte Carlo based dose calculation algorithm (RS-MC). The purpose of this study was to provide a validation of the two dose calculation algorithms against phantom measurements in the presence of complex geometries. Focus was given on heterogeneous tissue interfaces: bone-lung, bone-soft tissue, representing common scenarios for the lung or head cancer patients. Treatment plans for the target of 4x4x4 cm[up3] located behind the interface of the heterogeneous tissues were generated in the TPS and optimized using the RS-MC algorithm. Dose measurements in and behind the target were performed in a water phantom using a 3D holder with 24 PinPoint chambers (T31015, PTW, Germany) and compared to the TPS. To mimic complex geometries, tissue equivalent slabs (bone-lung or bone-soft tissue), enclosed in a watertight holder, were placed side by side into the water phantom. RS-MC calculated dose agreed with measurements in the target as well as behind the target within [+-]3% for both heterogeneous scenarios (bone-lung, bone-soft tissue). The treatment plans subsequently recalculated with the RS-PB algorithm, agreed with measurements within [+-]5% inside the target, however up to 18% and 10% differences in dose were observed behind the target for the bone-lung and bone-soft tissue, respectively. The performed validation indicates significant dose discrepancies when using the RS-PB dose calculation. The use of RS-MC algorithm leads to more accurate dosimetric results. Consequently, RS-MC should be preferred when dealing with complex geometries.

(P82) Fibroblast growth factor receptor 4 modulates glioma cell trans-differentiation

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(P83) T cell antigen recognition in health and autoimmunity

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

(P84) The Role of Lysosomal Transcriptional Control in Adipose Tissue Macrophages

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The regulation of the immune system within adipose tissue AT) is crucial for its metabolic function. Especially in pathogenic conditions such as obesity and type 2 diabetes mellitus T2DM), this multifunctional endocrine organ relies on macrophages buffering unduly large amounts of lipids released from overloaded adipocytes. Macrophages in turn try to cope with this load by activating their autophagic system and lysosomal biogenesis. Data from this lab show an upregulation of lysosomal metabolic activity in adipose tissue macrophages ATMs) in the context of obesity. More specifically, members of the CLEAR Coordinated Lysosomal Expression and Regulation) gene expression network that command lysosomal biogenesis and activity are regulated by obesity. On the other hand, upregulation of lysosomal metabolism in ATMs appears to have a crucial effect on inflammatory and metabolic control in obese AT which might beneficially affect diabetes. However, molecular mechanisms underlying upregulation of CLEAR genes and lysosomal metabolism in macrophages in the context of obesity has not been fully elucidated yet. Transcription factors responsible for lysosomal biogenesis further engage in cancer-related processes. With the objective of getting deeper insight into how ATM lysosomes operate, the corresponding transcriptional network was evaluated and vital factors highlighted. Using an appropriate macrophage cell culture model, quantitative and qualitative evaluation of lysosomal activity is currently performed under pathophysiologically relevant conditions. In summary, I aim to identify molecular players to ameliorate inflammatory-derived AT dysregulation which might lead to novel therapeutic strategies.

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(P85) Elucidating the functional role of EL4 of SERT by employing antibodies and F(ab)s

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(P86) Assessment of Vorinostat as an acute treatment option in the neuroinflammatory form of X-linked Adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD) is a neurometabolic disease caused by loss of function of the peroxisomal transporter ATP-binding cassette subfamily D member 1 (ABCD1), leading to impaired degradation and, thus, accumulation of very long-chain fatty acids (VLCFAs). The most severe form of X-ALD, childhood cerebral ALD (CALD), results in rapidly progressive inflammatory demyelination with impaired blood-brain-barrier (BBB) permeability. Hematopoietic stem cell transplantation (HSCT) or gene therapy (HSCGT) can halt the inflammation and demyelination but is only beneficial if applied at an early stage of the disease. Hence, there is an urgent need to develop alternative treatments for patients who are diagnosed with CALD too late to undergo HSCT or HSCGT. We hypothesize that the upregulation of the related ABCD2 gene, functionally redundant with ABCD1 represents an alternative therapeutic approach. Our in vitro results indicate that the histone deacetylase (HDAC) inhibitor Vorinostat induces ABCD2 expression and increases peroxisomal VLCFA degradation in primary macrophages derived from X-ALD patients. In addition, Vorinostat lowers the expression of the pro-inflammatory gene IL12B in activated X-ALD macrophages. Based on these findings, three boys with advanced inflammatory demyelinating CALD lesions and, thus, not eligible for HSCT or HSCGT received daily Vorinostat for 20 to 90 days. Clinically, the disease progressed in all three patients. Data from one patient after Vorinostat treatment were collected and analyzed. In this CALD patient, Vorinostat normalized the permeability of the BBB, as shown by decreased albumin and immunoglobulin Ig) cerebrospinal fluid-serum ratios and abrogated the intrathecal synthesis of Ig. These results indicate that HDAC inhibitors could be beneficial for CALD patients and prompt further research to discover more specific HDAC inhibitors with lower cytotoxicity in X-ALD.

(P87) A mutation of A1-adenosine receptor (A1R-G279S) associated Parkinson's disease

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Parkinson's disease is caused by a loss of dopaminergic input from the substantia nigra to the caudate nucleus and the putamen. This results in increased muscle tone, tremor and the difficult to initiate a movement (hypokinesia/akinesia). Up to 18 gene loci have been implicated in Parkinson's disease. Recently, a mutation in the gene encoding the A1-adenosine receptor (A1R-G279S) was identified by exome sequencing in an Iranian family, which affected two male off-springs of a consanguineous couple. The affected individuals suffered from early onset L-DOPA-responsive Parkinson's disease. In the striatum, the A1-receptor is known to be co-expressed with the D1-receptor in the medium size spiny neurons of the direct pathway, where the receptors control mutually antagonistic signaling pathways. Accordingly, we investigated the impact of the variant A1R-G279S on D1-receptor mediated cAMP elevation by coexpressing the receptors in HEK293 cells. As an internal control we also expressed the wild type A1-receptor and the mutant version with the _2-adrenergic receptor. The mutant A1R-G279S did not differ from the wild type A1-receptor in its ability to inhibit isoproterenol-stimulated cAMP accumulation, when co-expressed with the _2- receptor: the agonist affinity (EC50) was comparable and the maximum inhibition (by about 80%) was similar. In contrast, co-expression with the D1-receptor indicated a complex interaction, because co-expression of A1- and D1-receptor affected cell surface receptor levels and wild type and mutant receptors differed in their ability to modulate the D1-receptor-induced cAMP response. In addition, the thermal stability of the mutant A1R-G279S was higher than that of the wild type A1R. This increased stability is likely to contribute to enhanced inhibition of D1-receptor-mediated signaling. Taken together, these data point to a causative link between the mutation and a change in dopaminergic signaling.

(P88) Does neuroinflammation drive spinal sensory activity towards patterns associated with allodynia? A voltage-sensitive dye imaging study in rat spinal cord

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(P89) Brown fat activity in mood regulation

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There is a strong link between mood and metabolic disorders Pan et al., 2012). Since the discovery of leptin Zhang et al., 1994), adipose tissues are no longer considered as passive stores of energy. Instead they actively secrete a number of factors that can modulate several physiological functions Trayhurn, 2005). Two different types of adipose tissues exist, the white adipose tissue WAT) and the brown adipose tissue BAT). It was only recently that the presence of metabolic active BAT was confirmed in adult humans Cypess et al., 2009) and increasing evidence suggests a prominent role for brown adipose tissue BAT) in the regulation of patho)-physiological metabolic processes. Given the strong bidirectional association between mood disorders and metabolic disturbances we set to explore whether BAT represents a link possibly accounting for the interconnection of emotional and metabolic disturbances. For these purposes, animal models of both increased and decreased BAT activity are employed and the emotional behavior is assessed in several behavior paradigms. We report, for the first time, that brown adipose tissue activity is associated with emotional behavior. More specifically, low brown adipose tissue activity is accompanied by increased anxiety- and depression- like behavior, while increased brown adipose tissue activity is associated with lower levels of anxiety and depression. Current ongoing work is aiming at elucidating the exact molecular mechanisms linking BAT activity and emotional behavior.

(P90) Toll-like receptors: Deciphering their role toward Borrelia spp.

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Lyme borreliosis (LB) is the most common tick-borne disease in the Northern hemisphere. Humans can act as accidental hosts when spirochaetes are transmitted during a blood meal of an infected hard tick. In Europe the species B. afzelii, B. bavariensis, B. burgdorferi, B. garinii and B. spielmanii are known to cause LB. In the United States B. burgdorferi is – to the largest part - the sole agent of disease. Little is known about the complex interactions of innate immune cells toward Borrelia spp.. Especially the sensing via pattern recognition receptors (PRRs) is of major interest. Hence, this project aims to shed light on the role of different Toll-like receptors (TLRs) during the induction of an early inflammatory response toward Borrelia spp.. Initially, the T-lymphocytic Jurkat cell line is transduced with a NF_B-inducible enhanced green fluorescent protein (GFP) reporter gene and genes for respective TLRs. Subsequently, various Borrelia spp. strains are used for co-cultivation with the reporter cells at different Multiplicities of Infection. After 24 hours the GFP-expression is analyzed by flow cytometry. Our results confirm the predominant role of TLR1/2 as a sensor toward triacylated lipoproteins. Furthermore, we were able to proof for the first time the action of TLR2/6 in detecting Borrelia spp.. It was frequently speculated that also Borrelia-specific flagellin is recognized via TLR5, which we were able to disproof. Moreover, a recently described LPS-like protein found in Borrelia spp. could not trigger a strong immune reaction via TLR4/CD14. Overall, the reporter cells provide a fast and reliable screening method for various PRRs and we were able to identify relevant TLRs for the detection of Borrelia spp. Moreover, we could pinpoint major differences between Borrelia species. Supported by the PhD program Cell Communication in Health and Disease (CCHD) by the Austrian Science Fund (FWF)

(P91) Fundamental sex differences in morphine withdrawal-induced synaptic plasticity

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Introduction: Long-term potentiation induced upon withdrawal from systemic opioids is considered a cellular mechanism underlying opioid-induced hyperalgesia (OIH). Opioids can activate microglia leading to the release of pro-inflammatory mediators, which can influence synaptic plasticity and may thus contribute to OIH. Recent studies suggest that microglia play a sex-dependent role in the development of hyperalgesia. Therefore, we tested whether sex-differences exist with respect to withdrawal-LTP in male and female rats.
Methods: We performed in vivo recordings of C-fibre evoked field potentials at the spinal cord dorsal-horn in deeply anaesthetised male and female Sprague-Dawley rats. LTP was induced by a one hour infusion of morphine (14 mg/kg i.v.) followed by abrupt withdrawal induced by spinal application of the μ opioid receptor (MOR) antagonist CTOP (10 μM).
Results: Withdrawal-LTP was robustly induced in rats of both sexes. The spinal application of the glia cell inhibitor fluoroacetate fully blocked the induction of withdrawal-LTP in males, but was ineffective in female animals. In line with this, the blockade of TNF and Il-1 receptors prevented the induction of withdrawal LTP in males, but not in female rats. The spinal blockade of MOR fully blocked the induction of withdrawal-LTP in females, but not in males. In male rats, a systemic blockade of MOR was necessary to inhibit the induction of the withdrawal-LTP, suggesting the involvement of supra spinal descending mechanisms.
Conclusion: The present study revealed substantial differences in the mechanisms of withdrawal-LTP between males and females: While withdrawal-LTP in females requires the activation of spinal MORs but not spinal glial cells, withdrawal-LTP in males depends on activation of spinal glial cells as well as TNF- and Il-1 receptors. Additionally, withdrawal-LTP in males depends on descending modulation in comparison to a purely spinal mechanism in females.

(P92) GPR55 controls functional differentiation of self-renewing epithelial progenitors for salivation

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GPR55, a lipid-sensing receptor, is implicated in cell cycle control, malignant cell mobilization, and tissue invasion in cancer. However, a physiological role for GPR55 is virtually unknown for any tissue type. Here, via in situ hybridization and immunohistochemistry we localize GPR55 to self-renewing ductal epithelial cells and their terminally differentiated progeny in both human and mouse salivary glands. Immunohistochemically we find GPR55 expression downregulated in salivary gland mucoepidermoid carcinomas and GPR55 reinstatement by antitumor irradiation, suggesting that GPR55 controls renegade proliferation. Indeed, GPR55 antagonism increases cell proliferation and function determination in primary salivary gland cultures. In addition, Gpr55-/- mice present ~50% enlarged submandibular glands with many more granulated ducts. Electron microscopy was used to visualize disordered endoplasmic reticuli and glycoprotein content. Next, we hypothesized that GPR55 could also modulate salivation and glycoprotein content by entraining differentiated excretory progeny. Accordingly, in vitro Ca – imaging was performed from the cultured salivary glands to prove that GPR55 activation facilitates glycoprotein release by itself, inducing low-amplitude Ca2+ oscillations, as well as enhancing acetylcholine-induced Ca2+ responses. Topical application of GPR55 agonists, which are ineffective in Gpr55-/- mice, into adult rodent submandibular glands increased salivation and saliva glycoprotein content in vivo. Overall, we propose that GPR55 signaling in epithelial cells ensures both the life-long renewal of ductal cells and the continuous availability of saliva and glycoproteins for oral health and food intake.

(P93) Effects of cold exposure and adrenergic activation on bone metabolism

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Brown adipose tissue (BAT) is specialized in energy expenditure through adaptive thermogenesis which is activated by cold exposure or administration of _-adrenergic agents resulting in increased energy dissipation. Chronic cold exposure has been proposed as therapeutic approach to recruit BAT and counteract weight gain in humans. However, the effects of such interventions for other organs responsive to adrenergic signals, such as the bone, are completely unknown. Previous controversial reports have found an association between bone mineral density (BMD) and brown fat activity suggesting a potential interaction between bone and BAT. Hence, we set out to investigate the effects of ß-adrenergic stimulation on bone metabolism by means of cold exposure or direct ß3-AR stimulation. C57Bl/6J mice were exposed to cold (4°C) for a short period of time (24h or 48h) which resulted not only in significantly increased thermogenic gene expression in BAT, but also affected molecular and circulating parameters of bone turnover suggesting a shift towards an osteoclastic phenotype and increased bone resorption. Although not as pronounced, subcutaneous treatment with the specific ß3-agonist CL316,243 (via osmotic mini pumps) for 7 days induced a similar switch towards enhanced bone resorption with decreased markers of osteoblast activity and increased markers of osteoclast activity as seen during short-term cold exposure. To determine the effects of chronic adrenergic stimulation on BMD, mice were exposed to cold for 6 weeks. µCT image analysis of tibiae revealed a loss of the trabecular bone volume accompanied by an increase in trabecular porosity after prolonged cold exposure. In summary, our results show that elevated adrenergic stimulation enhances bone turnover presumably through increased osteoclastic activity with deleterious effects on BMD.

Group 8: 94 - 109
Disease Development/ Tumor biology

(P94) Dissecting the role of the Calcium-Sensing Receptor in colorectal cancer

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(P95) Residual repair of Cas9-induced DNA double strand breaks in cells deficient for non-homologous end joining and alternative end joining

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During CRISPR-Cas9 gene editing, Cas9 introduces a double strand break into the genome which can be repaired by either error-free homology directed repair or one out of two error-prone repair pathways: classical non-homologous end joining or alternative end joining. Surprisingly, we observed residual error-prone repair in cells deficient in both non-homologous end joining and alternative end joining suggesting the presence of another error-prone repair pathway. To identify candidate genes whose knock-down results in abrogation of error-prone repair, we are setting up a FACS-based CRISPRi screen. By targeting Cas9 to a fluorophore and monitoring fluorescence upon CRISPR-Cas9 editing, we can distinguish cells which underwent error-prone repair by their loss of fluorescence. By further validation of candidate genes, we aim to identify an additional DNA repair mechanism that deals with Cas9-generated lesions.

(P96) Mechanism of Axl-mediated tumor progression in metastatic Colorectal Cancer

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Background: In an attempt to gain a deeper understanding of factors driving primary and metastatic colorectal cancer (CRC) recurrence and resistance to treatments, we found the protein AXL as a potential oncogenic driver in CRC patients. Based on preliminary results from a clinical phase I trial, we hypothesize that AXL has an influence on conferring resistance of colorectal cancer cells to epidermal growth factor receptor (EGFR) inhibition, a key cell growth regulator highly activated in CRC. Material and Methods: *in silico* analysis, Western Blot, RT-qPCR, Immunostainings, GEMM Results: AXL expression is associated with a mesenchymal / invasive phenotype in CRC patients and correlates with an anti-inflammatory gene signature. CRC patients with a high expression of both AXL and EGFR show a significantly worse survival compared to patients with high AXL or EGFR expression, respectively. AXL expression is predominantly found in areas adjacent to the tumor (stroma + immune cells), while EGFR expression is found in both tumor and immune cells of untreated patients. EGFR expressed by myeloid cells of the CRC stroma rather than by cancer cells themselves contribute to tumor progression [2] therefore we hypothesized that patients with stromal cells double positive for EGFR and AXL would have a worse prognosis. We identified an AXL/EGFR double-positive subset of innate immune cells infiltrating the colorectal tumors in mice. Furthermore, to investigate a potential crosstalk between AXL and EGFR, we performed a time-depended EGF stimulation experiment and could show that AXL is activated upon EGF-mediated EGFR activation. This points at an alternative ligand-independent route of Axl kinase activation, which could be one major limitation in ligand-blocking therapies. Conclusions: Our data indicates a potential crosstalk between AXL and EGFR in CRC. Therefore, we want to further characterize the role of AXL in both the clinical and preclinical setting by analyzing human CRC patient material and establishing a CRC mouse model that allows the deletion of EGFR and AXL.

(P97) Modelling co-morbidity patterns and risk in patient trajectories in lung cancer patients

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Lung cancer is the leading cause of cancer related death in Austria. Prognosis of lung cancer depends mainly on the extent of disease at the time of diagnosis, the histology of the tumor and the performance state of the patient. To further improve the prediction of prognosis in individual patients and to tailor treatment, more refined prediction models are desirable. The aim of this thesis is the development of a reliable estimation model to detect and model co-morbidity patterns and to estimate the corresponding risk in lung cancer patients from an observed patient state.
A data-driven analysis of temporal disease progression patterns will be presented using anonymized data from the Wiener Krankenanstaltenverbund (KAV) which contains radiology images, lab data and complete medical history including treatment and diagnosis information between 2010 and 2018. The entire spectrum of lung cancer and associated co-morbidities will be investigated which covers about 19 000 patients in Vienna. Calculating comorbidity indexes, diagnosis correlation measure and diagnosis trajectory clustering along with appropriate statistical tests will be used to reach goals. Furthermore, an effective algorithm to analyze radiology images will be developed using methods in the areas of statistics and pattern recognition. A strong correlation between disease occurrences and age, gender and hospital type has been found. Finding diseases playing a basic role in disease progression, analyzing disease correlation and temporal disease trajectories, estimating and preventing future disease, reducing adverse events and improving treatment plans will be the most important results.

(P98) NUP98-fusion proteins cause altered Biomolecular Condensation in Leukemia

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Biomolecular condensates (BCs) can dynamically coordinate compartmentalisation of macromolecules, which orchestrate biological processes. Their formation is often regulated by proteins with intrinsically disordered regions (IDRs), which are able to undergo liquid-liquid phase separation (LLPS).

Fusion proteins (FPs) are often strong oncogenic drivers. In acute myeloid leukemia (AML), sequences of IDR-containing proteins are frequently fused to transcriptional or epigenetic effectors. The IDR-containing Nucleoporin 98 (NUP98) protein is fused to >30 partners, and NUP98-fusion-expressing AML is associated with poor prognosis.

We hypothesised that IDR-containing NUP98-FPs alter the genesis, composition and function of nuclear condensates to induce cancer. Using biotinylated-isoxazole (b-isox)-mediated precipitation, which can be used to enrich BCs from cellular samples, we found that NUP98-FPs were highly enriched in b-isox precipitates. To investigate global, NUP98-fusion-dependent changes in the cellular condensome, we analysed b-isox-precipitates from NUP98-fusion-expressing- and control cells by Mass Spectrometry. While more than 2200 proteins were identified in all samples, known proteins capable of LLPS (FUS, EWSR1, TAF15) were strongly enriched in b-isox precipitates in a dose-dependent manner, validating the feasibility of the approach. Approximately 100 proteins were selectively recruited to BCs upon NUP98-fusion expression, including several proteins of high relevance to leukemia, such as RUNX1 or TET2. These data show that NUP98 FPs have profound effects on the global composition of BCs.

We currently use CRISPR/Cas9-mediated loss-of function screening in combination with imaging read-outs to identify critical candidates within the NUP98-FP-dependent condensome. Results from this work might help to establish altered biomolecular condensation as a novel mechanism of oncogenic transformation to pave the way for more potent treatment approaches in cancer.

(P99) Nutritional and pharmacological modulation of the Calcium-Sensing Receptor in the dextran sulphate sodium- induced colitis mouse model

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(P100) Monitoring evidence on overall survival benefits of anti-cancer drugs approved by the European Medicines Agency between 2009 and 2015

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Objective: The introduction of fast-track licensing strategies increases the approval of anti-cancer drugs with ambiguous benefit-risk profiles. Thus, in many instances there is lacking evidence about overall survival (OS) at the time of marketing authorisation. Our objective was to monitor and characterise therapies with ambiguous benefit-risk profiles and identify any post-approval updates on median OS after at least three years of approval by the European Medicines Agency (EMA). Methods: We included all originator anti-cancer drugs with initially ambiguous benefit-risk profiles that received marketing authorisation by the EMA between Jan 1, 2009 and May 31, 2015. Our monitoring timeframe was at least three years after EMA-approval. To identify study updates, the following three sources were included: clinicaltrials.gov, EPARs, and PubMed. Results: In total, we identified 102 eligible approval studies. Out of these, a negative difference in median OS or no information was available in 43 (42.2%) instances. During monitoring, 11 updates with accessible information on median OS could be identified. Including monitoring results there are still 32 remaining therapies (31.4%) where no or negative information (n=27 [26.5%] and n=5 [4.9%], respectively) regarding median OS is present at least three years after EMA approval. Conclusion: One-third of oncology drugs with ambiguous benefit-risk profiles fail to demonstrate a survival benefit even after several years of marketing authorisation. Systematic and transparent post-approval monitoring mechanisms will be of high relevance to assure a clinically relevant patient benefit, since the trend towards faster access to medicine with uncertain benefit is increasing rather than declining.

(P101) Evaluation of ILEI/FAM3C as a prognostic marker and therapeutic target in head and neck and cutaneous squamous cell carcinomas

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Background: Though squamous cell carcinomas (SCC) of different body sites are clinically treated as separate entities, genetic studies evidence their common determinants. Head and neck SCC (HNSCC) is a highly deadly cancer with limited treatment options seeking for novel prognostic markers, therapeutic targets and predictability of responsiveness towards immunotherapy. However, we lack good preclinical models of this cancer. Thus, translational research on HNSCC could benefit from findings on other SCC entities. Attractive cutaneous SCC (cSCC) murine models could serve as suitable validation tool for HNSCC. Our focus is to evaluate the prognostic role of Interleukin-like epithelial-to-mesenchymal transition (EMT) inducer (ILEI)/FAM3C in SCC. ILEI is part of the metastatic secretome of tumor cells and its increased expression and secretion stands for poor prognosis in several carcinomas. Using the HNSCC data set from TCGA, we found that high ILEI mRNA expression correlates with poor survival (n=518), and while not expressed in normal skin, we observed a differential upregulation of the protein in human cSCCs (n=230). These data indicate that ILEI may represent a prognostic marker and potential therapeutic target in SCCs. Methodolgy and Results: To validate above data in a preclinical cSCC model, we generated mice with a deletion of the *ILEI* gene in basal keratinocytes of the skin epidermis *(ILEIΔEp*) and used the DMBA/TPA chemical protocol of skin carcinogenesis. In correlation with our earlier findings, *ILEI* deletion resulted a delay in tumor on-set and a decrease in tumor number and burden. For therapeutic interventions, we also performed inducible deletion of *ILEI* in the same compartment *(ILEIΔEpER*). *ILEI* deletion in pre-existing tumors lead to a deceleration of tumor growth and to tumor shrinkage. We found that ILEI deletion affected basal keratinocyte differentiation. Since the DMBA/TPA carcinogenesis protocol depends on the pro-inflammatory action of TPA, we are currently analyzing whether *ILEI* deletion reshaped the tumor immune microenvironment. We also aim to perform expression profiling of these tumors and study the cross-relevance of ILEI in cSCC and HNSCC via meta-analysis with human HNSCC transcriptome data. We expect that our approach will accelerate translational research on ILEI in both HNSCC and cSCC.

(P102) GATA6 suppresses pancreatic cancer progression and metastasis

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and lethal epithelial cancer having a reported 5-year overall survival of only less than 5%. Although the vast majority of PDAC patients develop and die with metastasis, only very little is known about the driving molecular mechanisms. We have identified the zinc finger transcription factor GATA6 as a tumor suppressor in PDAC. Low expression or loss of GATA6 provides tumors with more aggressive features and an undifferentiated EMT-like or basal-like phenotype. In order to recapitulate the human situation of the disease we developed a novel, sophisticated GEMM for PDAC. To reproduce how genetic alterations are accumulated during the process of tumor formation, the novel model is equipped with Flp-dependent KRas[upG12D] and Cre-dependent Gata6 KO alleles. Thereby it becomes possible to delete Gata6 at various time points. Analysis of cohorts of early-, mid- and late Gata6 KO mice revealed that tumors from late KO are less differentiated basal-like phenotype indicated by ectopic expression of Tp63 and Krt14 and significantly more metastatic. These observation reproduce well the situation seen in patients with low GATA6 expressing PDAC. Gata6 KO cells isolated from murine tumors are more proliferative, have a higher migratory potential and are more invasive. As observed in GATA6 low human tumors, Gata6 KO cells respond differently to chemotherapeutic drugs. For the first time we can thereby show that GATA6 has a crucial role in blocking tumor progression and formation of metastases and change towards the more aggressive basal-like phenotype. Using this novel model we are well equipped to elucidate the complex functions of GATA6 in PDAC progression.

(P103) The role of interleukin-8 in the pathogenesis of systemic mastocytosis

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(P104) Chimeric antigen receptor (CAR)-T cell therapy for myeloproliferative neoplasms with mutated calreticulin

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Oncogenesis in the three classical BCR-ABL negative myeloproliferative neoplasms (MPNs), polycythemia vera (PV), essential thromocythemia (ET) and primary myelofibrosis (PMF) is driven by mutations in three different genes (Jak2, Mpl and Calr). Here, we focus on MPNs with mutations in the calreticulin (Calr) gene, which encodes for a chaperone that is responsible for quality control of protein folding within the endoplasmatic reticulum (ER). In MPN, however, frameshift mutations drastically change the amino acid composition and enable new interactions of mutant calreticulin (CALRmut) with the thrombopoietin receptor (MPL). The CARLmut-MPL complex is transported to the plasma membrane, where CALRmut activates MPL, leading to signaling cascade activation and clonal hematopoiesis. We aim at exploiting the presence of CALRmut at the surface of malignant cells to generate new therapy approaches for CALR driven MPNs. By developing a chimeric antigen receptor (CAR) against CALRmut, we intend to direct T cell responses towards tumor cells. Therefore, we plan to produce a CAR library using single chain variable fragments (scFvs) from different species covering a broad range of epitopes and affinities. This library will be the basis for empirical CAR development. We will screen for receptors that engage with CALRmut at the target cell surface and lead to activation of Jurkat T cells. Functional CARs will further be tested for their potential to elicit cytotoxicity in vitro using both human and mouse primary T cells. Finally, promising candidates will be applied in vivo in an immunocompetent chimeric mouse model. Currently, MPN patients lack disease-modifying therapies with the only curative measure being allogeneic hematopoietic stem cell transplantation. Hence, high-risk patients urgently need targeted treatment. Here, we intend to provide the first proof for the efficacy of CAR-T cell therapy in MPN with mutated Calr.

(P105) Expression of chondroitin sulfate proteoglycan 4 CSPG4) is influenced by the inhibition of MAPK signaling in BRAF-mutant melanoma cells

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Chondroitin sulfate proteoglycan 4 (CSPG4) is a multifunctional transmembrane proteoglycan involved in spreading, migration and invasion of melanoma. In addition to the activating BRAFV600E mutation, CSPG4 was shown to promote MAPK signaling by mediating the growth-factor induced activation of receptor tyrosine kinases. The aim of the present study was to reveal the effect of BRAF/MEK inhibition on the protein expression of CSPG4. A panel of BRAF-mutant CSPG4-positive or -negative melanoma cell lines was exposed to the BRAF inhibitors Vemurafenib (PLX4032) and Dabrafenib (GSK2118436) as well as the MEK inhibitor Trametinib (GSK1120212). The appropriate concentrations of the drugs were determined for individual experiments and the level of CSPG4 was evaluated by flow cytometry (FACS), immunofluorescence microscopy (IF), quantitative PCR (qPCR) and western blotting. The mean fluorescence intensity of the CSPG4 signal was lower in WM9, WM35 and WM164 cells exposed to PLX4032, compared to untreated cells. This was confirmed by IF analysis of PLX4032-treated WM164 cells. Western blotting revealed that CSPG4 was expressed in WM9 and WM164 cell lines as a major protein of 250kDa. The exposure of cells to BRAF/MEK inhibitors resulted in much lower levels of the CSPG4 protein, as well as decreased levels of mRNA. Interestingly, CSPG4 mRNA was significantly upregulated in PLX4032-resistant WM164 cells. In conclusion, our results indicate that the inhibition of MAPK signaling influences the expression of CSPG4. This provides the basis for further investigation of the role of CSPG4 in the development of drug-resistance in melanoma cells.

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(P106) Thiosemicarbazone copper complex stability and its influence on paraptosis induction

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(P107) Mutational Processes due to High Fat Diet Induced Sterile Inflammation

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Mutational signatures provide a new way of investigating the evolution of cancer. However, many etiologies of known signatures remain unidentified, while endogenous factors involved in DNA damage and mutagenesis remain understudied. This project aims to study how DNA repair pathways are impaired in inflammatory conditions by identifying and characterizing mutational signatures associated with high fat diet induced sterile inflammation. To do so, wild type C57BL/6J mice are placed on a normal or high fat diet and mutational signatures are tracked over time by sequencing clonally derived intestinal organoids. Analysis of the observed signatures reveals which mutational processes were at play and thus which DNA repair pathways were dysregulated under inflammatory conditions. In the second part of the project, we will validate the dysregulation of predicted pathways and generate CRISPR-Cas9 mediated knockout organoids, targeting genes in candidate pathways. In this in vitro system we can investigate functional consequences of inflammation on DNA damage repair and mutagenesis. Mutational signature analysis of knockout organoids will confirm the mechanistic connection between mutational process and resulting mutational profile. By studying the DNA damage response under inflammatory conditions, we expect to bring new understanding to the molecular basis of cancer associated inflammation and identify new markers and targets for treatment of pre-cancerous lesions.

(P108) Proteomic profiling to predict therapeutic response to anti-PD1 therapy in melanoma

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Immunotherapy gives new hope to patients with metastatic melanoma. By antibody-blockade of PD-1, T cell function can be enhanced and the immune system can selectively attack tumor cells. This shows highly durable responses, but so far no biomarkers accurately predict if patients with metastatic melanoma will respond to immunomodulatory therapy or not. The proteome of 57 baseline serum samples and 8 tumor-biopsy-derived cell cultures was analyzed. To concentrate low abundant serum proteins we performed two approaches: depleting high abundant serum proteins and enriching glycoproteins. For the tumor tissue, whole cell lysates out of cell culture pellets were processed. Comparison between responders and non-responders helped to uncover possible predictive factors. By applying a two-sided t-test, significantly up- and down-regulated proteins were determined and considered for subsequent annotation enrichment analysis, based on GO terms using DAVID Bioinformatics Resources. We were able to detect 330 proteins with serum depletion, 169 with glycoproteomics and 3341 in the cell lysates. After statistical testing we selected proteins that were over- or underrepresented with a fold change of at least 2 and a p-value below 0.05 and finally focused on the proteins that were overrepresented in the group of non-responders. We could identify an involvement of the immune response to acute phase and inflammatory processes, as well as leukocyte cell-cell adhesion and migration of leukocytes, scavenger receptor activity and RAGE receptor binding, platelet aggregation and integrin signaling. For the validation and quantification of these proteome profiles, targeted proteomics will be performed, complementing the discovery capabilities of our shotgun strategy. By identifying protein signatures representing the underlying pathophysiological processes, our findings might help to facilitate individualized therapy and avoid toxicity of ineffective therapy for patients in the future.

(P109) Establishing a model of Ewing Sarcoma in zebrafish

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Ewing sarcoma (EwS) is a malignant bone and soft tissue tumor in children and adolescents. In 85% of all cases the formation of EwS is caused by a chromosomal translocation, leading to the expression of the oncogene EWS-FLI1. This aberrant transcription factor is the main driver of the disease and leads to massive transcriptional deregulation. Although the genetic mechanism that drives EwS is well understood, an animal model adequately mimicking the disease is still lacking. One reason, why modeling attempts have remained difficult, is the elusive cell-of-origin in EwS. Several cell types including neural crest progenitor cells and mesenchymal stem cells have been proposed, but none of them have been confirmed as cell-of-origin of EwS yet. Zebrafish expressing human EWS-FLI1 in an untargeted way have been reported to develop EwS-like tumors, however at very low frequency. We reason, that targeting EWS-FLI1 to the cell-of-origin cell type will greatly enhance tumor formation. Towards this goal, we have already established a Cre-inducible zebrafish effector strain, harboring human EWS-FLI1. By crossing this strain to different Cre-driver strains, we will target EWS-FLI1 expression to distinct cell types, including neural crest and mesenchymal stem cells. In addition, we are currently following a novel approach exploring the use of regulatory elements, identified to be specifically active in EwS cells, to target EWS-FLI1 expression in zebrafish to putative cell-of-origin cells. If successful, a zebrafish EwS model will help to understand tumor initiation and progression in EwS and furthermore, will be a valuable tool to develop novel therapeutic strategies.

Group 9: 110 – 124
Thematic Programs: Preclinical and Clinical Research for Drug Development/ Organ failure-, replacement and Transplantation

(P110) Intestinal biofilms are an endoscopic feature of irritable bowel syndrome

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IBS is a multifactorial disorder with potential involvement of the gut microbiome. Recently, biofilms have been implicated in a variety of GI-diseases. Endoscopically-visible biofilms were defined as cohesive layer on the intestinal surface, which either resist detachment by jet-washing or detach in a film-like manner. The presence of biofilms was scored prospectively at the Vienna General Hospital between May and August 2018 (n=446 cases). Cases with a BBPS <6 (n=43), with non-PEG-based cleansing (n=9), or with cecum not reached (n=9) were excluded from the analysis. Mucosal/biofilm biopsies were collected from patients (n=24) and controls (n=33) and subjected to scanning electron microscopy (SEM), fluorescence in situ hybridization (FISH) and 16S-sequencing. Biofilms were identified in the ileum or colon in 61/385 colonoscopies (16%). Biofilms were prevalent in patients with IBS (47%), after transplantation (40%), ulcerative colitis (27%) and portal hypertension (19%; table 1) and were commonly located in the cecum (72%), terminal ileum (62%), ascending colon (44%) and to a lesser extent in distal parts of the colon (9%). Ileal biofilms concurred with right-sided-colonic biofilms in 78%. Two out of three patients kept their biofilm phenotype upon a follow-up colonoscopy. Bacterial origin of biofilms was validated by 16S-sequencing, FISH and SEM. 16S-analysis showed a reduced bacterial diversity and increased abundance of *Bacteroides* and *E.coli* in biofilms. FISH and SEM revealed a dense bacterial aggregate in contact with the intestinal epithelium with a thickness of up to 15 µm. Bacterial composition was similar in biofilm biopsies and flushes and distinct from normal mucosa. In our cohort endoscopically-visible biofilms are a characteristic feature of patients with IBS and after transplantation and likely caused by dysbiosis. We speculate that intestinal biofilms are causatively involved in the pathogenesis of IBS.

(P111) Cell-free DNA analysis for sensitive follow-up monitoring and early relapse detection in MYCN amplified high-risk neuroblastoma patients

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Background: To learn about the disease stage at diagnosis, the response to treatment and the occurrence of minimal residual disease (MRD) during therapy, different detection methods are applied on leukemia and solid tumor entities. More than 95% of high-risk neuroblastoma patients show disseminated tumor cells (DTCs) in the bone marrow (BM) at diagnosis. Thus, DTC detection in BM samples from these patients can be used for sensitive MRD detection. However, in case of relapses outside the BM, disease detection in this compartment may fail. Due to the minimally invasive nature and availability (e.g. left over samples), peripheral blood (PB) plasma based liquid biopsy samples can be taken repeatedly during the course of disease, enabling a precise monitoring of the disease evolution. We investigated the feasibility of MYCN copy number change detection for MRD monitoring in PB cell-free DNA (cfDNA) of neuroblastoma patients with proven MYCN amplification and compared this method to the detection of DTCs within the BM compartment.
Results: MYCN copy number changes, investigated by ddPCR, correlated with treatment response and the clinical course of 10 neuroblastoma patients. Further, MYCN copy number detection in the plasma was compared to the highly sensitive and specific detection of GD2[uppos]/CD56[uppos]/DAPI[uppos] DTCs in BM aspirates. Remarkably, in one case, despite the absence of tumor cells in the BM, elevated MYCN copy numbers (10 copies/reference) were detected in two plasma samples already six weeks before the clinical manifestation of a local relapse.
Conclusion: cfDNA from blood plasma can be used for sensitive disease monitoring and early relapse detection also in patients with localized relapse, when no BM disease can be measured.

(P112) STAT3β is a tumor suppressor in acute myeloid leukemia

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STAT3, a multifunctional regulator of transcription, is expressed in two alternatively spliced isoforms, STAT3α and truncated STAT3β. Although formerly postulated as a dominant negative form of full-length STAT3, STAT3β has been shown to have various regulatory functions and recently gained attention as a powerful anti-tumorigenic molecule in cancer. STAT3 has been found to be constitutively active in acute myeloid leukemia AML) patients, however, the role of STAT3 isoforms in AML remains elusive. Thus, we analyzed the STAT3β/α mRNA expression ratio in AML patients, where we observed that a higher STAT3β /α mRNA ratio correlates with a favorable prognosis and increased survival. Additionally, we engineered a transgenic mouse allowing for transgenic STAT3β expression resulting in balanced STAT3 isoform levels. Increased STAT3β expression resulted in delayed disease progression and extended survival in two independent AML mouse models. Our RNA-seq findings further indicate that the tumor-suppressive function of STAT3β derived from the tumor-intrinsic regulation of a small set of genes, involved in cell surface interactions at the vascular wall and mobilization. In conclusion, we demonstrate that STAT3β plays an essential tumor-suppressive role in AML.

(P113) SERUM AND URINARY BIOMARKERS FOR THE PREDICTION OF LATE ANTIBODY-MEDIATED KIDNEY TRANSPLANT REJECTION

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(P114) Development of donor-specific antibodies in murine cardiac transplant model during CTLA4 immunoglobulin treatment

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Costimulation blockade targeting B7.1/2 (Belatacept, CTLA4 immunoglobulin) is already EMA approved as immunosuppression therapy for kidney transplant patients, leading to an increased long-term graft and patient survival and an improved glomerular filtration rate. Some case reports additionally already exist for the use of Belatacept in cardiothoracic transplant patients. Patients treated with Belatacept show a significant decrease in the development of de novo donor-specific antibodies (DSA). We hypothesized that this decrease in DSA production might be due to a reduced class switch. For further understanding the impact of this costimulation blockade on the development of de novo DSAs, we investigated the effect of CTLA4Ig monotherapy in a dose dependent manner in a murine model using fully mismatched cardiac allograft (BALB/c onto C57BL/6). CTLA4Ig was injected chronically either at low dose (LD) or high dose (HD) after cardiac allograft transplantation. Endpoints of our investigations involved the observation of graft survival, graft histology and semi-quantitative analysis of MHC class I and class II specific antibodies using a MHC-specific ELISA. Treatment with LD CTLA4Ig led to an increased graft survival (MST= 36d vs. MST= 10d untreated), treatment with HD CTLA4Ig resulted in long-term graft survival (80% upon take down at d100) and inconspicuous histology. We could show that CTLA4Ig monotherapy interferes with DSA production in a dose dependent manner. In mice treated with LD CTLA4Ig the peak of donor-specific IgM was delayed compared to untreated mice and no development of donor-specific IgG1 occurred. In mice treated with HD CTLA4Ig the production of donor-specific IgM was significantly decreased and no class switch to IgG1 took place. This might indicate that CTLA4Ig significantly decreases not only the class switch from IgM to IgG1 when given at low doses but might also effectively inhibit the production of DSA at all when given at high doses.

(P115) Evaluation of the feasibility of an in-house ex-vivo cytokine release assay in the Vienna cohort of peritoneal dialysis patients.

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Complications related to peritoneal dialysis (PD) occur in a significant proportion of PD-patients, limiting the long-term applicability of this renal replacement therapy. Reduced peritoneal immunocompetence, caused by the continuous exposure to PD-fluids, prompts the need for a tool to assess peritoneal immune function. In 2 clinical trials, we established an ex-vivo stimulation assay as a method for immune function testing in PD-effluent (PDE).In this study we aim to broaden the applicability of the assay to the general PD-population by using the Vienna PD cohort and correlating the cytokine levels to clinical outcome parameters. Supernatants of 298 PDE samples from 108 patients, which had been stimulated ex-vivo with the toll-like receptor-agonists lipopolysaccharide and Pam3Cys, were available for cytokine quantification by commercially available tumor necrosis factor-a and interleukin-6 assays. Unstimulated samples of each PDE were used as control. Cytokine concentrations were analyzed using Wilcoxon matched-pairs signed rank test and a mixed model logistic regression analysis was applied to investigate the predictive value of the assay for clinical outcome parameters. Ex-vivo stimulation of peritoneal cells resulted in a dwell-time dependent increase of cytokine release. The highest cytokine levels were observed in PDE samples of patients with concurrent peritonitis, while the cytokine levels of unstimulated controls were found to be below the level of quantification of the assay. Correlation of the cytokine levels with the predefined clinical parameters revealed a certain predictive potential of the assay for the occurrence of infectious complications, as well as of ultrafiltration and technique failure. This study provides further evidence of the utility of ex-vivo induced cytokine release in PDE as a surrogate of the functional peritoneal immune competence and yields promising results concerning the value of the assay as a tool to predict clinical outcomes.

(P116) Connecting the proteome and metabolome of peritoneal dialysis effluent – influence of dwell duration and cytoprotective intervention

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Peritoneal dialysis (PD) effluent represents a rich but underexplored source of molecular markers for the prediction of clinical outcome and therapy monitoring. Novel PD fluids may enable patient-tailored interventions, such as peritoneal immunomodulation. High performance chromatographic coupled to high-resolution mass spectrometric methods allow monitoring of a myriad of analytes in parallel. In this study, we investigate the proteome and metabolome of PD effluent (PDE) samples after different dwell times and with or without intervention by alanyl-glutamine (AG) addition to PDF. The proteomes of 24 samples from six patients obtained after two PD dwell durations (16h overnight followed by 4h PET) with and without addition of 8mM AG were assessed using a refined recently established proteomics workflow. Proteome analysis and quantification was performed using isobaric mass tags and included internal standardization. Small molecule concentrations were determined by targeted metabolomics at four different dwell time points (0h, 1h, 4h, 16h) and from plasma at the 2h PET time point. We identified and quantified approximately 2700 proteins and 300 metabolites in PDE. Molecular properties correlating with membrane transport characteristics were defined by comparison with plasma proteome reference values and metabolomic plasma samples from the same PD dwells. Bioinformatic analysis of proteome-metabolome interference was employed to discriminate local and systemic regulation and transport. Furthermore, we propose mechanisms explaining the protective effects of short and elongated periods of AG-supplementation to PDF. This combinatorial investigation of proteomic and metabolomic properties of PDE represents the first cross-omics based investigation of peritoneal pathomechanisms and transport characteristics during PD. The results enable a further step to investigate the protective potential of AG-supplementation and its molecular mode of action in PD therapy.

(P117) Use of hiPSC derived podocytes for disease modelling of Drug Induced Phospholipidosis

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Drug-induced phospholipidosis (DIP) is induced by cationic amphiphilic drugs (CAD) and it is characterized by the accumulation of undegraded lipids in the form of concentric electrodense structures within the lysosomes, termed lamellar bodies. Chloroquine is a commonly used CAD for the treatment of malaria. It is also used to treat certain forms of autoimmune diseases in which a localized form of DIP in glomerular epithelial kidney cells (podocytes) can be observed that can cause proteinuria and acute kidney failure. The pathogenesis of DIP is incompletely understood and the low incidence mainly in females with systemic autoimmunity indicates a potential genetic factor. Here we use patient derived induced Pluripotent Stem Cells (hiPSCs) to investigate, in a personalized disease model, the etiology and pathogenesis of DIP. hiPSCs from a patient with DIP and a control subject were differentiated into podocyte-like cells and treated with a dose of Chloroquine similar to the drug plasma concentration found in patients (200nM) for an extended period (7 days). We confirmed by light, fluorescence and transmission electron microscopy the presence of phospholipids and lamellar bodies in iPSC from the patient but not control. Proteomic data from MALDI/TOF analysis indicated an accumulation of glycerophosphates. These results are used to investigate the metabolic pathways under treatment conditions in patient and control iPSC and to correlate the data with those from genomic and transcriptomic analysis. In conclusion, we have established a cellular model of DIP derived from an affected patient that allows to dissect the pathomechanisms and genetic background of this condition.

(P118) Generative Adversarial Nets for the Prediction of Lesions in High Risk Breast Cancer Patients

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The early recognition of breast lesions in high risk breast cancer patients is necessary to take appropriate steps towards the cure of a patient as soon as possible. High risk patients therefore have the opportunity to take part in regular screening sessions during which DC-MRI data is generated and has to be inspected by radiologists. So as to support radiologists in their decision making process AI takes on an increasing role. Here, we propose an AI – algorithm, a so-called Generative Adversarial Network [GAN] to detect lesions in high risk breast cancer patients. Longitudinal T1-weighted DC-MR images serve as data source for the GAN.

After the alignment of images belonging to the same patient, the GAN learns a distribution of healthy breast tissue in the first step. Then it is used to create an anomaly score which represents the divergence from the learned distribution of healthy tissue. This anomaly score is calculated based on difference images between time points of a patient, by a comparison with the GAN model of normal breast tissue. On image patch level, the score allows the identification of lesions in images, which have been acquired one time point earlier then they have been identified by a radiologist. The sensitivity in this case is 92.7% and the specificity is 78.6%.

(P119) Re-epithelialization studies in a novel ex vivo human skin wound-healing model

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Numerous models have been established for human wound healing studies. Here we present a novel human ex vivo wound model based on application of negative pressure inducing blistering and consequently dermal epidermal separation, to study re-epithelialization without disrupting the underlying dermal compartment. Upon experimentally induced blisters, epidermis was cut and used for staining. Wounded skin was cultivated for up to 12 days and analysed at selected time points. The effects of negative pressure on immune cells in the wounded area and on keratinocyte migration and proliferation were investigated by haematoxylin & eosin and immunofluorescence staining. We found that type IV collagen staining was confined to the base of the blister, indicating that the basement membrane remained intact. Basal keratinocytes were absent in the wounded area as evidenced by a lack of K14 staining. A single layer of keratinocytes expressing Ki67 and K14 closed the wound gap as early as 6 days after wounding and differentiated during the following days to form a multi-layered epidermis. While application of negative pressure affected neither the morphology nor numbers of Langerhans cells in the blister epidermis, we observed significantly more $CD3^+$ T cells as compared to normal controls. Additionally, we identified clusters of $CD207^-CD11c^+CD83^+$ dermal dendritic cells, which correlated with their decrease in the dermis, indicating an influx upon pressure. This model recapitulates the main features of epithelial wound regeneration, and thus can be applied for testing wound healing therapies and investigating the underlying mechanisms.

(P120) Intra bladder wall mesenchymal stem cell transplantation in management of neurogenic bladder dysfunction: a translational study

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(P121) Modelling of brain activation during a psychopharmacological challenge based on the topology of molecular targets

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Pharmacological magnetic resonance imaging (phMRI) allows the study of brain activation and network changes in response to drug application. However, fMRI is rather unspecific and does2not yield information on the specific involvement of different neurotransmitter. Considering that psychiatric disorders are causally related to alterations of a complex interplay between neurotransmitter systems, it is important to develop novel models, based on neurotransmitter action that are able to catch this complexity. The aim is to develop an approach for the characterization of the phMRI signal while including multimodal parameters, such as receptor distribution and affinity. Thirty-seven healthy volunteers (30.43 ± 9.23 years, 21 female) completed two PET/MR sessions mapping the serotonin transporter with [^{11}C]DASB. Subjects were randomly assigned to receive an intravenous selective serotonin reuptake inhibitor (SSRI) challenge (citalopram 8 mg/8 min) or placebo in a cross-over study design. Resting-state phMRI measurements were performed over a 40-minute period. To characterize the phMRI data, different models with an increasing complexity will be employed: (1) phMRI kinetic regressor approaches. (2) Inclusion of receptor distributions. (3) Inclusion of receptor affinities. No significant results were found (FWE-corrected $p<0.05$) after analyzing the drug effects when using model 1. Models 2 and 3 will be systematically implemented to better characterize the phMRI signal and thus derive a clearer picture of the molecular-functional coupling of the human brain. The potential of pharmacological imaging in humans is far from exhausted and current limitations can be overcome by developing novel modelling approaches. Furthermore, establishing models for integrated analysis of pharmacological effects based on brain activation responses, regional expression of molecular targets and their occupancy is a valuable research paradigm that may aid drug development and clinical practice.

(P122) Strategy for high-throughput chemical screening targeting solute carrier membrane transporters

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Solute carrier membrane transporters (SLCs) represent a class of around 450 proteins, acting as gatekeepers between the cell and its environment, as well as between different cellular compartments. They are involved in uptake or sensing of small molecules, such as nutrients, cofactors, some signalling molecules but also a removal of waste products of metabolism. Emerging evidence is showing that many SLCs also play a role in different diseases, such as cancer, and thus could serve as drug targets. Although SLCs are in general exquisitely druggable, setting up high-throughput (HTP) chemical screen targeting SLCs could be challenging mainly due to possible redundancies between different SLCs. Here we are presenting a strategy combining cellular thermal shift assay (CETSA) and complementation of split Nano Luciferase - This strategy is based on a shift in the temperature of protein unfolding caused by the binding of the chemical compound. A protein of interest is tagged by a small fragment of Nano Luciferase in the cells, after which the chemical compound is applied. This is then followed by heating, which causes unfolding of unbound protein, while proteins with bound chemicals remain stable. Stable protein is then quantified by a complementary part of the Nano Luciferase. This approach is suitable as a primary HTP chemical screen targeting SLCs, which could lead to developing novel specific inhibitors.

(P123) The ER (endoplasmic reticulum) in the ER (emergency room): effect of ER Stress modulation on organ injury and blood circulation in Traumatic Hemorrhagic Shock

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Traumatic hemorrhagic shock (THS) is accompanied by circulatory failure causing hypoxia and inflammation, which has been shown to induce endoplasmic reticulum (ER) stress. ER stress triggers the unfolded protein response (UPR) aiming at resolving ER stress or inducing cell death if ER stress cannot be solved. Thus, the activation of the UPR can either rescue injured cells and organs or further aggravate their dysfunction. We questioned if UPR modulation can influence the developing of organ damage following THS. THS was induced in male Sprague Dawley rats by a median laparotomy and blood withdrawal until the mean arterial pressure dropped to 35-40 mmHg. The animals were treated at the beginning of resuscitation with an activator (Tunicamycin) or an inhibitor (TUDCA) of the ER stress. Blood and tissue samples were collected 24 hours after shock for determination of UPR activation (GRP78, spliced XPB1, and CHOP). In the THS group, markers of liver damage (ALT and AST), cell/muscle injury (CK, LDH), as well as cardiac injury (troponin I and FABP3) were increased but the UPR markers analyzed were neither up-regulated at mRNA, nor at the protein level. In contrast, the treatment with Tunicamycin strongly increased UPR genes and proteins in liver, kidney, and lungs but not the heart tissue. Surprisingly, Tunicamycin treatment showed an increase in peripheral resistance and a consistent tendency to decrease organ injury. In addition, we found an inverse correlation between the release of cTnI and the spliced XBP1 expressed in the liver and the kidney. The treatment with TUDCA did not result in any remarkable alteration. In vitro, inhibition of spliced XBP1 promoted increased of liver cells injury in normoxic conditions, and after 2h of hypoxia Tunicamycin decreased AST. Our findings suggest that UPR activation has a double beneficial effect at the local and systemic levels ameliorating cell injury caused by hypoxia and improving circulation impaired by THS.

(P124) The potential influence of perfluoralkyl substances on fetal growth

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Perfluoralkyl substances (PFAS) are a group of man-made chemicals used since decades in a variety of industries around the globe. There are more than 3000 different compounds on the market and their numbers are rising. The majority of PFAS have fatty-acid-like structures. They are also known as extremely persistent. Epidemiological data show that PFAS serum concentrations of pregnant women are associated with a reduction in birth weight. Reduced birth weight can lead to serious health problems in the later life of these children. This study aims to prove the evidence for the effect of prenatal PFAS exposure to growth restricting effects on the fetus. In the Austrian cohort study NEWDA, maternal blood, cord blood and placental tissue are analysed for the concentrations of 40 different PFAS. To reduce the risk of confounding, creatinine levels in maternal serum (to control changes in renal clearance) as well as albumin levels of maternal serum and cord serum (albumin is the major ligand of PFAS) will be determined. In addition, comprehensive in vitro toxicity tests in primary human trophoblasts (hTCs) treated with five prevalent PFAS (PFOS, PFOA, PFTrDa, PFHxS and GenX) are currently performed.